PHOTOSYNTHESIS

BY
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CARNEGIE INSTITUTION OF WASHINGTON

American Chemical Society
Monograph Series

BOOK DEPARTMENT
The CHEMICAL CATALOG COMPANY, *Inc.*
19 EAST 24TH STREET, NEW YORK, U. S. A.
1926

Printed in the United States of America by
J. J. LITTLE AND IVES COMPANY, NEW YORK

GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coördinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of coöperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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Preface

The popularity which the general subject of the utilization of solar energy has enjoyed within recent years has led to much speculation regarding means of accomplishing this. These speculations have concerned themselves not only with mechanical contrivances designed to utilize the energy of the sun, but in many cases have also endeavored to explain the manner in which the green plant plays the rôle of a converter of radiant energy. In this book only the latter subject is discussed. Unfortunately much of the speculation regarding the manner in which the green plant utilizes solar energy has not been restrained by a knowledge of certain facts concerning the process. It is quite true that the subject with which this monograph deals is still in a condition of development; our knowledge of many phases of the subject is fragmentary and incomplete. There are nevertheless certain well established facts which cannot be disregarded in any consideration of the problem.

In the following pages the results of experimental investigations have been stressed rather than the conclusions which have been arrived at by observations in the field or by empirical methods. There can be little doubt that the greatest advances in our knowledge of the phenomenon of photosynthesis have been made through experimental study, that is, through analysis of the phenomenon by the exact control of the different factors which affect the process. These experimental studies have also served to emphasize the complexity of the subject and to demonstrate that great experimental skill is required in order to penetrate more deeply into the nature of the phenomenon. While the direct object of this book is to give the present status of the subject, it is hoped that it may serve to stimulate the interest of those familiar with allied branches of science and bring other and more refined methods to bear on the problems involved.

The problem of photosynthesis borders on so many sciences that there have developed a number of avenues of approach. Each of these presents an aspect somewhat different from the other, each sees certain features in relief which from another approach are only vaguely discernible. It is very much like looking at a mountain from different sides. It is as yet impossible to determine which is the surest path of ascent. But of this we can be quite certain, that a single approach will not give us a complete view of our objective. Only from knowledge obtained by a study of the different aspects can we construct a true picture.

The sciences of chemistry, plant physiology, physics, geology, oceanography and others have offered a view of the phenomenon of photosynthesis. To many viewing this process from one such restricted field it has appeared that they have obtained a perfect impression and they have endeavored to construct a complete picture on the basis of their knowledge. Very few of these pictures, which have taken the form of theories of the mechanism of photosynthesis, have stood the test of time. They have served by pointing out the need of accumulating more precise knowledge and by emphasizing the necessity of applying a wider range of vision in formulating conceptions of the working of organic nature.

Because of the fact that the phenomenon of photosynthesis has been considered from many different sides, publications on the subject are scattered through many different scientific journals. The literature of the subject is also very extensive. It has not been the object to achieve the inclusion of full references on all of the subjects discussed. Nor are all papers which have been published given discussion; this would result in great prolixity. An endeavor has been made to discuss the salient features of the subject with some consideration of those theoretical aspects which offer promise of fruitful development on the basis of experimental study.

H. A. S.

Carmel-by-the-Sea
California
April, 1926

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Chapter I

The Origin of Organic Matter and the Cosmical Function of Green Plants

1. Historical Introduction

It is the history of almost every science that in the course of its development the carefully executed and accurately recorded experiments have stood out as bright beacons to guide workers in later generations. So does a perusal of the early attempts to understand the phenomena associated with the nutrition of plants clearly reveal this fact.

Prior to 1779, the date of the publication of "Experiments upon Vegetables," by Ingen-Housz, the subject was in a highly confused condition. Practically all of the work before this time was influenced by the Aristotelian dictum that plants derive their nutrition from the soil. Against this mass of incongruous speculation there stand a few clear and beautiful observations.

The great iatro chemist van Helmont (1577-1644), endowed with an experimental attitude of mind and extraordinary clearness of perception, denied the Aristotelian doctrine of the composition of organic matter and of plant nutrition. One of his experiments has become a classic. In a pot he placed 200 pounds of thoroughly desiccated soil and planted therein a willow twig weighing 5 pounds. This was protected from dust and watered daily with rain-water. The twig took root, and after five years had grown into a small tree. It was then carefully removed from the soil and the latter was again thoroughly dried. The willow showed an increase of 164 pounds while the soil had lost only 2 ounces.

On the basis of this experiment van Helmont concluded that the great increase in the weight of the plant had not been taken from the soil. His further deductions, however, were not so fortunate. He concluded that the soil contributed nothing to the growth of the plant, that water was the only true element and that the substance of the plant had been formed entirely from the water with which it had been irrigated. Later Mariotte (1621-1684) showed that the mineral constituents of a plant which are found in the ash thereof are taken up from the soil by the plant.

That the leaves are the organs which produce the substances necessary for the development of the plant was suggested by the Italian anatomist Malpighi in the seventeenth century. By removing the cotyledons (which he regarded as true leaves) he demonstrated the importance of the leaves to the development of the plant. He noticed, moreover, that in leaves there are small openings, "which," he says, "pour out either air or moisture," though it is quite evident that Malpighi did not recognize the other function of the stomata, namely, the absorption of gases. The existence of stomata was also pointed out by Grew in 1676.

In considering the attempts which were made at this time to unravel the mystery which surrounds the growth of plants, it becomes manifest that the development of the subject was largely dependent upon that of physics and chemistry. The most confused and contradictory opinions prevailed upon such fundamental questions as the composition of the atmosphere, of water, the nature of combustion, etc. All the more remarkable are the observations of that brilliant investigator, Stephen Hales (1677-1721). On the basis of his extensive experiments he concluded that plants draw some part of their nourishment through their leaves from the atmosphere. He was probably also the first to suggest the influence of light. A contemporary of Newton, Hales regarded light as a substance and asks, "may not light which makes its way into the outer surfaces of leaves and flowers contribute much to the refining of substances in the plant?"

To these observations are to be added those of Bonnet who undoubtedly noticed the emission of bubbles of oxygen from a submerged, illuminated leaf (1754). He further established that this emission of gas ceased after dusk. Unfortunately, however, he was unable to follow up his experiments or to interpret properly the observations he had made.

These are very briefly the outstanding observational facts which had been established before the discovery of oxygen. There is no attempt made here to review the early historical development of the subject. Owing to the lack of chemical knowledge the early dissertations now naturally appear exceedingly confused and mingled with much irrelevant matter. But this was all before the discovery of oxygen and the overthrow of the phlogiston theory.[1]

In November of 1773, Pringle, then president of the Royal Society, in presenting Priestley with the gold medal of the society, delivered an address in which he, with great perspicuity, discussed the importance of Priestley's discoveries and their relation to life on the planet in supplying oxygen. This address caused widespread comment in the scientific circles of Europe and was destined to exert a great influence on the development

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[1] For the early history of photosynthesis see: Sachs' History of Botany. Translated by H. E. F. Garnesey, Oxford, 1890. Meyer, Ernst H. F., Geschichte der Botanik, Koenigsberg, 1854. Wiesner, J., Jan Ingen-Housz, Vienna, 1905. Hansen, A., Geschichte der Assimilation und Chlorophyllfunction, Leipzig, 1882. Gibson, R. J. H., Pioneer Investigators in Photosynthesis. New Phytologist, 13, 191-205 (1914). Kimpflin, G., Essai sur L'assimilation Photochlorophyllienne du Carbon. Lyon, 1908. Wittwer, W. C., Geschichtliche Darstellung der verschiedenen Lehren über die Respiration der Pflanzen. München, 1850. For accounts of van Helmont's life and work: Kopp, H., Geschichte der Chemie. 1843, I, p. 117. Meyer, E. S. C. von, A History of Chemistry. Translated by G. McGowan. London, 1891, p. 72.

of our knowledge of photosynthesis. Priestley had noticed that plants confined in an atmosphere rich in fixed air (carbon dioxide) produced in the course of several days large quantities of dephlogisticated air (oxygen). Priestley explained the phenomenon as caused by the *growth* of the plants.

About the same time Scheele, working in Sweden, occupied himself with the same subject. He arrived at results which were quite the opposite to those of Priestley. While Priestley's plants improved the air with oxygen, Scheele's plants produced carbon dioxide. As we now know, the cause of this contradiction lay in the fact that neither Priestley nor Scheele realized clearly under what external conditions the plant emitted carbon dioxide and when it emitted oxygen. Priestley industriously repeated his investigations but became confused through the irregular outcome of his experiments.

In 1768 Jean Ingen-Housz, a Dutch physician, who had been studying vaccination against small-pox in London, was called to Vienna to combat this disease which was raging in that city and had claimed many victims from the royal family. Ingen-Housz had wide scientific interests and knowledge. It was through reading the address of Pringle on Priestley's discoveries, which has already been referred to, that Ingen-Housz became filled with the desire to repeat the experiments on the production of oxygen by plants.

Through his successes with vaccination he soon won the favor of the Empress Maria Theresa and was finally given an annuity from the state which permitted him to follow his own scientific investigations. Ingen-Housz was at first primarily interested in the influence of foul and pure air on the health of man. The discoveries of Priestley, already referred to, served as a great stimulus to his studies and were the beginning of his fundamental discoveries. Thus, in the preface to his "Experiments upon Vegetables" (1779) he wrote: "The discovery of Dr. Priestley that plants thrive better in foul air than in common and in dephlogisticated air, and that plants have a power of correcting bad air, has thrown a new and important light upon the arrangements of this world. It shews . . . that the air, spoiled and rendered noxious to animals by their breathing in it, serves to plants as a kind of nourishment."

Ingen-Housz was much more fortunate in his experimentation than either Priestley or Scheele. He soon saw that the mere growth of a plant had nothing to do with the purification of the air. His experiments are masterpieces of manipulation and self-criticism. It should be remembered that this was before Lavoisier had established the nature of combustion. Step by step Ingen-Housz approached the correct interpretation of the phenomenon. The plants were able to purify bad air in a few hours when subjected to sunlight. He discovered that they absorb the air and exhale oxygen; that this action is the more active the brighter the sunlight, that in the shade the activity is less while in deeper shade and at night, plants, far from purifying the air, contaminate it as animals do; that only leaves and petioles can accomplish this, and that mature leaves give off more oxygen than young ones; that all plants in darkness, especially such parts as roots, flowers, fruits render the air impure; that high concentrations of carbon dioxide are poisonous even to plants and that the sunlight itself has no effect on the air without acting in conjunction with the plants. Besides, Ingen-Housz established a number of minor points which were all most logically presented in his publication of about 150 pages. The contradictory results of Priestley and of Scheele were explained and thus did Ingen-Housz grasp the very fundamentals of the process.

In 1784 Lavoisier established the composition of carbon dioxide and the nature of combustion. At this time the battle of opinions regarding this process was at its height, and the tremendous importance of Lavoisier's discoveries was at first not realized even by Ingen-Housz. But in his last important publication: "An Essay on the Food of Plants and the Renovation of Soils" (London 1796) he had quite freed himself from the phlogiston conceptions and succeeded in interpreting his discoveries according to the newer school of Lavoisier. Ingen-Housz now saw clearly the cosmical function of green plants, the relation between animal and plant nutrition, the closed cycle of carbon dioxide and water → organic matter + oxygen, of the organic matter as food for animals + oxygen → carbon dioxide + water. The source of the oxygen was the carbon dioxide; the combustible matter remained in the plant and served as food for plant and animal, when it was again converted into carbon dioxide by the combustion process.

There were at this time also some contributions from other workers; none of them, however, had the same clarity of vision as Ingen-Housz and could distinguish between the two functions proceeding simultaneously, photosynthesis and respiration, nor did they make use of the correct conception of the composition of carbon dioxide. Thus there arose heated arguments and polemics, more especially between Ingen-Housz and Senebier. The historical writings regarding these discussions have in general favored the contentions of Ingen-Housz. The publications of the latter are marked by a clear style of writing, succinct expression and logical sequence of reasoning. Senebier, on the other hand, while an industrious experimenter, accumulated a large mass of observational data which was published in an exceedingly prolix and poorly ordered manner and does not support a logical sequence of thought. He repeated many of the experiments of Ingen-Housz, and succeeded in demonstrating that only the green portions of leaves were photosynthetically active. He also showed that it was the light and not the heat from the sun which induced the emission of oxygen, and by use of his well known double walled bell-jars filled with colored solutions, he was able to ascribe the chief action to the red rays of the spectrum.

Thus Priestley, Ingen-Housz and Senebier may be considered the pioneers who discovered this new domain and made the first surveys thereof. Considering the status of the knowledge of chemistry at the time, their accomplishments were highly meritorious.

With the publication of "Recherches chimiques sur la végétation" in 1804 by de Saussure a further step was marked in the development of the subject. These investigations clearly indicate the tremendous change which had been wrought by the new chemistry of Lavoisier. De Saussure worked quantitatively, he asked certain questions and from his experiments received definite answers; he spoke a new language and followed a new system of thought. He definitely established the necessity of carbon dioxide for the development of plants and showed that a plant in a confined space in which the respiration carbon dioxide was absorbed by potassium hydroxide, soon succumbed. He also showed that plants in the light were capable of using more carbon dioxide than is normally in the atmosphere and that under such conditions showed increased growth—also that high concentrations of carbon dioxide are deleterious to the plant. He further demonstrated that oxygen is essential to the life of the plant; in an atmosphere of nitrogen and carbon dioxide the plant cannot survive. The quantitative relations between the amount of carbon dioxide fixed and oxygen emitted were also studied by de Saussure; he thus introduced the conception of the respiration and photosynthesis quotient. He also showed the importance of water in the photosynthetic process, and was probably the first to claim that carbon monoxide could not supplant carbon dioxide in photosynthesis. His extensive investigations of the complex gaseous exchange of succulent plants, such as cacti, were of fundamental significance in elucidating the nature of the photosynthetic process under these conditions and brought to light the intricate relations between organic acids found in these plants and the apparently abnormal conditions of the photosynthetic quotient. Unfortunately de Saussure treated the earlier literature of his subject very carelessly, so that from the reading of his works only, one would be apt to gain a rather distorted conception of the historical development of the subject.

The quarter of a century ending with the publication of "Recherches chimiques sur la végétation" in 1804 in many ways witnesses greater interest in the subject of photosynthesis than it has ever enjoyed. The general interest in the subject waned decidedly. The botanists exhibited very little regard; under the sway of the Linnéan system, they occupied themselves primarily with the describing and naming of new plants. While Linné himself probably had higher aims, he had devised a method which gave occupation to scores of botanists in the classification of plants, probably largely stimulated by the hope of the everlasting glory which falls to the discoverer of a new species. Although from a broad viewpoint photosynthesis is undoubtedly the most important function of the vegetable kingdom, from the botanists it has never received the consideration its importance demands. Hales, Priestley, Ingen-Housz, de Saussure were not botanists in the sense of their day, they were chemists. They founded the science of plant physiology, a division of the knowledge of plants which until recently has been tolerated rather than encouraged by botanists.

But it is not to any single cause or condition that the slow development of photosynthesis can be attributed. The great complexity of the subject itself is largely responsible, coupled perhaps with the unfortunate results arising from the academic division of the sciences. Photosynthesis is a phenomenon of plant life and thus naturally falls in the domain of botany. But the botanists (with a few exceptions) have either not exhibited any interest or have not been in possession of sufficient experimental skill and knowledge of other sciences to make any great advances. Photosynthesis as a chemical reaction has been dealt with by the chemist in a purely theoretical manner, in that he has endeavored to apply the knowledge gained from his chemical experiments to the reactions taking place in the green leaf with utter disregard of many very important biological facts.

Historically the next impetus which was given to the subject were the investigations and writings of Liebig in Germany and of Boussingault in France. The publication of Liebig's "Die Organische Chemie in Ihrer Anwendung auf Agricultur und Physiologie" in 1840 exerted profound influence on the application of the young science of organic chemistry to various phases of life activities. Through Liebig's clear exposition the origin of carbon compounds in plants was again correctly explained. The humus theory, according to which the brown amorphous substance of the soil afforded the chief source of nutrition, was refuted. It was shown that the humus of the soil comes from the decomposition of carbon compounds of plants and the chief source of carbon in plants is the carbon dioxide of the atmosphere which is absorbed by the leaves and through the action of sunlight is converted into compounds of high complexity. These latter substances in turn serve the plant as material for its respiratory activity and for its growth. Similarly the plant is capable of synthesizing complex organic nitrogen compounds from the simple compounds such as ammonia and nitric acid. The animal, unlike the plant, is incapable of effecting any of these syntheses, of the inorganic to the organic. The animal is therefore entirely dependent for its existence upon the synthetic activities of plants. Thus did Liebig clearly outline the function of plants on the earth and their relation to animal life, particularly as expressed in the importance of agriculture to human existence and civilization. He further elucidated the importance of mineral fertilizers, rotation of crops and many other fundamental principles of agriculture and showed clearly that the new science of chemistry was destined to be of enormous importance in the physiology of both plants and animals.

Liebig's views rested principally on the results of his predecessors, Priestley, Ingen-Housz, Senebier, de Saussure, on general considerations and observations and on calculations. He followed rather a deductive method than an experimental one.

In France at the same time Boussingault was patiently following the course of pure induction. Sometime before the appearance of Liebig's book Boussingault had begun experiments on the nutrition of plants. His first results were not decisive, but he persisted and between 1851 and 1855 perfected his method of water cultures. He was thus able definitely to demonstrate that higher plants are not capable of assimilating atmospheric nitrogen, but that when they are supplied with nitrogen from the nitrates of the soil they develop normally. He further showed that plants can develop in a soil in which all traces of organic substance have been removed and that all of the carbon in such plants is derived from the atmosphere. This was an experimental refutation of the humus theory and clearly showed that the favorable effect of a soil rich in humus must be due to other causes than those which were maintained by the proponents of the humus theory. Boussingault's greatest service, however, probably was in the demonstration of the fact that all of these questions are amenable to experimental treatment, difficult and time-consuming though the method may be.

Great as the influence of Liebig's writings was in demonstrating the application of organic chemistry to biological problems, the development of organic chemistry was destined to follow a course which for a long time neglected a consideration of the synthesis of organic substances by the plant. Through the discovery by Wöhler of the synthesis of urea from ammonium cyanate the theory was refuted that the carbon compounds found in plants and animals, the "organic" compounds, were the result of a vital force peculiar to living things. Thereafter a continuously increasing number of such organic compounds was synthesized in the laboratory and it was concluded that the intermediary action of living things was not essential to the production of most of the substances found in nature. The chemist had devised a number of short cuts and could, in fact, greatly improve on nature in the production of the multifarious substances of use in industry and the arts. Under the leadership of men like Dumas, Laurent, Gerhardt, Kekulé, Hofmann and Baeyer the primary interest was centered about considerations of constitution, structure and synthesis. The tremendous development which the study of carbon compounds has enjoyed since the middle of the nineteenth century together with the stimulus of the commercial application of synthetic products and the development of new processes, for a considerable time forced the study of the chemistry of living things into second place. At the same time, however, the tremendous mass of information regarding carbon compounds which was thus accumulated served as a logical prerequisite to a rational study of the chemistry of living things and as the most valuable tool in the unravelling of the tangle of chemical reactions which go to make up the various life processes.

It is, of course, true that the study of the chemical changes taking place in living things has been assiduously pursued for many years. When, with the development of organic chemistry this discipline became a separate division of science, the study of biological chemistry also enjoyed a rapid and fruitful development. Its efforts were, however, both in research and teaching, very largely devoted to investigations on animals and relatively little attention was devoted to the chemistry of plants. This fact undoubtedly, to a large measure, accounts for the difficulty of enlisting the interest of chemists in the subject of photosynthesis and the slow progress which has been made in arriving at a clearer understanding of the chemical reactions involved.

In certain respects the present has its points of similarity with the time of Wöhler and Liebig. Through the exhaustive studies of the nutritional requirements of animals it has been found that besides the materials which supply energy for the maintenance of bodily activity, the nitrogenous materials necessary for the building of tissue and the mineral nutrients, there are certain essential accessory factors which, as it were, serve to maintain the "spark of life." These substances called vitamines have been variously classified according to their origin, solubility in various solvents and their specific action in preventing certain pathological conditions and stimulating particular functions of the body. The vitamines from the chemical viewpoint have proven to be highly elusive substances and their presence can be determined only from the effects which certain materials exert on various functions of the animal organism. Thus far it has not been possible to synthesize vitamines or substances which play a similar rôle or to prepare any mixtures which produce the same effects as do these substances. It appears, therefore, that we are dealing with substances which can be produced only by the activity of the living plant. The sources are not confined to the chlorophyllous plants; yeasts have been found to contain certain vitamines. However, the chlorophyll-bearing plants not only are a direct source of vitamines, but as the ultimate source of all food material must be considered as of fundamental importance in the production of all vitamines in nature. From a nutritional viewpoint, therefore, at present it seems that we shall have to depend upon the natural photosynthetic process for the production of man's food supply on account of the essential vitamines which can be supplied in no other manner. This is quite aside from the many practical difficulties which any scheme for the artificial production of foods entails. Such schemes must still be regarded as the fanciful dreams of the popularization of science.

The more obvious significance, however, of the phenomenon of photosynthesis centers about the supplying of our earth with energy.

2. Sunlight: the Prime-mover of Civilization

Our early progenitors were at some period of their development most of them sun-worshipers, regarding the sun as that from which all blessings flow. The human mind has wandered far in its groping for fundamental truth and has constructed for itself many divers gods and idols. Scientific investigation is showing that the instincts of our sun-worshiping forefathers were well founded. The sun is in fact the source of all our material wealth, our power, our life. The full realization of this fact has been very slow in penetrating our minds.

Numerous have been the attempts to differentiate and classify peoples, to ascribe the success of one group or the failure of another in meeting its problems, to specific characteristics or attributes of the mind. In establishing a criterion of what constitutes progress and development, the emphasis has usually been placed upon the possession of material contrivances and the utilization of natural resources. The past one hundred years, truly called the scientific era, have brought many drastic changes in our material environment, accompanied by corresponding social and political alterations. There are many able men who deny us the right to call this progress, arguing that not until it has been established in what direction these changes are carrying us can it be determined whether the term progress may be applied. But it must be evident to the careful observer that these changes have not only wrought marked alterations in our social and political institutions, but perhaps to a greater, though a less noticeable degree are affecting our mental and spiritual outlook.

The dispelling of superstitions, the method of seeking for every effect a discernible cause is probably the essence of the scientific attitude of mind. Fundamentally it is the spiritual outlook which determines the destiny of a people, and their material activities and accomplishments must be taken as but the manifestations of their mental attitude.

Whatever may have been the urge which prompted the explorer to penetrate unknown lands, the chemist to seek out the materials our world is made up of and the biologist to study the nature of living things, it has resulted in a better understanding of the world we live in. We have learned that the exercise of foresight pays, that the scientist is capable of viewing the present on the background of the past and is constantly gaining in the power of prediction. We are learning, in brief, that mind has control over man's material environment: that, to a large measure, progress consists in the demonstration of this mastery. It now appears that this assertion of mastery over the material environment on the one hand, and the complete submission thereto on the other, constitutes the greatest difference between peoples. So also does the demonstration of this mastery mark a notable step in mental evolution.

For hundreds of centuries man, in his struggle with his environment, relied upon his own physical strength. In peace and war strength was measured by the physical unit of man-power. Slowly he devised for himself various tools and simple machines which increased his own power and that of the slaves and beasts he had forced into his service. Constantly he was engaged in devising means of increasing the efficacy of his strength. The knowledge of handling fire was a great step toward making man independent of his environment and master of the forces of nature. Soon he realized that the power of the elements in wind and waterfall would serve him, thereby lightening his burdens and widening his horizon. For many more centuries he plodded along, sowing and reaping, spinning and weaving in the sweat of his brow.

Fire had served man primarily by enabling him to keep warm in the colder regions of the world. He had long burned wood and already in the sixteenth century in England considerable coal was used. But a full realization of the power of fire was slow in coming. Not until the middle of the eighteenth century did man comprehend the value of the great patrimony which had been stored for him in the earth. And then by an intricate interplay of social and economic circumstances man-power was suddenly replaced by steam-power. The spinners and weavers of England were the first to abandon man-power as a prime mover, and then in a hurried succession the kindling of coal-fires caused revolution in one industry after another. Through the production of energy in the form of steam and its use in the smelting of ores, coal brought about the greatest revolution in human history.

Similarly petroleum, known to man in small quantities for centuries, in recent years has brought about a revolution in his methods of transportation. The great success of internal combustion engines in automobiles, airplanes, tractors, etc., as well as the many uses of the Diesel engine, has very greatly influenced our economic life. Not only as fuel in the many uses of the internal combustion engine but as the main source of lubricants has petroleum become one of the most important commodities on which our present civilization depends. So that in our modern life the physical strength of man, the unit one-man power, is an almost insignificant factor. A single machine can accomplish the work of a whole army of men, so that not only have many burdens been lifted from the shoulders of man, but the world's output of work has been enormously increased.

Animate energy has to a very large extent been replaced by inanimate laborers, and the true strength of man is measured by the intelligence he exercises in utilizing inanimate forces. For this reason scientists have for some time been occupied with the problem of our resources of inanimate energy. Thus already in the last century the eminent physicist Bolzmann pointed out that the struggle for existence is essentially not a fight for raw materials which are abundant in earth, sky and sea, nor for the energies as such, but for the potential energies as in coal, oil, sugar and meat.

While, then, to a considerable extent there is a strong tendency to emancipate man from his rôle as a beast of burden, he still requires his daily ration of energy. In fact, all living things on the earth demand for their maintenance and propagation a continuous supply of energy. The source of this energy for living things is derived from food. All animals, including man, are fundamentally dependent upon plants for their food. The object of agriculture is essentially to provide man with such materials from which he is able to derive the energy necessary for the maintenance of his bodily activities, his growth and propagation.

So far as the matter is concerned of which the food material is composed, there exists a closed cycle. Man partly feeds on animals, and animals on plants; the plants feed on the carbon dioxide given to the air by the animals as a result of the latter's use of food. Thus the plant reconverts the waste products of animal metabolism into food. The latter process is called Photosynthesis.[2] The plant absorbs through its leaves the carbon dioxide which is universally present in the atmosphere and which is formed by the burning of coal, fuel oil and so forth, and is also exhaled by animals. By means of the light from the sun the carbon dioxide thus absorbed by the leaves is changed into material such as sugar or wood, which can again be used as food for animals or as fuel. Van Helmont, whose classical experiment was described in the previous section, knew nothing of the composition of the air and never suspected that the plant absorbed from the atmosphere the major portion of the material which went to make up the 164 pounds which his willow twig had gained in weight in five years' time. As far as the changes of materials are concerned in this interrelation of plants and animals, the net result is *nil*. Thus, in brief, a plant yields a certain amount of substance which can be used as food. The food is consumed by man and thus enables him to do some work. Thereby the food material is burned in the body and is exhaled as the gas, carbon dioxide. Or the fuel is burned and the products of combustion, carbon dioxide, escape into the atmosphere.

The fundamentally important point is in relation to the energy changes. The energy expended by the man has been permanently lost to a large extent. Similarly that obtained from fuel. The reconversion of the carbon dioxide into food or fuel material can be accomplished only by the use of a great deal of energy. The cycle is made possible only by the introduction of energy from without. This energy is derived from the sunlight which the plant, unlike the animal, is able to utilize and so convert the waste carbon dioxide again into food or fuel material.

If our earth were an isolated system in which there were no imports and no exports, our state of affairs would be very different from that which now presents itself to us. According to our experience formulated in the laws of thermodynamics, in all naturally occurring transformations the tendency is to arrive at a condition of stable equilibrium. Thus the substances on the earth are constantly tending to arrive at a condition of greatest entropy, meaning "rundownness." Most of the metals, for in-

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[2] As yet no term has been proposed for this process which is entirely satisfactory. This is due largely to the difficulty of expressing adequately in a succinct term a process which appears to be highly complex and about which we have as yet incomplete information. *Carbon assimilation* used very generally by British writers, describes the process but very incompletely, especially as the word assimilation has been employed in so many different connotations. Furthermore the light factor is in no way suggested, nor that of chlorophyll. The same criticism applies to the German *Kohlensaeureassimilation.* On the other hand the French *assimilation chlorophyllienne* is cumbersome and not very much more expressive. The term *photosyntax* suggested by Barnes, Bot. Gaz. **18**, 403-411 (1893), has found little favor. While *photosynthesis* is not an altogether adequate expression, it is not too narrow and has come into very general use to mean the synthesis of complex carbon compounds out of carbon dioxide and water, in the presence of chlorophyll, through the action of light. The following have been suggested as more definite phrases: "Photosynthesis of carbon compounds," "Chlorophyll-photosynthesis of carbohydrates," "Photosynthetic utilization of carbon dioxide," "Photochemical synthesis of carbo-hydrates," "Photosynthetic assimilation of carbon," "Photosynthetic appropriation of carbon."

stance, are oxidised to their most stable oxides and converted into other compounds which under existing conditions are extremely stable. Our ores are those stable oxides or salts. Although this condition has not been uniformly attained in the earth, while there are still, for example, natural deposits of metallic copper and silver, yet, unquestionably that is the direction in which the chemical changes are proceeding.

Now most of these substances before they can be made use of require certain chemical changes which are a reversal of the naturally occurring ones. The ores, oxides or salts of the metals, must be reduced to the elemental metals. This, of course, is the reversal of the processes occurring in nature, and to accomplish such a reversal, work must be done, energy must be supplied.

If then, the tendency is to attain the dead level, this state of equilibrium on our earth, what are the agencies or sources of energy which counteract this tendency and make possible the reverse reaction, the pumping of water up-hill, as it were?

In searching for such possible sources of energy which might serve this purpose, we find that a little heat is probably given to the surface of the earth from the interior, another very small amount is the result of certain radio-active chemical changes, the action of the tides contributes some, and a further amount is received from radiation from the stars and moon. But these amounts are quite inadequate and insignificant when compared with the primal source of our energy, the sun. The radiations from the sun constitute our main source of energy. This is our main and most consequential import, the only potent factor which counteracts the tendency of complete running down. It is important not only in such reactions as the smelting of ores, but equally to the life on the planet.

All living things on the earth demand for their maintenance and propagation a continuous supply of energy. In final analysis, plants are the fundamental source of energy of all animals as well as man. Just as the herbivorous land animals are the source of food of the carnivoræ, the diatoms are the fundamental source of food of the sea.

It thus becomes evident that all life on the planet depends upon the energy derived from the sun through the intermediary of the plant, i.e., through the process of photosynthesis. Mankind lives entirely on the energy derived from the sun through the pursuits of agriculture.

But in addition to this we are squandering the principal of an enormous legacy of solar energy accumulated during the past ages. The plants, which alone are capable of utilizing the enormous floods of solar energy pouring upon the earth, have been at work for many ages prior to man's appearance upon the earth, and have, during time which would make the total span of human history appear as but a moment, built the foundations upon which all his present eminence rests. This fossil vegetation, preserved as coal and oil, represents a very small fraction of the energy which has been falling upon the earth and which has been conserved for man. It is kindled, its energy liberated and used in a thousand ways, and the rays of sunlight stored beneath the earth for millions of years give birth to a civilization such as the world has never known.

But this great civilization of coal and steel is at the same time a most squandrous and profligate one; it is using the principal of its legacy in numberless new ways. A year's consumption of coal at the present rate represents the accumulation of hundreds of years. The quest for further sources of energy in the form of coal and petroleum is being pushed with a feverish intensity revealing at times man's least attractive nature in personal and national greed. Our civilization is dependent upon the amount of available energy in the form of food and fuel. Fundamentally, our source of food and of fuel is the same. Photosynthesis supplies us with food directly. The accumulation of the products of photosynthesis during the past ages represents our present fuel supply. When these accumulations are exhausted or impracticable to utilize, our daily ration of solar energy will represent almost our entire means of livelihood. Our civilization now based upon the inanimate forces, must give way to one in which human physical effort is again the driving force.

It is one function of the scientist to care for the material welfare of man. His horizon should extend beyond the domain of the present, his view penetrate the future and by the exercise of his foresight guide us to ever increasing assurance of mastery over the world. And therefore the scientific world is realizing the necessity of considering our available sources of energy. Our ever increasing population and the development of civilization demand an ever increasing supply of energy in the form of food and fuel. National power is pre-eminently dependent upon such commodities. Liquid fuel, especially in the form of petroleum, is rapidly becoming the most prized possession.

Our main source of energy is coal and, although it is less than one hundred years since it has been put to extensive use as fuel, the present annual consumption is stupendous, about 650,000,000 tons. Each decade has brought a very decided increase in the rate of consumption.

Another very considerable source of energy is that developed from the water powers. Theoretically, this is virtually an inexhaustible supply and one of relatively high efficiency. The late Mr. Charles P. Steinmetz[3] has calculated on the basis of every raindrop which falls in the United States being collected and all the power it could produce on its way to the ocean being developed, that there would be possible about three hundred million horse-power. This enormous figure represents about the amount received from our present total consumption of coal. Thus this theoretical hydro-electric power would just about cover our present coal consumption, but leave nothing for future increased needs nor to cover other sources of energy now in use. Moreover, this figure for hydro-electric power is purely hypothetical, of which only a small fraction represents that actually available, which, when united with other difficulties

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[3] Steinmetz, C. P., *Survey Graphic*, **1**, 1035 (1922).

such as cost of equipment and limitations of distribution, shows very clearly that all the water powers of the country cannot suffice.

Much attention has been given to the production of liquid fuel other than petroleum. Thus far the investigations along these lines have almost universally led to the opinion that the substance best suited to these methods is alcohol. This is on the basis that alcohol can be produced from vegetable material and is the most direct route from solar energy. It is thus proposed to develop a photosynthetic industry on the basis of agriculture, the products of which are to be converted into alcohol by means of fermentation.

It is characteristic of most discussions of energy that they finally revert to a consideration of our primal source of energy, the sun. It is equally significant that when we arrive at this stage we realize that the green plant still remains the only large scale converter of solar energy through the process termed photosynthesis.

In the process of photosynthesis nature has worked out a method of utilizing solar energy. In principle this method is probably the most effective imaginable. It is the only chemical reaction we know, induced by visible light, in which there is a great accumulation of energy. The products are easily stored, transported and capable of transformation in numberless ways. As we shall see, the natural process of photosynthesis is not very efficient; but it can serve as a most valuable guide to the development of a method of the utilization of solar energy. The chemist need not be timid about competing with nature. He has many cases to his credit in which he has learned to surpass nature both in efficiency and reliability.

3. The Green Plant as a Converter of Solar Energy

Photosynthesis is a complex process; for its successful operation a number of elements or factors are essential. These include light, carbon dioxide, water, oxygen, the minute corpuscles called chloroplasts which contain the chlorophyll, and a temperature at which the plant is able to exist. It must also be realized that the process of photosynthesis is intimately connected with the life processes of the plant. It is not merely a manufacturing of food which makes possible the life activities of the organism, but the operation of the photosynthetic process is apparently as much dependent upon the interplay of enzymatic reactions and the structure of the organism as is respiration. When we consider the difficulties which have been encountered in unravelling the chemistry of the respiratory process, as for instance the oxidation in the organism of glucose to carbon dioxide and water, it is not surprising that the synthetic reaction, starting with the relatively inert carbon dioxide and involving photochemical reactions about which until very recently we knew practically nothing, should appear as such a difficult task.

Of all the factors which are essential to photosynthesis, light has probably received the most thorough study. This is the case not only on the part of the physicist in the laboratory but as well by the astronomer and meteorologist for solar radiation. The measurement of the total supply of solar radiation as well as the wave lengths and wave frequencies which constitute this radiation have been the subject of careful and exhaustive investigation. The pioneer work in this field was done by the American physicist, Langley, and has been greatly extended by the investigations of Abbot and his collaborators. In most of these investigations[4] the intensity of solar radiation has been measured by means of the heat produced when the radiation is absorbed on a black surface at right angles to the rays, and has been converted into calories per square centimeter per minute. The values thus obtained depend, among other factors, upon elevation above sea-level, i.e. the thickness of atmosphere which the rays must penetrate, and the angle distance which the sun is from the zenith. Thus Abbot states ("The Sun," p. 284) "The maximum intensity of solar radiation as measured near sea level at Washington when the sun is not more than 45° from the zenith usually ranges from 1.15 to 1.45 calories per square centimeter per minute on cloudless days, depending on the clearness and dryness of the air. At Mount Wilson in California, over one mile above sea level, the values observed range from 1.45 to 1.62 calories, and on Mount Whitney in California, nearly three miles in altitude, the observed values reach 1.75 calories."

Observations have been made over extended periods of time at many different points on the earth with a number of different instruments. The total annual insolation (for clear sky) varies with the latitude, is greatest at the equator and diminishes toward the poles. It should be emphasized that the values of total insolation have only an indirect bearing on possible photosynthetic activity, because the latter activity depends upon a number of factors besides insolation which greatly complicates the situation.

In Figure 1 is shown the maximum radiation on normal and horizontal surfaces during the course of the year. The lower maxima at normal incidence during the summer are due to the dust and moisture in the atmosphere during this season.[5]

Solar radiation is greatly affected both in intensity and composition by its passage through the earth's atmosphere. This is due to the fact that the lower layers of the air contain dust particles and water vapor which absorb and refract the light rays. Because of the fact that the quantity and quality of the dust and the distribution of water vapor are changing from day to day it is not possible to predict accurately the in-

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[4] Annals of the Astrophysical Observatory of the Smithsonian Institution, **2** (1908); **3** (1913); **4** (1922). Abbot, C. G., The Sun, Appletons, New York, 1911. Bigelow, F. H., Treatise on the Sun's Radiation and Other Solar Phenomena, New York, 1918. Kimball, H. H., Bull. Mt. Weather Observatory, **1**, parts 2 and 4; **2**, part 2; **3**, part 2 (1910). Pulling, H. E., Sunlight and Its Measurement. The Plant World, **22**, 151-171; 187-209 (1919). Dorno, C., Studie über Licht und Luft des Hochgebirges. Braunschweig, 1911.

[5] Kimball, *Monthly Weather Rev.*, **43**, 102 (1915).

crease in total solar radiation with increasing elevation. Abbot[6] states that the loss of radiation in passing through the last mile of air is almost as great as the entire loss sustained above Mount Wilson (5,675 feet).

In their passage through the earth's atmosphere some wave lengths are more absorbed than others. The absorption bands of oxygen, ozone, water vapor and carbon dioxide are chiefly in the infra-red and some in the extreme ultra-violet portions of the spectrum. The solar radiation received on the earth lies almost entirely within the wave lengths 0.29 μ and 2.5 μ. The spectral energy curve shows that the maximum intensity is at .47 μ, decreasing to almost zero in the ultra-violet and infra-red regions beyond the limits mentioned and exhibiting a number of depressions at the Fraunhofer lines and in the regions where the earth's atmosphere exercises selective absorption. Abbot[7] has made extensive studies of the energy of the solar spectrum and by means of the spectrobolometer has prepared energy curves, called bolographs, which show the distribution of solar radiation and the transmission of the atmosphere at all parts of the spectrum. These investigations are of fundamental importance for every phase of the question of solar energy. He has also prepared a table of the transmission of the atmosphere at various wave lengths. This is reproduced in Table 1. "The[8] values represent the fraction of intensity of the solar beam outside the atmosphere which would remain in a direct beam transmitted vertically to the earth's surface. Average values for cloudless (but not necessarily hazeless) days are given, as

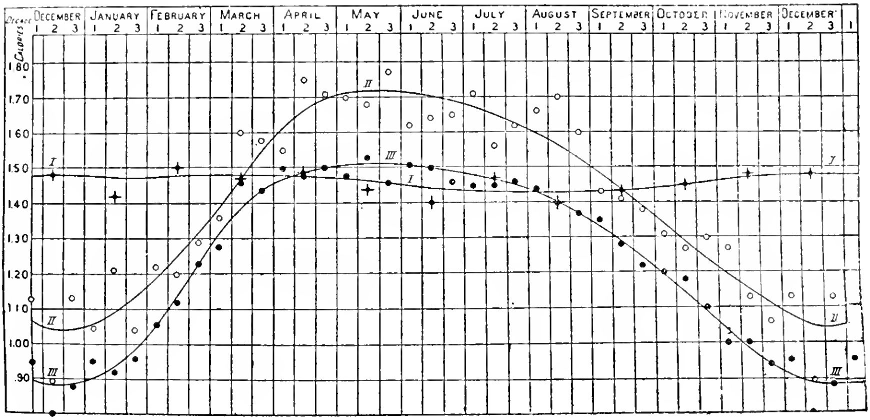

FIG. 1.—Maximum solar radiation per minute in gram calories per square centimeter at Washington, D. C. I. Solar radiation at normal incidence. II. Solar and sky radiation on a horizontal surface, with clouds near the sun, but not obscuring it. III. Solar radiation on a horizontal surface, with cloudless sky. (From Kimball.)

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[6] Abbot, *The Sun*, p. 293.

[7] Abbot, *Smithsonian Inst. Ann. Astrophysical Obser.*, 3, 21 (1913).

[8] Abbot, *Ibid.*, 197.

they have been found at Washington, Mount Wilson and Mount Whitney, for various wave lengths. To compute the transmission for other than zero zenith distances, the coefficients here given must be raised to a power equal to the secant of the zenith distances. This does not hold closely for zenith distances above 75°."

TABLE 1

ABBOT'S TABLE OF MEAN COEFFICIENTS OF ATMOSPHERIC TRANSMISSION.

Wave length	Washington 1902-1907	Mount Wilson 1909-1910	Mount Whitney 1909-1910
0.30	....	....	0.70?
0.325	....	(0.550)	0.635
0.35	....	0.612	0.715
0.375	....	0.662	0.776
0.39	0.445	0.694	0.800
0.42	0.586	0.764	0.831
0.43	0.600	0.778	0.844
0.45	0.640	0.800	0.875
0.47	0.671	0.827	0.902
0.50	0.705	0.858	0.919
0.55	0.739	0.876	0.930
0.60	0.760	0.890	0.940
0.70	0.839	0.942	0.964
0.80	0.865	0.964	0.976
1.00	0.901	0.973	0.975
1.30	0.916	0.972	0.967
1.60	0.930	0.975	0.963
2.00	0.909	0.957	0.932
2.50	0.870	(0.900)	0.945
3.00	....	....	0.916

The intensity and composition of solar radiation varies considerably with the altitude; and also, at a given place, the composition of the light varies considerably, almost from minute to minute. This is especially true in the violet end of the spectrum.

Probably of even greater significance than the gaseous constituents of the atmosphere for the absorption of solar radiation is the dust in the upper atmosphere. The origin of this dust is usually volcanic. Benjamin Franklin already suggested that the hard winters which had been recorded in history may be due to the decrease in solar radiation by the fine volcanic dust and that these abnormally cold periods may be synchronous with volcanic activity in different parts of the world. That such is the case, at least to a considerable degree, now seems highly probable. Thus, Abbot [9] found, following the volcanic eruption of Mount Katmai, Alaska, June 6-7, 1912, that evidence of the dust appeared at Bassour, Algeria, on or before June 19, and at Mount Wilson, California, on or before June 21. The effect reached its maximum in August and reduced the total direct radiation of the sun by nearly 20 per cent. The complicated effects produced by such layers of volcanic dust on solar radiation have been studied also by Kimball [10] and by Humphreys.[11] The latter has compiled

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[9] Abbot, *Smithsonian Inst. Ann. Astrophysical Obser.*, **3**, 214 (1913).

[10] Kimball, H. H., *Monthly Weather Review*, **46**, 355 (1918).

[11] Humphreys, W. J., *Physics of the Air.* Philadelphia, 1920, p. 569.

very interesting data on the relation of volcanic disturbances to solar radiation and atmospheric temperatures. It appears that not all eruptions result in a decrease in surface temperatures; usually only those in which a large quantity of fine dust is thrown into the high isothermal layer of the atmosphere. The end results depend upon a number of factors, chiefly the absorption by the dust of the direct solar radiation and the effect of the dust on the earth's radiation into space. But there seems to be little room for doubt that both volcanic dust and solar activity, the latter evidenced by sun-spots, have decided effects both on the direct solar radiations and on atmospheric temperatures. The effect of such changes is discussed by Humphreys: "For instance, during the summer, or growing season, a change of 0.5° C. produces a latitude shift by fully 80 miles. Hence, if there is but little or no volcanic dust to interfere, during sun-spot minima cereals and other crops may be successfully grown 50 to 150 miles farther north (or south in the southern hemisphere) than at times of sun-spot maxima. This alone is of great practical importance, especially to those who live near the thermal limits of crop production.

"In addition to changing the area over which crop production is possible, a change of average temperature also affects, in some cases greatly, the time of plant development. Thus Walter [12] has shown that a change of only 0.7° C. may alter, and in Mauritius has been observed actually to alter by as much as an entire year, the time required for the maturing of sugar cane. Hence the temperature changes that normally accompany sun-spot variations, though small in absolute magnitude, are of great importance, and, by availing ourselves of the reasonable foreknowledge we have of these changes, may easily be made of still greater importance."

The astronomer is particularly interested in the amount of solar radiation received at the outside of the earth's atmosphere. This quantity he obtains by making determinations of the amount of radiation received at the surface of the earth and calculating the amount of loss sustained by passage through the atmosphere. The value thus obtained is called the "solar constant" of radiation. For this measure Abbot [13] has given the value of 1.946 calories (15°) per square centimeter per minute for the epoch 1912 to 1920 determined by 1,244 observations. Bigelow [14] arrived at another value by means of different methods; his results are summarized below, in values of gram calories per square centimeter per hour:

1.	True Solar intensity of radiation	5.85	calories
2.	Effective Solar intensity at the distance of the earth	3.98	"
3.	Effective intensity by the bolometer	3.98	"
4.	Effective intensity by thermodynamics	3.98	"
5.	Extrapolated intensity by the pyreheliometer	1.95	"
6.	Intensity at sea level by the pyreheliometer	1.50	"

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[12] "On the Influence of Forests on Rainfall and Probable Effect of 'Deboisement' on Agriculture in Mauritius" (1908), quoted from Humphreys. l. c., p. 602.

[13] Abbot, *Smithsonian Inst. Ann. Astrophysical Obser.*, 4, 192 (1922).

[14] Bigelow, Treatise on the Sun's Radiation. New York, 1918, p. 211.

It is important to bear in mind that the value for the solar constant (Abbot 1.95 and Bigelow 3.98) refers to the intensity at the outside of the atmosphere. While the establishment of the true value of this influx of energy is of very great importance, it has only indirect bearing on the problem of photosynthesis. In calculating the amount of solar energy available on the earth, the value of the solar constant has occasionally been used. That this is erroneous is evident. Fortunately there appears to be little dispute regarding the amount of solar energy received at the surface of the earth and it is this factor which is of more immediate importance to the problem of photosynthesis.

Abbot has summarized the total solar radiation on normal incidence and horizontal surface assuming the sun to shine 261,000 minutes per year and has calculated the square feet required per horse-power on the basis of complete absorption and transformation. Table 2 is taken from Abbot's "The Sun," page 386.

TABLE 2

	Normal Incidence		Horizontal Surface		
Latitude	Sea level	6,000 feet	Sea level	6,000 feet	
20°	292,000	362,000	185,000	226,000	Calories per sq. cm. per year.
30°	287,000	355,000	170,000	203,000	
38°	271,000	342,000	152,000	185,000	
45°	270,000	340,000	137,000	169,000	
20°	10.5	8.5	16.6	13.6	Average sq. ft. per horse-power.
30°	10.7	8.8	18.1	15.1	
38°	11.3	9.0	20.2	16.6	
45°	11.4	9.1	22.4	18.2	

Abbot [15] makes the following statement to give an idea of the total amount of energy received by the earth from the sun: "Expressed in another way, the measurements indicate that if the sun's rays could be completely employed to melt ice exposed continuously to them at right angles, they would suffice to melt a layer 426 feet thick in a year. Such a layer at the earth's mean distance, if it entirely surrounded the sun, would weigh 4×10^{25} (4 followed by 25 ciphers) tons, and the complete melting of it each year would represent as many heat units as the burning of 4×10^{23} tons of anthracite coal. This, then, is a measure of the sun's yearly output of radiation."

It is a very interesting fact that the value of the solar constant is subject to certain periodic fluctuations. These are, firstly, long period variations which indicate that the emission of radiation varies with solar activity as made evident by sun-spots and other phenomena. With increasing number of sun-spots solar radiation increases, though the relation is not a direct numerical one. Thus Abbot reports that the increasing solar activity of 1914 resulted in a 3.5 per cent increase in the value of the solar constant over 1913. The solar constant is also subject to short-period variations.

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[15] Abbot, *The Sun*, p. 299.

Thus from day to day there have been extreme fluctuations observed ranging over nearly 10 per cent. These short-period variations are apparently associated with changes in the opacity of the outer solar layers.[16] The correlation between the variations in solar activity and climatic conditions on the earth may be of great significance for the photosynthetic activity of plants. It should be stated, however, that there is no direct correlation between variations of the solar constant and terrestrial temperatures and that the interrelations appear to be very complex.

The foregoing is of necessity only a very brief consideration of the factor of solar radiation. Many phases have not been touched upon but the reader will find in the literature cited a wealth of material for this most fascinating study. We have, however, sufficient data to enable us to draw some general conclusions on the relation of solar energy to photosynthetic activity; a more detailed consideration of the light factor in photosynthesis will be found in Chapter 2.

In order to form a conception of the amount of solar energy received at the surface of the earth and the proportion of this energy which is utilized by the plant and which thus represents the amount of energy now available for use by man, let us make the following calculations. Instead of 1.5 calories per square centimeter per minute as the amount of solar energy received at the surface of the earth we shall take 1.35 calories. Such a calculation should be based upon good agriculture conditions and should exclude, naturally, extreme conditions of solar intensity such as exist on the desert. In a six hour day at 1.35 calories per square centimeter per minute there would be received 486 calories per square centimeter. From Kimball's [17] values, obtained with a Callender pyreheliometer, at Washington, D. C., and Mount Weather, Va., the mean daily normal for the 92 days of May, June and July, 1910-1914, is 522 gram calories per square centimeter of horizontal surface. This somewhat higher value is due to the fact that it represents the amount of radiation received during the entire day instead of only six hours, but it serves to show that we have not taken too high a quantity in the 1.35 gram calories. We shall take 90 days as the growing season, and convert the total amount of solar radiation received on an area of one U. S. acre into terms expressed by the energy derived from the combustion of coal. Thus, taking the heat of combustion of anthracite coal equal to 8,000 kilogram calories per kilogram, we conclude that the solar energy received on an acre of land during a growing season of 90 days is equal to the energy contained in 243 tons of anthracite coal.

For the purpose of comparison, we can calculate on the same basis, the amount of this total energy which the plant fixes and which is found in the potential energy of the substances which have been elaborated by the plant in the photosynthetic process. We shall take a yield of 25 bushels

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[16] Abbot, C. G., *Smithsonian Inst. Ann. Astrophysical Obser.*, **4**, 16, 177, 184 (1922). Humphreys, W. J., *Astrophy. Jour.*, **32**, 97 (1910).

[17] Kimball, *Monthly Weather Review*, **43**, 108 (1915).

of corn (bushels of 56 pounds) per acre and consider the heat value of the grain only. De Baufre[18] reports the heat value of fully cured corn, containing 10 per cent of water, as 6,700 B.t.u. per pound. The energy of the crop of corn from an acre of land is thus equivalent to 0.325 of a ton of anthracite coal. That is, by raising corn on a given area of land about .13 per cent of the total amount of solar energy received is "fixed" by conversion into potential energy of carbon compounds.

If the yield of corn from one acre were fermented in order to obtain alcohol, the energy obtainable would be still further reduced. Thus, from 25 bushels of corn at 2.7 gallons per bushel there would be obtained 67.5 gallons of alcohol. The heat of combustion of this amount of ethyl alcohol corresponds to about 0.20 tons of anthracite coal or about 0.08 per cent of the total energy of solar radiation per acre for a period of 90 days.

On the basis of a conversion factor of 10 per cent, the yield of corn from one acre, when fed to steers, would produce meat corresponding to about 0.033 tons of coal per acre.

A similar calculation can be made of the production of material synthesized by forest trees.[19] We shall take one of the fastest growing trees, the redwood (*sequoia sempervirens*). The average annual growth of 20-60 year trees is about 300 cubic feet, or 75 cubic feet for a period of three months. This includes the stem and top but not the bark and limbs. The heat of combustion of one cubic foot of redwood is 159,000 B.t.u. This would yield an energy equivalent of 0.41 tons of anthracite from one acre of redwood in a period of three months. While this does not represent the entire yield of material synthesized by the trees, it is a liberal accounting of the merchantable timber. Another very rapid growing tree is *Eucalyptus globulus*. This tree averages about 355 cubic feet of wood per year for 20 year trees, or 89 cubic feet for three months. The heat of combustion of one cubic foot of this wood is 268,000 B.t.u. On this basis an acre of *eucalyptus* trees would yield energy corresponding to 0.826 tons of coal in a period of three months. This is about double the quantity obtained from redwood; the latter is notoriously poor fuel while *eucalyptus* though fast growing, is a hard wood of high heat of combustion.

The foregoing figures have been given in order to present an approximate idea of the plant as a converter of solar energy. They cannot be taken as a true index of the efficiency of the photosynthetic process; this will be discussed in a later chapter. The values have a "practical" rather than a strictly scientific meaning. For instance, in the determination of the per cent of conversion in corn we did not include the stalks, which would probably double the total value. Yet the use of such material for fuel or for the production of alcohol presents many difficulties which make them of questionable value. One of the practical questions of the

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[18] De Baufre, *Power*, **56**, 212 (1922).

[19] Bruce, University of California, Agricultural Experiment Station. Bulletin No. **361**, Metcalf, Ibid., **380**. U. S. Dept. of Agriculture, Agriculture Bull., **753** (1919).

photosynthesis problem is whether the production of industrial energy from solar radiation through the intermediary of the plant seems feasible.

In Figure 2 is shown graphically the relation of the total energy of solar radiation to that portion which is fixed or transformed into usable form by the plant. The great difference in these quantities is very evident. We are now utilizing about 0.1 per cent of the solar energy falling on a given area of the earth's surface, and this only during a fraction of the year. For this utilization we are still wholly dependent upon the intermediary of the chlorophyllous plant. The causes which account for this low order of efficiency are rather complicated. Moreover, different species of plants vary greatly in the various factors which must be taken into account in analyzing the effectiveness of plants as storers of solar energy. We have prepared what may be termed a composite picture of the relation of plants to solar energy.

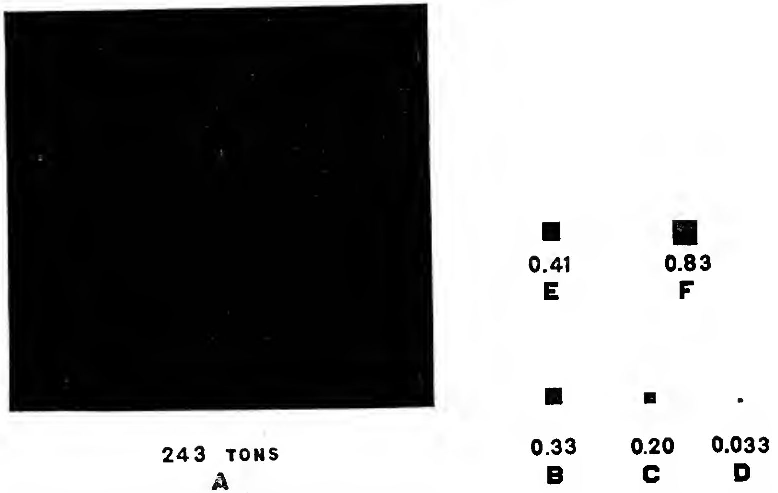

FIG. 2.—*A*, amount of solar energy received on an area of one acre in a growing season of 90 days expressed in tons of anthracite coal. *B*, 25 bushels of corn from an acre of land, energy obtainable therefrom expressed in tons of coal. *C*, energy of alcohol obtained by fermentation of 25 bushels of corn. *D*, energy from meat when corn obtained from an acre is fed to steers. *E*, energy in terms of coal from an acre of redwood in 3 months. *F*, energy in terms of coal from an acre of Eucalyptus.

In Figure 3 is shown the disposal by the leaf of the energy incident on it. All of this energy is not absorbed by the leaf; approximately 70 per cent is absorbed, 30 per cent being transmitted or reflected. In land plants a very considerable portion of the energy absorbed by the leaf is dissipated through evaporation of water from the leaf. This has the effect of cooling the leaf. The quantity of energy thus lost naturally varies greatly with external conditions. We can place this amount at 50 per cent. When there is relatively little evaporation the absorption of solar radiation raises the temperature of the leaf above that of its surroundings. The leaf thus also loses energy by reradiation and convection cooling. With a loss of energy by transpiration of 50 per cent, that lost by reradiation can be placed at 19 per cent. As directly determined the amount of energy used up in the endothermic process of photosynthesis is relatively very small. It also varies considerably with external conditions and in different plants, but a fair value for the present purpose is about 1 per cent. This value of 1 per cent of the total incident solar radiation used in photosynthesis represents the value for mature active leaves.

In Figure 4 is shown the distribution of material which has been manufactured in the photosynthetic process. The plant can, of course, produce carbohydrates only during the hours of illumination. For its life activities a certain amount of the materials elaborated are used up by the process of respiration. This in a sense represents the operating cost of the manufacture and amounts to approximately 20 per cent. The material of the plant can be divided into the crop, the leaves and stalks, and the roots. Here again there are great variations in different species of plant. For corn which has served as an example we can take the ratio as given in Figure 4. Thus we can account for a further reduction in the ratio of total incident energy to energy recovered in the plant crop. The 1 per cent of the incident solar energy used in photosynthesis is the ratio of the energy utilized in photosynthesis to the total radiation falling on the leaf.

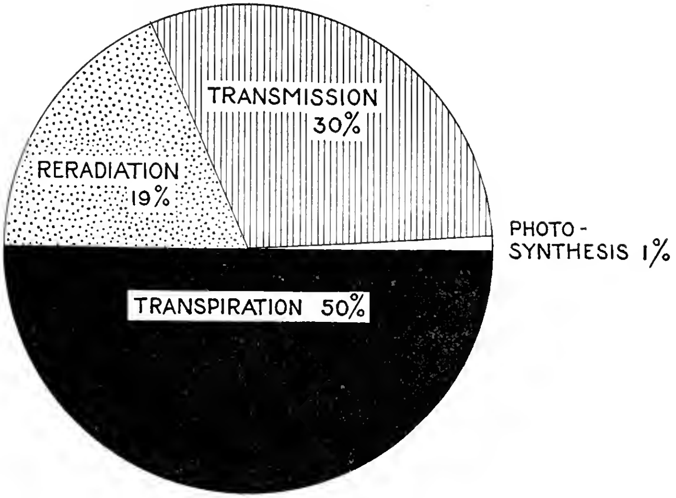

Fig. 3.—What happens to solar radiation incident on a chlorophyllous leaf. The values indicated give approximate disposal of the energy; the ratio varies with changes in external conditions.

In our first analysis we concluded that about 0.13 per cent of the total incident energy was recovered in the crop over a period of 90 days. The

1 per cent used in photosynthesis represents a value determined for the absorbing leaves and not for an entire plant. Moreover, in a growing period of 90 days we include the plant from the seedling stage to the time of harvest. During this period the photosynthetic activity of the entire plant varies from virtually nothing to maximum and then decreases again. Also the entire acre of land on which we based our calculation is not covered by leaf surface. These various factors, then, contribute to reducing the net conversion by the plant to about 0.1 per cent of the total incident solar radiation.

The foregoing brief discussion gives a general idea of the efficiency of the green plant as a converter of solar energy. Under natural conditions the rate of photosynthetic activity undergoes wide fluctuations according to the changes in external conditions to which it is subjected. Before entering upon an analytical study of the influence of each of these various factors we shall consider the function of green plants on our earth in its more general aspects.

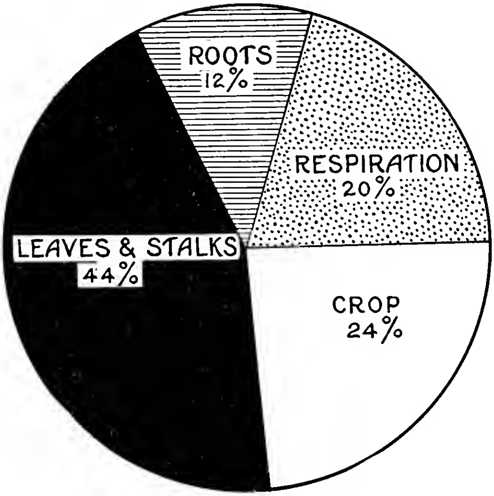

Fig. 4.—Approximate distribution of material manufactured in the photosynthetic process.

The composition of the atmosphere is, of course, of very great importance in relation to the photosynthetic activity of plants. While there is still considerable discussion regarding the composition of the atmosphere to its outer limits, we are concerned primarily with the composition at the surface of the earth. Here the different gases are present in the following volume percentages:

Nitrogen	78.03	Hydrogen	0.01
Oxygen	20.99	Neon	0.0012
Argon	0.94	Helium	0.0004
Carbon dioxide	0.03		

In view of the fact that the amount of oxygen required for photosynthetic activity is relatively very small we can confine our attention to the carbon dioxide. The calculated difference in the percentage distribution of gases in the atmosphere with increasing altitude[20] are also of no immediate significance to our subject, as we must naturally confine our attention to land areas capable of supporting vegetative growth.

There is still some debate regarding the exact value of the carbon dioxide content of the atmosphere, more particularly the constancy of the amount present with change in location and weather conditions. It should be stated immediately that it is highly probable that many of the discordant results unquestionably are due to the fact that different methods of analysis were used and, simple as the problem may seem, thoroughly reliable methods of air analysis have been worked out only relatively recently. The tension of the water vapor in the atmosphere may cause a considerable variation and some of the analyses have apparently been corrected for moisture while others have not. Moreover, the place of taking the sample whether this is, for instance, close to the ground or not may be of considerable influence. Sources of carbon dioxide even when these are some distance away may influence results. Thus Reiset[21] took samples 8 kilometers from the city of Dieppe and found a mean value of 0.02917 volume per cent CO_2. The proximity of a drove of sheep raised this value to 0.03178.

Benedict[22] reports remarkably constant values for carbon dioxide and oxygen. He concludes: "The results of analyses of air taken near the laboratory showed no material fluctuations in oxygen percentage during a period extending from April 15, 1911, to January 30, 1912. This constancy was maintained in spite of all possible alteration in weather conditions, changes in barometer, thermometer, humidity, and wind direction and strength; furthermore, the experiments were made before, during, and after the vegetative season. The average result of 212 analyses showed 0.031 per cent of carbon dioxide and 20.938 per cent of oxygen. The analyses of air collected over the ocean, at two different times of the year, and on top of Pike's Peak, gave essentially similar results." The carbon dioxide-content in crowded city streets was found to be slightly higher than normal, while samples taken in New York and Boston subway stations contained about twice the normal amount.

Among the older analyses reported one finds very considerable variations in the amount of CO_2. By some writers much importance has been attached to these variations. In fact Reinau[23] has developed an elaborate theory on the basis of these observations, in which he stresses the significance of periodic variations in carbon dioxide content of the atmosphere.

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[20] Humphreys, F. J., *Physics of the Air.* Philadelphia, 1920, p. 60.

[21] Reiset, J. A., Compt. rend., **88**, 1007 (1879).

[22] Benedict, F. G., The Composition of the Atmosphere with Special Reference to its Oxygen Content. Carnegie Inst. of Washington. Pub. No. 166 (1912), p. 114.

[23] Reinau, E., Kohlensäure und Pflanzen. Halle, 1920.

Without entering here upon a discussion of this theory, it is nevertheless well to bear in mind that the older analyses were carried out by means of different methods and at a time when all the precautions essential for accurate air analyses were not yet realized.

Lundegårdh,[24] working on the island of Hollands Väderö, 3.2 kilometers from the mainland of Sweden, reported the following analyses for carbon dioxide during the summer months:

1920	1921	1922	1923	
0.612	0.5603	0.5267	0.5565	mg. per Liter
0.03295	0.03031	0.02843	0.0300	vol. per cent
19.97	17.3	16.48	15.39	mean temperature

The mean variation during any one summer was ± 14 to ± 16 per cent.

That there is a variation in the CO_2-concentration during day and night has been reported by a number of investigators. Thus Reiset,[25] Münz and Aubin,[26] and Letts and Blake [27] conclude that the diurnal CO_2 content is about 12 per cent lower than the nocturnal when the analyses were made at some distance from the sea. Such variations were not observed in analyses made at sea (Thorpe) [28] or on a small island (Lundegårdh).[29]

Schulz [30] reports variations ranging in extremes from 2.7 to 3.2 in the air over the North Sea and Baltic. He considers these as due to inaccuracies in analyses rather than actual differences in the carbon dioxide content of the atmosphere and regards a mean value of 0.000293 atmosphere as the partial pressure of carbon dioxide over these bodies of water.

The entire question of the carbon dioxide content of the atmosphere is in need of a thorough investigation with uniform methods at different points on the earth and extending over a number of years. It is only in this way that a solution of such questions as convection, the complete mixing of the atmosphere and the influence of land and sea is to be hoped for. Kendall [31] concludes his discussion of the carbon dioxide content of the atmosphere with the following comment: "The fact that exceedingly large variations (as much as 700 per cent) have been recorded by numerous observers is probably due to the faulty methods of estimation that have been generally employed. The most careful and recent analyses of pure out-door air indicate relatively constant values. Unfortunately most of the observations on record have been obtained in connection with non-chemical investigations, and these frequently betray the fact that at

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[24] Lundegårdh, H., Der Kreislauf der Kohlensäure in der Natur. Jena, 1924, p. 9.

[25] Reiset, J. A., Compt. rend., **88**, 1007 (1879).

[26] Münz, A., and Aubin, E., Compt. rend., **92**, 247, 1229 (1881), **93**, 797 (1881), **94**, 1651 (1882), **96**, 1793 (1883).

[27] Letts and Blake, Roy. Soc. Dublin Proc. N. S., **9**, 107 (1899-1902).

[28] Thorpe, *Ann. Chem.*, **145**, 94 (1868).

[29] Lundegårdh, l.c., p. 16.

[30] Schulz, Arch. deutsch. Seewarte, **40**, 16 (1922), **41**, 6 (1923).

[31] Kendall, J., *Jour. Amer. Chem. Soc.*, **38**, 1490 (1916).

least a little knowledge of chemistry would have been of great assistance to the experimenter, both in the choice of method and in the necessary manipulations."

Many years ago Regnault[32] inaugurated an extensive international investigation of the composition of the atmosphere. While the results from this elaborately planned cooperative investigation were considerably curtailed by the political disturbances existing in Europe at the time, they are sufficient to demonstrate the value of such an undertaking. With the advance in methods of analysis, of communication and of transportation a similar undertaking now ought to be capable of execution and would certainly justify itself.

If photosynthesis actually has an appreciable influence on the carbon dioxide content of the atmosphere, this could only be detected because of imperfect and slow mixing of the gases of the air.[33] As nearly as can be calculated there are about 2.2×10^{12} tons (about 2×10^{15} kilograms) of carbon dioxide in the atmosphere. The total photosynthetic carbon dioxide consumption can be put at about 54×10^{12} kilograms per year on the basis of the calculations of Sachs given below. The annual consumption therefore would be roughly only one fiftieth of the total supply of atmospheric carbon dioxide. These figures are obviously very rough, but indicate that if *perfect mixing* took place, the difference in carbon dioxide concentration of the atmosphere, due to photosynthesis, would be so slight that it could not be detected by analyses. Day and night variations in carbon dioxide content, if they exist, are therefore probably due to a combination of local conditions. On the other hand, with no wind, when there is imperfect mixing it is highly probable that densely vegetated areas show a carbon dioxide content below the "normal."

A larger reservoir of carbon dioxide than the atmosphere is the sea.[34] Sea-water contains approximately 50 cc. of carbon dioxide per liter, but varies with the depth at which the water is drawn. Moreover, the oceans comprise fully 70 per cent of the surface of the earth, so that they play an important rôle in the carbon dioxide cycle. In absolute amount also the sea contains considerably more carbon dioxide than the atmosphere; the latter contains approximately 2100 billion kilograms while the former contains about 16,000 billion kilograms, though these figures can be taken only as approximations. Different estimates give the oceans eighteen to twenty-seven times as much carbon dioxide as the atmosphere.

Just how great are the potentialities of the sea for food it appears difficult to determine. In limited areas the sea produces as much or slightly

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[32] Regnault, *Ann. Chim. et Phys. III*, **36**, 385 (1852).

[33] Clarke, Data of Geo-chemistry. Bul. U. S. Geological Survey No. 770, 1924, p. 50.

[34] Krogh, A., Compt. rend., **139**, 896 (1904). Krümmel, O., *Handb. der Ozeanographie*, Vol. I, 312 (1923). Schulz, A., *Naturwissenschaften*, **12**, 105-113, 126-133 (1924). Politzsch, S., *Compt. rend. des travaux du Laboratoire de Carlsberg*, **11**, 199 (1916). Wells, R. C., U. S. Geol. Survey, Professional Paper **120**-*A*, 1-16 (1918).

more food than an equal area of land. East[35] considers that most of the plants, which are as much the primary source of food of sea animals as of land animals, are found in waters not over 200 feet in depth, and that such areas of the sea would approximately equal 5 per cent of the land area of the globe. With the possible exception of certain rather restricted regions as in the North Sea and Baltic, where the economic importance of sea life has long been recognized, few careful surveys have been made of the production of the sea. In most cases the products of photosynthesis in the sea are harvested indirectly. That is, while some algae are used as a source of food in China and Japan, this is not very general and man confines himself largely to the consumption of animals which feed upon sea vegetation or other sea animals. In many cases this is several steps removed from the original plant source. The result is a tremendous waste of energy, so that man finally obtains an exceedingly small quantity of food from a very large amount of carbon dioxide reduced by the marine plants. The diatoms and holophytic peridines are the great marine converters of solar energy. Upon these and other sea plants we depend for our sea food; the very intricate relationships between the various forms of marine life make the determination of the "food producing value" of an area of water an exceedingly complex problem.

The chemistry of the sea and its relation to the gases of the atmosphere has been the subject of much experimental and theoretical study.[36] While at first these problems appeared to be relatively simple, when studied more minutely, particularly in relation to the activities of living organisms, many complex conditions became apparent. The solubility of the gases of the atmosphere, the influence of the salinity of the ocean and of temperature on this and similar conditions determine the fitness of the ocean for plant and animal life. While most of the constituents of the ocean are maintained in relatively constant proportions the concentration of carbon dioxide and to a lesser degree also oxygen are found to undergo considerable variation. These variations together with the intimately associated hydrogen ion concentration of the sea are of paramount importance to living organisms.

Schulz[37] has made a very careful study of the aeration of the North Sea and Baltic Sea and his recent results are here given to illustrate the conditions existing for life in the sea. For more detailed information the literature cited must be consulted. Table 3, taken from Schulz's publication shows the percentages of the different gases of the atmosphere in natural waters.

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[35] East, *Mankind at the Crossroads,* p. 71.

[36] Murray, J., and Hjort, J., *The Depths of the Ocean.* London, 1912. Forchammer, Phil. Trans. Roy. Soc., **155**, 203-262 (1865). Dittmar, *Challenger* Report, Physics and Chemistry, vol. **1**, 1-251 (1884). Quinton, R., *L'eau de Mer.* Paris (1904). Clarke, *Data of Geochemistry,* Bull. U. S. Geol. Survey No. 770 (1924). Stieglitz, Carnegie Inst. of Washington Pub. **107** (1909). Schulz, *Arch. deutsch. Seewarte*, **40**, 1 (1922), **41**, 1 (1923).

[37] Schulz, B., *Die Naturwissenschaften*, **12**, 105-113, 126-133 (1924).

TABLE 3

Gases of the Atmosphere Contained in Fresh and Sea-water. (From Schulz.)

			Nitrogen + Argon	Oxygen	Carbon Dioxide	Nitrogen + Oxygen + Carbon Dioxide
A, in the Atmosphere		In 1,000 cc.	790 cc.	210 cc.	0.3 cc.	
		In per cent	79	21	0.03	
B, In Fresh water	At 0°	In 1,000 cc.	18.64 cc.	10.29 cc.	0.51 cc.	29.4 cc.
		In per cent	63	35	1.7	100
	At 20°	In 1,000 cc.	12.59 cc.	6.57 cc.	0.26 cc.	19.4 cc.
		In per cent	65	34	1.3	100
C, In Sea-water with Salt Content of 3.5 per cent	At 0°	In 1,000 cc.	14.42 cc.	8.04 cc.	0.44 cc.	22.9 cc.
		In per cent	63	35	1.9	100
	At 20°	In 1,000 cc.	10.42 cc.	5.36 cc.	0.23 cc.	16.0 cc.
		In per cent	65	34	1.4	100

Owing to differences in the solubility of the gases of the atmosphere the ratios of oxygen, nitrogen and carbon dioxide are different in water than in air. The amounts of the gases present in the water are, of course, greatly influenced by temperature, as well as by the salinity; both of these factors vary greatly in different parts of the world. It has been found that in the summer the oxygen content of sea-water at depths of about 20 meters is greater than that obtaining with sea-water and air in equilibrium. The explanation offered for this is that during the summer months photosynthesis by marine plants is in excess of respiration and the oxygen liberated during photosynthesis accumulates in the water. At the surface of the sea where there is opportunity for diffusion into the atmosphere,

TABLE 4

Increased Oxygen Content of Sea-water Due to Photosynthesis in Summer. (From Schulz.)

"Poseidon." Station 13.

56° 40′ N, 2° 14′ E.

July 19, 1921.

Depth m.	Water Temperature ° C.	Salt content °/00	Oxygen cc./L.	Oxygen Relative Content
0	14.60	34.25	5.77	100
5	14.53	34.25	5.87	101
10	13.78	34.60	6.05	103
20	12.17	34.99	6.46	107
30	11.56	34.99	6.13	100
40	10.96	35.01	6.08	98
50	6.71	35.07	5.34	79
60	6.70	35.03	5.36	79
70	6.72	35.07	5.34	79
78	6.74	35.08	4.66	69

the oxygen-content is equal to that of sea-water in equilibrium with air. In Table 4 are given the results of one of Schulz's analyses. By "relative oxygen-content" is meant the ratio in per cent of sea-water in equilibrium with air to the amounts actually found.

In the winter the conditions are changed; due to cooling, convection currents are set up which result in more thorough mixing of the water. The changes throughout the year are, however, greatly affected by local conditions.[38]

There is a slight increase in the amount of free carbonic acid in sea-water with depth though there is apparently no invert ratio between the oxygen-content and that of carbon dioxide. The quantitative relation of these two gases is of great importance to the life of plants and animals in the sea and the relative content in the sea is probably to a considerable extent also determined by the activity of plants and animals.

TABLE 5[39]

AVERAGE AMOUNT OF FREE CARBONIC ACID IN SEA-WATER AT VARIOUS DEPTHS IN MG. PER LITER.

Surface	42.6	300 fathoms	44.0
25 fathoms	33.7	400 "	41.0
50 "	48.8	800 "	42.2
100 "	43.6	More than 800 fathoms	44.6
200 "	44.6	Bottom	47.4

One of the most striking properties of sea-water is the constancy of its hydrogen ion concentration. Thus Politzsch[40] found that in samples of water taken from the Atlantic, the Mediterranean, the Black Sea and the Baltic the hydrogen ion concentration varied only from $10^{-7.98}$ to $10^{-8.35}$. Atkins[41] has made determinations of the hydrogen ion concentration of sea-water over several years and found that the changes in the pH values are associated with a variety of factors but that the variations are very small. The variations in pH are somewhat greater where there is abundant plant and animal life, as near the coast. Photosynthesis causes the water to become more alkaline, while the carbon dioxide liberated in respiration tends to decrease the alkalinity. Where large streams meet the sea the water may, of course, vary in composition. Also in some regions sulphurous acid is found in the lower depths of the sea which naturally increases the hydrogen ion concentration in that locality.

McClendon[42] and his collaborators have made a careful study of hydrogen ion concentration and carbon dioxide-content of sea-water. They conclude that the pH of sea-water is determined "solely by the ratio of the concentration of buffers, including carbon dioxide and other weak

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[38] Schulz, *Arch. deutsch. Seewarte*, **41**, 8 (1923).
[39] From Clarke, Data of Geochemistry (1924), p. 146.
[40] Politzsch, S., *Biochem. Zeit.*, **37**, 116 (1911).
[41] Atkins, W. R. G., *Journ. Marine Biol. Ass.*, **12**, 717-771 (1922), **13**, 93-118 (1923), **13**, 437-446 (1924).
[42] McClendon, Carnegie Inst. of Washington, Pub. No. **251**, 23-69 (1917).

acids, to the concentration of bases combined with them (excess base over strong acid)." They have worked out methods of determining the pH of sea-water and therefrom the carbon dioxide-tension thereof.

For many years the influence which the oceans may exert on the carbon dioxide-content of the atmosphere has been a subject of much discussion. If chemical reactions in the sea or the activity of living organisms therein influence the carbon dioxide-content of the atmosphere, it is evident that such factors may be of great significance in the photosynthetic activity of land plants. Carbon dioxide is continually being added to and lost from the sea. Submarine volcanic springs and the action of marine animals, the latter forming normal calcium carbonate from bicarbonates, both are among the factors which tend to increase the carbon dioxide-content, while the development of the marine flora tends to remove carbon dioxide. According to a theory of Schlössing[43] the oceans serve as the great regulators of the carbon dioxide-content of the atmosphere. According to this view, due to the presence of carbonates and bicarbonates of the alkaline metals and alkaline earths, sea-water has the capacity of absorbing or giving off carbon dioxide, depending upon the partial pressure of this gas in the atmosphere. If the carbon dioxide-content of the atmosphere increases, the excess will be taken up by the sea-water and if there is a decrease in atmospheric carbon dioxide, this gas will be liberated from the sea-water until equilibrium is reached again. In view of the fact that complete concordance has not been attained in regard to the carbon dioxide-content of the air over the sea and on land, there still exists some difficulty in establishing the validity of Schlössing's theory.

Krogh[44] reported the carbon dioxide-tension of the water of the North Atlantic corresponded to a carbon dioxide-content of the atmosphere of 0.023 per cent. This has been interpreted by Reinau to mean that the carbon dioxide equilibrium is shifting in favor of sea-water and that the oceans are actually absorbing carbon dioxide, although Schlössing stated that the sea does not continuously absorb the carbon dioxide and that the adjustment is a very slow one. Reinau[45] concludes that there is little connection between the carbon dioxide reservoir of the atmosphere and that of the sea. In fact, he considers that the only regulator of carbon dioxide is the life activity of plants and animals, and that the variations in the carbon dioxide-content are to be ascribed entirely to the factor which regulates "assimilation" and "dissimilation" of carbon, namely, light and heat, or, ultimately the sun.

There appears to be little doubt as to the possibility of an interchange of carbon dioxide between the sea and the atmosphere. The conditions affecting this equilibrium over long periods of time are, however, numerous and apparently impossible to evaluate and correlate in a quantitative sense. The possible significance of variations in carbon dioxide-content of the

[43] Schlössing, Compt. rend., **90**, 1410 (1880).
[44] Krogh, Compt. rend., **139**, 896 (1904).
[45] Reinau, E., *Kohlensäure und Pflanzen*, p. 140.

atmosphere in relation to the great climatic alternations as evidenced by glacial periods and epochs of luxuriant vegetative growth, is, after all, a matter of speculation upon which we cannot enter here. These are questions which involve thorough discussion from the geological viewpoint [46] and upon which there still exist divergent opinions. The conditions of interchange of carbon dioxide between atmosphere and sea as they exist at present are sufficiently complex. The biochemistry of the sea is of itself a chemical constellation.

The living organisms in the sea exert a decided influence on the composition of the water. The plants absorb large quantities of carbon dioxide and convert it into organic compounds. United with these are hydrogen, nitrogen, sulphur and phosphorus. These compounds are in turn appropriated by the animals of the sea, constituting a system fully as complex as the life of land plants and animals. Many of these animals liberate carbon dioxide from bicarbonates, precipitating calcium carbonate, and thus enormous calcareous deposits of tufa and travertine are formed. After death the bodies of both plants and animals decompose yielding ammonia, hydrogen sulphide and carbon dioxide. These compounds are again drawn into the metabolism of other organisms. The whole forms an enormously complex series of cycles involving fine adjustments and stupendous quantities of material, an evaluation of which has not yet been attained.

The whole question of the carbon dioxide-content of the atmosphere in different parts of the world, under varying conditions and its relation to the sea and the life therein is in need of thorough study over a longer period of time. It is only within recent years that reliable methods of analysis have been perfected. Such a survey should include studies not only at marine stations near the coast but also at localities far distant from the mainland and with consideration of the effect of the great streams.

McClendon [47] has made some very valuable contributions to the methods of analysis of sea-water. Some of his conclusions are here quoted. "It has been supposed that the amount of CO_2 in sea-water regulates the growth of seaweed, but the reverse is probably more nearly correct. The respiratory quotient of marine organisms seems to be about 0.7 to 1.0 and the respiration of animals and plants reciprocal. Some marine bacteria take their oxygen from nitrates, but this effect must be minute, since the supply of nitrates is small. The atmosphere cannot be the chief regulator of the CO_2 of the sea, since there is about 30 times as much CO_2 in the sea as in the air. There is always a superabundance of CO_2 in sea-water to supply the needs of green, red, or brown seaweed, but by using it the plants increase the pH of the water. It seems probable that the plants grow rapidly until the pH that is most favorable to them is ex-

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[46] Chamberlin, T. C., and Salisbury, R. D., *Geology*. New York, 1907. Vol. I, 640. Arrhenius, *S. Phil. Mag.* (5), 41, 237 (1896). Abbot and Fowle, *Ann. Astrophys. Obs. Smithsonian Inst.*, 2, 172, 175 (1908).

[47] McClendon, J. F., Carnegie Inst. Wash. Pub. No. 251, p. 37 (1917).

ceeded. This is in harmony with the fact that the pH of the great oceans to the depth penetrated by light is more constant than the CO_2 tension, the pH varying from about 8.0 to 8.25 and the CO_2 tension from about 0.00015 to 0.0005 atmosphere. The sea may be compared to the body of one of the higher vertebrates. The mammal regulates the pH of the blood through the action of the respiratory center. The sea regulates the pH of its surface-water most probably through the action of seaweed. The limit in the supply of oxygen probably prevents animal life from getting the upper hand temporarily and thus endangering the communal life in the sea.

"It seems probable that seaweeds regulate the CO_2 of the atmosphere. The gaseous exchange between sea and air is necessarily at the surface and is comparatively slow. Bohr observed that the absorption of CO_2 from an atmosphere of the pure gas by CO_2—free water that is stirred (probably more vigorously than the sea ever is) is about 0.1 cc. per square centimeter of surface per minute. Since the difference in CO_2 tension between air and sea seems never to exceed 0.02 per cent of that in Bohr's experiment, except in the polar regions, the rate of diffusion would not exceed 0.00001 cc. per square centimeter per minute or 0.1 cc. per square meter per minute in a storm, and necessarily much less in calm weather on account of the lessened rate of stirring at the surface. When we consider the volume of the sea and air compared to the sea-air surface, the fact becomes intelligible that the CO_2 in the air is relatively constant (3 per 10,000) in the different regions of the world where it has been accurately measured, whereas the CO_2 tension of the sea-surface varies from 1.5 to 5 per 10,000. The air is stirred more rapidly than the sea, and the CO_2 of the air seems to be determined by an equilibrium between gain in CO_2 over some regions of the sea-surface and loss over others. The partial pressure of CO_2 in the air is therefore the average CO_2 tension of the sea-surface. The burning of billions of tons of coal per year is probably changing the CO_2 content of the sea and of the rocks and not of the atmosphere."

McClendon's analysis of the relation of the carbon dioxide in the atmosphere and in the sea is much more thorough than that of Reinau. McClendon points out that Krogh's results show that the carbon dioxide-tension of sea-water increases with depth and that the tension of the sea-surface is not in equilibrium with the air. This is also evident from the work of Schulz already cited. Moreover, the carbon dioxide-tension of sea-water varies; Krogh found it to vary from 1.5 to 3 per 10,000 in the North Atlantic and McClendon from 3.3 to 4.7 per 10,000 at Tortugas near the Gulf Stream. McClendon assumes that the water maintains a relatively constant carbon dioxide-content and that on its flow northward the carbon dioxide-tension decreases with the lower temperatures. In general it would appear that McClendon's investigations support the general theory of Schlössing.

It has been assumed, on the basis of some analytical data, that there is a cap or belt of air of low carbon dioxide-content at the poles of the earth. The mixing of a portion of this mass of cold air is supposed to be intimately associated with formation of cyclones. It might be expected therefore that the passage of a "low" would give some indication in the carbon dioxide-content of the air. Some evidence of this is claimed by Lundegårdh,[48] though a clear demonstration thereof is made difficult on account of the influence of other factors.

The carbon dioxide-content of the sea and atmosphere represents a dynamic equilibrium. There are, on the one hand, sources which are producing carbon dioxide in prodigious quantities and, on the other hand, agents which are removing the carbon dioxide from air and sea. The nature of these sources and agents differs widely. The situation can be schematically represented about as follows:

CO_2 PRODUCTION				CO_2 CONSUMPTION
Springs Volcanoes Combustion of Coal, Wood and Petroleum Respiration of lower organisms, plants and animals	⟶	Atmosphere and Sea	⟶	Photosynthesis by Plants $CaCO_3$-forming organisms Chemosynthesis of certain bacteria The weathering of rocks

It is not possible to discuss here exhaustively all the factors which effect this equilibrium. We are dealing with chemical changes taking place on an enormous scale and in many cases involving extensive periods of time. Nor are we probably familiar with all the factors which have a part in this great drama in which the life of man plays but an insignificant rôle. For this reason most calculations are but rough approximations.

Let us consider first the sources of carbon dioxide. What may be termed the inorganic sources are perhaps of the greatest influence. Thus, there are ancient craters (e.g. Agnano near Naples) and gas wells which have been discharging carbon dioxide since time immemorial. The composition of the gases of volcanic eruptions varies greatly but carbon dioxide is a frequent component.[49] The observations of Lewy [50] on the island of Guadaloup, though not very accurate, indicate that after the eruption of the volcano the atmosphere contained fifty times the normal amount of carbon dioxide. No consistent efforts have been made to determine whether the great volcanic disturbances result in changes in the atmospheric carbon dioxide-content at distances from the place of eruption. Even less is known concerning the volcanic eruptions at the bottom of the sea. Mineral springs constitute a very important source of carbon dioxide; the waters of some of them are supersaturated with the gas as they issue

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[48] Lundegårdh, *Der Kreislauf der Kohlensäure*, p. 38.

[49] Allen, E. T. J., Franklin Inst., **193**, 29 (1922) for bibliography and chemical analyses of volcanic gases.

[50] Lewy, M., *Ann. Chim. Phys.*, **8**, 425 (1843).

from the earth. However, of none of these sources can an approach at quantitative estimation be made.

More accurate estimations can be made of carbon dioxide produced through the combustion of mineral fuels. A detailed compilation of the world's coal production for 1920 has been made by Sievers.[51] According to these figures the annual production of coal amounted to a total of 1,317,000,000 metric tons (2204 lbs.) or $1,317 \times 10^9$ kilograms. This includes anthracite, bituminous and lignite or corresponds at the most to about 70 per cent carbon which when burned would give about 338×10^{10} kilograms of carbon dioxide. Thus we have 338×10^{10} kilograms carbon dioxide from the burning of coal compared to the 21×10^{14} kilograms of carbon dioxide in the atmosphere, or an annual production of 0.16 per cent of the existing carbon dioxide. At this rate, other conditions remaining the same, it would take about 650 years to double the concentration of carbon dioxide in the atmosphere.[52]

If the amount of carbon dioxide produced annually by the burning of coal were distributed through the entire atmosphere it would exert but a very slight effect and could not be detected by our present methods of analysis. It is possible, however, that the mixing above about 100 meters is slow, so that it may be expected that the carbon dioxide-concentration near large industrial centers is higher.

Coal has been used on a large scale for only about one hundred years. If the rate of increase in consumption continues it might be expected that this would result in an accumulation of atmospheric carbon dioxide. On the other hand such an increase in carbon dioxide-content of the atmosphere would result in augmented photosynthetic activity, which in turn would tend to reëstablish the equilibrium. To what extent such an increase in carbon dioxide-content would be noticeable in the vegetation of the earth it is difficult to calculate.

Increase in the rate of photosynthesis is about directly proportional to increase in carbon dioxide-concentration under what may be termed natural conditions. That is, for instance, with bright sunlight, at 20-25°, and ample water supply a plant will about double its photosynthetic rate when the carbon dioxide concentration is raised from 0.03 to 0.06 per cent. However, the influence of external factors on the rate of photosynthesis is very complex, as will be shown later; moreover, photosynthesis is but one factor affecting the development of a plant, so that it is very difficult to make predictions regarding the final effect of any single factor. Moreover, an increase in the carbon dioxide-content of the atmosphere would in time be largely equalized by the influence of the sea.

During the last few years there has been much discussion and specula-

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[51] Sievers, E. G., *Gas Age Record*, **51**, 757-761 (1923).

[52] The annual coal consumption has been constantly increasing so that various older estimations are at slight variance with this figure. Krogh, *Meddelelser om Groenland*, **26**, 419 (1904). Van Hise, Mon. U. S. Geol. Survey, **47**, 964 (1904). Dittmar, *Challenger Report*, Vol. 1, pt. 2, 954. Chamberlin, *Jour. Geol.*, **7**, 682 (1899).

tion regarding the influence of the carbon dioxide-content of the atmosphere on our economic plants. Many schemes have been advanced for increasing the atmospheric carbon dioxide. These have included the setting afire of the coal deposits of the polar regions. Nernst has suggested that this coal, which is inaccessible for mining purposes, be ignited and kept burning by means of shafts. It would thus become useful to man by increasing the carbon dioxide-content of the atmosphere, resulting in higher crop yields. It has also been suggested that powdered coal of low grade be spread over the cultivated land. Through auto-oxidation the coal would yield carbon dioxide which would be directly available for the plant.[53]

The respiration of plants including fungi and bacteria yields very considerable quantities of carbon dioxide. Fundamentally, of course, most of the material which is thus burned is of photosynthetic origin, so that these organisms are simply reconverting the products of photosynthesis into carbon dioxide and water. The rate of respiration of most of the lower organisms is relatively very high, so that even when the period of their activity is short the amount of work accomplished is great. The amount of carbon dioxide produced by micro-organisms per unit surface or weight is many times that of man.

The number of substances which bacteria can convert into carbon dioxide is very great; this assures the complete conversion of plants and animals after death to the simplest substances. The complete process of the degradation of carbon compounds by lower organisms presents in detail an exceedingly complex and intricate picture of the interrelation of these organisms. The chief product of photosynthesis is probably cellulose. This is converted by various organisms into soluble carbohydrates or in the absence of air into formic, acetic, butyric acids, carbon dioxide, hydrogen and methane. The latter two gases are further oxidized by special organisms to carbon dioxide and water, so that while there are enormous quantities of hydrogen and methane produced in this manner annually and have been for eons, only traces of these gases are found in our atmosphere. The soluble carbohydrates may be fermented to alcohols and fatty acids which in turn are converted by other bacteria and some fungi to carbon dioxide and water. The anaerobic cellulose-destroying organisms play an enormously important rôle in the carbon dioxide cycle, as much of the cellulose is finally buried under water or soil. Similarly other substances, such as proteins, which contain besides carbon, hydrogen and oxygen also nitrogen, phosphorus and sulphur are completely broken down to simple inorganic compounds. In these processes, many of which are analogous to step-reactions, there often exists a close interrelationship between aerobic and anaerobic organisms. Were it not for the lower organisms such as bacteria and fungi which rapidly convert the components of dead plants and animals to carbon dioxide and other simple compounds, it is conceivable that large quantities of carbon dioxide

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[53] Reinau, E., *Kohlensäure und Pflanzen.* Halle, 1920, p. 125.

would in time be withdrawn from the atmosphere, and that the rates of change in the carbon dioxide cycle would be greatly retarded. The effect of such a state of affairs on the life of our planet is impossible to imagine. An important factor in the production of carbon dioxide by decaying animal and vegetable matter is that it is produced under or on the surface of the ground where it soon becomes directly available again for photosynthesis. Air analyses show that the carbon dioxide content near the ground is often many times that at several feet higher. The amount of carbon dioxide produced by lower organisms cannot be calculated with any degree of accuracy. Over a period of years it would, of course, not be in excess of the amount of carbon dioxide reduced by the chlorophyllous plants, though probably it is greater than the amount produced by higher plants and animals.

The higher chlorophyll-bearing plants cannot be regarded as carbon dioxide producers, for in the total span of their life history they reduce carbon dioxide and convert it largely into carbohydrates. Of course all of the carbon dioxide reduced by a chlorophyllous plant is not permanently laid down as carbohydrate. The plant is a living organism and requires energy for its life processes: this it obtains from the oxidation of carbon compounds previously produced. Thus a portion of the material produced in photosynthesis is oxidized by the plant and is in a sense the operating expense of the factory. This factor varies with different species, but in most cases 15 to 20 per cent of the material synthesized is consumed in this manner. The carbon dioxide production of germinating seeds is very high. In some cases as much as 25 per cent of the dry material is consumed within 24 hours. In this manner most of the stored material is depleted by the time the plant becomes a self-supporting organism. But taken as a whole and during the entire course of its life the chlorophyllous plant is on the minus side of the carbon dioxide cycle.

The carbon dioxide production of animals is in a sense a reversal of the photosynthetic process. The food of animals is directly or indirectly a product of photosynthesis. The amount of carbon dioxide which can be produced by the respiration of animals is naturally dependent upon the quantity of material produced by photosynthesis. The respiration of animals, therefore, tends to maintain the constancy of the atmospheric carbon dioxide. Not all of the carbon in the food of animals is exhaled as carbon dioxide. In mammals about 5 per cent of the carbon is excreted in the form of organic compounds. The latter are in turn broken down by micro-organisms. The rate of carbon dioxide production and the degree of oxidation of the organic food materials varies greatly with different animals.

Some idea can be gained of this source of carbon dioxide from a calculation of the amount produced by man. While the amount varies greatly with weight and muscular work, we can take the value of 900 grams of CO_2 produced per individual per day. The 1750 million inhabitants[54] of the earth would thus produce 1575 million kilograms of carbon dioxide or 575×10^9 kilograms per year. Compared to the atmospheric supply of 2×10^{15} kilograms this is a very insignificant amount, about 0.02 per cent; it nevertheless corresponds to 156×10^9 kilograms of carbon or approximately 230,000,000 tons of coal.

It is impossible to estimate the quantity of carbon dioxide produced by other animals. In general, however, it is probably safe to state that of the total of what chlorophyllous plants produce in the form of carbon compounds through photosynthesis, the greater portion is reconverted into carbon dioxide not by animals, but by lower plants and microorganisms.

Turning now to the other side of the carbon dioxide cycle, that of CO_2-consumption, photosynthesis by the chlorophyllous plants is the factor of foremost importance. While some attempts have been made to estimate the amount of photosynthesis on the surface of the entire earth, this involves so many variables that the results of such calculations must be regarded as subject to more or less drastic revision. Such a calculation, it has been claimed, would be of value in determining the total amount of food the earth is capable of producing and consequently contribute to the general problem of world population. But different plants differ enormously in their behavior under like conditions which, together with differences between tropical and temperate regions, makes such a calculation, with the available data, impossible.

The amount of carbon dioxide which plants remove from the atmosphere is very considerable. Thus Noll[55] has calculated that a tree of 5000 kilograms dry weight contains about 2500 kilograms of carbon. In order to have obtained this amount of carbon the tree must have removed the carbon dioxide from about 12 million cubic meters of air. Similarly the old calculations of Sachs,[56] while they cannot be taken as an exact measure of photosynthetic activity, give an idea of the order of magnitude of the function of plants. The leaves of an ordinary sunflower plant have an area of about 1.5 square meters. Sachs found that such a plant absorbs 660 cc. or 1.3 grams of carbon dioxide per hour. In a ten hour day the plant would absorb about 400 grams of carbon dioxide per month. If the entire land area of the earth were covered with sunflowers so that on each square meter there was one plant or a million plants to each square kilometer, the plants covering the 135 million square kilometers of land would absorb 54×10^{12} kilograms carbon dioxide in a month. At this rate, the 21×10^{14} kilograms carbon dioxide of the atmosphere would last about 40 months. The limited significance of such calculations requires no further comment. The concentration of atmospheric carbon dioxide represents a condition of dynamic equilibrium in which photosynthesis is but one factor.

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[54] East, E. M., *Mankind at the Crossroads.* N. Y., 1924, p. 111.

[55] Noll, Strasburger, *Lehrbuch der Botanik.* Jena, 1906. 8th Edition, p. 181.

[56] Sachs, J., *Arbeiten aus dem bot. Inst. Würzburg,* **3**, 1-33 (1884).

The quantity of carbon dioxide consumed in the weathering of rocks can also not be given any precise valuation though undoubtedly considerable amounts are thus consumed. The carbon in the coal and petroleum deposits and probably also that in the sedimentary rocks was drawn from the atmosphere ages ago.

It has already been stated that photosynthesis constitutes the chief means of counteracting the "running down" in energy of our planet. Photosynthesis is essentially a process of reduction, in which a carbon compound containing the maximum amount of oxygen is reduced to compounds of carbon containing hydrogen and oxygen in the proportion in which the latter two elements are found in water, i.e. carbohydrates. This is an endothermal reaction and the energy for it is obtained from the radiations of the sun. Similarly, nitrates are reduced to amino compounds. Further reductions are carried on through the metabolic activity of the plant resulting in alcohols, fats and hydrocarbons. For the latter reactions, so far as we know, solar energy is not essential, the plant apparently being able to utilize the energy derived from the oxidation of a portion of its carbohydrate supply for these reductions. The chemical kinetics of the metabolic reduction reactions taking place in plants is still an unsolved problem. Largely by means of these reactions there are produced the enormous number of organic compounds found in plants and for which these have become valuable to man. The reducing power of the metabolic activity of plants is illustrated by the fact that a wide variety of compounds can be reduced, many of which never enter into the normal metabolism of plants. This is clearly demonstrated by experiments with the yeast plant.[57] If this plant has an ample supply of sugar it is capable of reducing many different compounds including aliphatic, aromatic and cyclic aldehydes, ketones, nitro-compounds, sodium thiosulfate and others.

Thus the plant in its photosynthetic and metabolic activities may be considered as a reducing machine, providing materials which are capable of combining with oxygen and thus serve man as food and fuel. The object of agriculture is, of course, the production of food materials for man by means of the photosynthetic process. Our present chief sources of energy in the form of coal and petroleum are likewise the product of photosynthetic activity, produced ages ago. It is only comparatively recently that man is realizing that he is using energy at a greater rate than it is being placed at his disposal, that his industries depend upon the accumulation of centuries. Especially in the case of petroleum is it being realized that, while depletion of the supply may not be imminent, nevertheless this source of energy is not in the form of a continuous flow, and that the question of its exhaustion should be given timely consideration.[58] While opinions are still divided as to the extent of the world's petroleum reserves,

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[57] Neuberg and Ehrlich, *Biochem. Zeit.*, **101**, 276 (1919-1920).

[58] White, D., *Sibley Journ. Eng.*, 1920, p. 156. Burrell, G. A., *Oil Gas Journ.*, 1920, p. 84. Smith, G. O., Amer. Petroleum Institute, Bull. **132** (1920).

the necessity of investigating other forms of fuel has been very generally recognized.

Probably foremost among these "synthetic fuels" is alcohol. This has been used in limited amounts for motor fuel for many years. Owing to a complex of economic reasons the development of the industry has had many difficulties to contend with. There are in general two methods of alcohol formation: in the first can be included those synthetic methods which start with ethylene or acetylene and in another the fermentation of various vegetable materials. Regarding the first of these Monier-Williams [59] concludes: "On the general question as to whether, apart from cost of production, it is sound policy to look to synthetic alcohol as one of the motor fuels of the future, the following points may be considered. The source of the carbon in synthetic alcohol is coal, while in fermentation alcohol it is derived from atmospheric carbon dioxide. Although the world's reserves of coal are ample, and there is little danger of their being exhausted within a reasonable period, it is likely that the cost of raising the coal will gradually increase as the more easily worked seams are used up and the accessibility of the material diminishes.

"Economy in the use of coal is a matter of national importance, and it is questionable whether alcohol is the most economic form in which the available carbon can be utilized as motor fuel. On the face of it, it would seem preferable to work in the direction of utilizing more directly the heat of combustion of acetylene, possibly by polymerizing it into hydrocarbons of higher boiling-point, rather than to add to it the elements of water which represent so much dead weight in the resulting fuel. Quite apart from this there is the question of the electrical energy required for the manufacture of calcium carbide from lime and coke. Where ample water power is available, the demands made upon coal are limited to that necessary for the actual formation of the carbide in the furnace. It is claimed, however, that under the most favorable conditions electrical power may be obtained almost as cheaply in the neighborhood of coal-fields as near waterfalls. Where coal is used as a source of power, it has been estimated that the amount consumed in the manufacture of synthetic alcohol is nine times as great as that required for the same quantity of fermentation alcohol.

"Another point which has been raised has reference to the value as a fertilizer of calcium cyanamide, prepared from calcium carbide by the Frank-Caro process. One ton of carbide will yield approximately 110 gallons of alcohol. If the carbide is used for the production of calcium cyanamide, Ca.N.CN, the nitrogen thereby made available for crops amounts to 550 to 600 pounds. Applied to potato land under normal weather conditions this quantity of nitrogen should result in an increase of over 20 tons in the crop yield. Twenty tons of potatoes will yield at

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[59] Monier-Williams, C. W., *Power Alcohol.* Oxford Technical Publications. London, 1922, p. 184.

least 400 gallons of alcohol, or nearly four times as much as could be obtained direct from one ton of carbide."

For our purpose we shall confine ourselves to a brief discussion of the production of alcohol through the fermentation of the products of photosynthesis. This in short depends upon the hydrolysis of various polysaccharides such as cellulose, starch or sucrose to monosaccharides and the conversion of the latter according to the general equation, $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$, by means of fermentation. From the standpoint of fuel the most important constituent is the carbon, of this about 66 per cent is converted into alcohol, the remainder passing into the useless CO_2. The amount of fermentable material contained in different vegetable materials varies greatly. Moreover, the cost of hydrolysis varies also

TABLE 6

ALCOHOL OBTAINABLE FROM VARIOUS VEGETABLE MATERIAL.

Material.	Total Fermentable Carbohydrates Per cent	Average Crop Yield per Acre in Tons	Yield 95 Per cent Alcohol per Ton		Yield 95 Per cent Alcohol per Acre	
			U. S. Gallons	Liters	U.S. Gallons	Liters
CEREALS						
Wheat	65	0.5	99.6	375	50	189
Barley	60	0.65	90	341	59	223
Rye	64	0.4	96	363	38	144
Oats	55	0.6	84	318	50	189
Maize	67	0.7	103	390	72	273
Sorghum grain	67	0.7	103	390	72	273
Rice (cleaned)	76	0.43	102	386	44	167
TUBERS						
Potatoes	17.5	6.5	25	95	164	621
Cassava	30	9	44	167	396	1,499
Jerusalem artichoke	17	7	23	87	160	602
Sweet potato	27	4	42	159	168	636
Yam	17	5	24	91	120	454
ROOTS						
Sugar beet	15	14	25	95	353	1,336
Mangolds	5	20	8	31	168	636
Sugar mangolds	8	20	13	49	264	999
Sugar cane	13	15	20	76	306	1,158
Sorghum cane	14	15	14	53	224	848
Molasses (cane)	58	..	88	333	...	...
Molasses (beet)	50	..	72	273	...	...
TREES						
Mowra flowers (dried)	50	1.3	83	314	108	400
Nipa palm (sap)	12	15	18	68	260	984
Zamia palm (pith)	13	..	22	83	...	...
Grass tree (Xanthorrhaea)	..	..	23	87	...	...
Horse chestnuts and acorns	..	..	43	163	...	...
Iceland and Reindeer moss	60	..	76	288		
Sea-weed (dry)	..	..	34	129		

with different raw materials. Table 6 is taken from the book of Monier-Williams; the values have been converted into U. S. gallons and liters.

There are, of course, a great many more plants than those given in Table 6 from which alcohol is produced, the plants used in different parts of the world depending upon a number of different agricultural and economic conditions. It is only the hexose sugars, d-glucose, d-fructose and d-mannose that can be converted readily into alcohol by the enzyme of yeast. Galactose is present in the products of hydrolysis of many plants, but this sugar is fermented with difficulty. The pentose sugars which comprise a considerable proportion of many plants are not fermented with pure yeast. Recently other organisms have been found which convert the pentose sugars into a variety of products, but this is not as yet carried out on a large scale.

The production of alcohol on a large scale has usually been considered from the economic viewpoint. Under present circumstances this is essential and must include the many agricultural conditions related to soil, climate, water supply, fertilizers, etc., as well as the factors of methods of production, labor, transportation, markets, by-products, etc. The problem therefore becomes an exceedingly complicated one. But here we are, for the moment, not concerned with alcohol as competitive or even supplementary to petroleum but rather with the perhaps somewhat speculative idea of whether alcohol can replace petroleum, i.e. whether it can serve as one of our principal sources of energy.

In view of the fact that the raw materials from which alcohol can be produced are all plant products the question immediately arises what effect such a gigantic undertaking would have on the production of foodstuffs. There is no sound indication that man will be able to derive his food from any other source but the soil. So that if his means of obtaining industrial energy is to be the same as that for obtaining foodstuffs, the question is raised whether there will be a competition for arable land to be devoted to food production on the one hand or fuel production on the other. Boyd[60] has made an interesting analysis of the possibilities of using vegetation as a source of motor fuel. The following figures give an idea of the situation:

Average annual U. S. production of corn, 1913-1919, bushels..	2,740,000,000
Average annual Acreage in corn, 1913-1919..................	106,000,000
Alcohol from corn at 2.75 gallons per bushel.................	7,500,000,000

Concerning this Boyd states: "The heating value of this amount of alcohol is about equal to that of 5,000,000,000 gallons of gasoline. The production of gasoline in the U. S. during 1920 was very close to this amount, it having been about 4,900,000,000 gallons. The average acreage in corn as given above is equal to 166,000 square miles, which is more than four times the total area of Ohio. In view of the fact the possible alcohol production from corn represents close to 60 per cent of the total

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[60] Boyd, T. A., *Journ. Ind. and Eng. Chem.*, 13, 836-841 (1921).

possible amount of alcohol that could be prepared from all the starch and sugar containing foodstuffs produced in the United States, and that such a large acreage is required for its production, the possibility of a sufficiently large increase in production of such materials to be diverted to the manufacture of motor fuel seems very unlikely. At any rate if large quantities of motor fuels are to be prepared from vegetation another material, if not instead of foodstuffs, at least in addition to foodstuffs, must be relied upon as a source."

A similar analysis has been made by Lane and Bauer[61] who discuss the various means of developing motor fuels. In considering alcohol as a possible motor fuel, they conclude: "For example, the corn that can be grown on an average acre of Oklahoma soil in a year will yield about 3 barrels of alcohol. Many of the oil fields of Oklahoma have an ultimate production of about 3,500 barrels of crude petroleum per acre, of which about 1000 barrels can be considered as gasoline. One would have to grow corn on an acre of Oklahoma soil land for about three hundred years to obtain a volume of alcohol equal in fuel value to a supply of gasoline produced in from five to ten years. . . . Of course, the area of oil fields in the country is but a small part of the total area of the United States, but this simply emphasizes the fact that while we can grow corn over an area many times as large as the area of the producing oil lands, it will then have to be harvested and brought to the distilleries, a laborious and expensive task. Furthermore, edible grain of any kind is usually too valuable to be used in the manufacture of alcohol. And even if we had a surplus it would be a mere drop in the bucket. A surplus of a 1,000,000,000 bushels of corn would sound tremendous to the farmers of this country, but it would yield only 250,000,000 gallons of motor fuel, enough to run our cars and trucks about three weeks."

There is no doubt that the growing of plants for the purpose of obtaining fuel would require such enormous areas of land that it would materially affect the production of foodstuffs under present systems of agriculture. It has happened that corn has been used directly as fuel. When prices for corn were exceedingly low (32 cents a bushel) it was an economy for the farmer to burn his corn rather than buy coal at 16 dollars a ton. Under such conditions it has happened that even large public utilities have been run with grain as fuel. But this represents extremely abnormal economic conditions and must be considered in the light of an emergency measure rather than as a contribution to the fuel problem. De Baufre[62] has determined the heat value of corn and concludes that with coal at $10.00 a ton, corn on the cob must be less than 20 cents a bushel of 70 pounds to make the use of corn as fuel economical.

At the same time in the production of food by means of agriculture there are tremendous quantities of waste materials. These have high energy content and many of them could be converted into fuel by alcoholic

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[61] Lane, F. W., and Bauer, A. D., *Ind. and Eng. Chem.*, **15**, 479 (1923).
[62] De Baufre, *Power*, **36**, 212 (1922).

fermentation and similar processes. From the waste molasses of the sugar industry alone there are some two hundred million gallons of alcohol produced annually. However, in the utilization of such surplus and waste materials for large scale production a number of difficulties are encountered. The supplies of such materials are often uncertain and irregular, so that operation of a plant is overtaxed at one time and idle at another. Storage of a great deal of such waste material is often impossible or expensive, which has made the continuous operation of the distilleries impossible. One of the requisites for the successful operation of an industry of this kind seems to be that the supply of the product is continuous or synchronous with the demand, thus obviating the expense and hazard associated with the storage of enormous quantities of alcohol. With seasonal crops this has been exceedingly difficult to accomplish.

Probably the most promising waste material of vegetable origin is the cellulose from the lumber industry. Such material answers many of the requirements of a source of fuel: it is easily produced and its supply can, with proper management, be continuous. The chemical methods for hydrolyzing the cellulose to fermentable sugars are, in principal at least, very simple and the waste products from the fermentation process can be returned to the soil to maintain its fertility.[63] Although this problem has been the subject of much intensive research there is still much to be done to assure good yields of alcohol. At present there are obtained from 15 to 25 gallons of 95 per cent alcohol from a ton of sawdust which is about 50 per cent cellulose or a yield of approximately 35 per cent of the theoretical amount. The increase in these yields is dependent upon the development of the chemistry of the processes involved.

Whether there is sufficient waste cellulose available to supply the needs is very difficult to ascertain. It has been estimated that there are approximately 5,000,000,000 cubic feet of waste in the woods and at the mills. Assuming a cubic foot of wood to weigh 30 pounds this would be 75,000,000 tons which at 20 gallons of alcohol per ton would yield 1,500,000,000 gallons of alcohol. This quantity represents about one third of our present motor fuel requirements. In all probability the quantity of waste material could be increased and it is also to be expected that the yield of alcohol can be increased. By making what appear to be rather generous allowances for increase in waste material by various means, Hibbert calculates that about 75 per cent of present requirements can be cared for. Calculations of this nature are, of course, of limited value, as they do not include the various economic factors of labor, transportation, interest charges, etc., which usually are the determining ones. But such approximations as can be made, even in their most sanguine aspect, do not assure a continuous supply of fuel in quantities to meet present petroleum consumption.

There is one other factor which may play a very important rôle in

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[63] Sherrand, E. C., *Chem. Age*, 29, 53 (1921). Hibbert, H., *Journ. Ind. and Eng. Chem.*, 13, 841 (1921).

supplying the needs for fuel through vegetable material.[64] This factor is the productivity of the tropics. While agriculturalists are apparently not in agreement on the potentialities of the tropics, there is no question but that these regions are very favorably suited for the conversion of solar energy through photosynthesis. But, on the whole, the conditions in the tropics are even less amenable to careful survey over a period of years than the temperate zones. The question of the kind of plants best suited for fuel production has received little attention, apparently even as to whether these should be sugar and starch or oil producing plants.

In the production of alcohol fuel is one of the most important items; every gallon of 95 per cent alcohol requires about 15 pounds of coal. In tropical countries refuse from crops must constitute the main fuel supply. In the cane sugar industry the residual fibre or "bagasse" constitutes about one fourth of the weight of the canes. This waste material is unsuitable for the preparation of ethyl alcohol, and as yet probably can be used only as fuel directly.[65]

If alcohol is used as fuel, starting with a 10 per cent solution of alcohol by weight and producing 91 per cent alcohol, about one third of the alcohol produced will be required to furnish the heat necessary for distillation.[66] While a solution of alcohol 91 per cent by weight would probably be satisfactory for fuel, considerably more heat would be required to produce 95 per cent alcohol. These and many other economic and technical problems such as transportation and water supply make the enterprise of fuel alcohol a very complicated one. But if in the future we must look to agriculture for our industrial energy, there is no doubt that the tropics will play a very important part.

How such a state of affairs would affect the production of food it is impossible to say. The temperate portions of Europe and Asia with the exception of Russia are already overpopulated. Tropical Asia largely is in the same condition. It is doubtful whether these as well as the arable portions of the United States and Canada can be counted on for anything but the production of foodstuffs. There still remain huge agricultural areas in tropical South America and central Africa where the population is relatively sparse. Whether these areas are destined to offer relief to the increasing population of Europe and to over-populated Asia, or can be put to other uses than food production raises economic and political questions far beyond the domain of this work.

But the relation of land areas to population is one of the most fundamental economic conditions affecting the lives and destiny of a people. Hence any new factor introduced into these conditions is certain to have profound consequences. The utilization of land areas in the United States has been the subject of much careful study particularly by the

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[64] Whitford, H. N., *Journ. Ind. and Eng. Chem.*, **14**, 151 (1922).

[65] Sherrand, E. C., and Blanco, C. W., *Journ. Ind. and Eng. Chem.*, **12**, 1160 (1920).

[66] From private communication of Dr. W. H. Rodebush.

Department of Agriculture.[67] We cannot here enter upon a discussion of the intricate problem of land requirements in relation to increasing population. Suffice it to state that from the surveys which have been made it appears exceedingly doubtful whether any considerable land areas in the United States could be spared from agricultural needs for the production of fuel. From the report of the U. S. Department of Agriculture, 1923, we learn that 94 per cent of all the land available for crops, pasture and forest are now employed for these purposes, although of these, large areas are and always will be of low productiveness and other areas are under-used. Table 7 is taken from the above-mentioned report.

TABLE 7

CROP AND PASTURE LAND THAT WOULD BE REQUIRED FOR 150,000,000 PEOPLE ASSUMING NO CHANGE IN PER CAPITA CONSUMPTION AND PRODUCTION PER ACRE, ALSO NO EXPORTS OF AGRICULTURAL PRODUCTS AND NO CHANGE IN PER CAPITA IMPORTS.

Use of Land	Area (Acres)
Crop land	431,000,000
Woodland pasture	237,000,000
Other humid pasture	336,000,000*
Semi-arid pasture	587,000,000
Total	1,591,000,000

* As a result of assuming the acreage of semi-arid pasture and woodland pasture to remain constant, the area of other humid pasture is increased in greater proportion than the increase in population.

The report continues: "It has already been noted that if the present policy continued the area of land in forests, beginning with approximately 402,000,000 acres of standing timber, will rapidly diminish until the point of approximate exhaustion is reached. On the other hand, if we wish to provide enough forest land to grow our timber, a much larger quantity of land will be required; at the present rate of growth and of waste and consumption per capita the enormous area of 1,465,000,000 acres would be needed for a population of 150,000,000 people. The impossibility of such an outlook is emphasized by combining this area with the 1,591,000,000 acres of crop and pasture land which, as shown above, would be required under similar assumptions. The total resulting requirement would be 2,819,000,000 acres after allowing for duplications, or about 48 per cent more than the present land area of the continental United States.

"The result suggests that if we are to maintain our present degree of self-sufficiency, for a population of 150,000,000 we must increase the average production per acre of our crop, pasture, and forest land, effect

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[67] Gray, L. C., Baker, O. E., Marschner, F. J., Weitz, B. O., Chapline, W. R., Shepard, W., Zon, R., U. S. Department of Agriculture, Yearbook for 1923, 415-506. Baker, O. E., and Strong, H. M., U. S. Department of Agriculture, Yearbook for 1918, 433-441. East, E. M., "Mankind at the Crossroads," N. Y., 1924.

marked reductions in per capita consumption of farm and forest products, or make changes in both regards."

It is at least highly probable that the relative increasing scarcity of land resources will in a measure be met by increased production per acre and readjustments of standards and habits of living. But if we accept the conclusions of the foregoing report, it certainly appears highly improbable that any considerable area of land can be devoted to the production of industrial energy.

There remains in this connection one factor the value of which it is as yet impossible to evaluate. This applies to the arid or desert regions. There are about 468,000,000 acres of arid grazing land most of which is of low productivity and about 67,000,000 acres of waste desert. It is calculated that there are about 30,000,000 acres capable of irrigation. But the greater portion of the arid lands will probably never be cultivated. Most of these regions are subjected to solar radiation of high intensity and have a large percentage of clear days. If science devises a means of directly utilizing solar energy, may not these be the regions of greatest value for such an undertaking?

Recognizing the difficulties which beset the production of fuel through the intermediary of crop plants, the suggestion has repeatedly been made that all that is necessary is that the botanist or horticulturalist create some kind of a "super-plant." That is, while it is realized that present crops are not adequate to supply the necessary amount of carbohydrate or oil, it ought not to be difficult for the biologist to produce a plant which instead of yielding 300 gallons of alcohol per acre would, for instance, yield 3,000 gallons. This is, of course, the same question which is constantly recurring in regard to the problem of food production: why does not the agricultural explorer bring in new plants which yield two bushels in place of one? The answer is the same for both cases, namely, that the food-plants which constitute our chief means of subsistence were already brought into cultivation by primitive man. While there is a large variety of plants which are used as food in one form or another, man almost the world over, derives most of his food from the grass family, the grains. These together with the seeds of some legumens and oil-bearing nuts constitute the mainstay of man's existence. Undoubtedly our standards of living will in time undergo some readjustments and the yields per unit area be increased somewhat, but it is doubtful whether these will more than keep step with the increasing demand for food occasioned by a growing population.

In advocating the production of material to replace our present sources of energy by means of agriculture not only is the question of area an important one but a number of other factors immediately present themselves. For the production of energy on a large scale reliance could not be placed upon extant material. Recourse would have to be taken to very extensive cultivation. While, as has been stated, there is still considerable arable land not yet under cultivation, it is very clear that any interference with the areas of land required for food production would lead to severe economic disturbances.

Moreover, the fact is too readily forgotten that agriculture is a very sensitive and highly complex industry. Agriculture deals primarily with biological processes involving all the fine adjustments and balances of a growing organism. In the growth of a plant sunlight is but one of a number of determining factors. The fact that during critical periods in the development of a plant slight changes in climatic conditions during a short time may greatly reduce or entirely destroy a crop, serves to emphasize the hazard of obtaining energy through the intermediary of plants. In agriculture water supply and temperature are far more variable and determining factors than light intensity. On the proper coördination of these two factors, probably more than any other, depends the success of crop production. The multiplicity of pests and diseases which annually destroy a large per cent of our crops greatly increase the hazards of this industry. It is evident, therefore, that the plant at best is not only very inefficient in storing solar energy but is also rather unreliable. The plant not only produces organic compounds of great complexity from carbon dioxide and water by the use of solar energy, but also uses a considerable portion of this material for its own life activities. Most plants are, however, thrifty beings. Of the products of their labor they lay by a small portion. From these savings the succeeding generation gains its strength for growth until it is a self-supporting organism. Man lives largely by taking this surplus from the plant for the maintenance of his own life. Agriculture has been largely concerned with the study of conditions of soil, climate and cultivation for the production of this surplus of the plant. It has given little consideration to the vital question, the process by which the plant manufactures its products, i.e., photosynthesis. As has been stated, a clearer understanding of this process is of importance not only that we may understand the internal working of the plant, but now also as a guide to accomplish outside of the living organism what the plant is doing.

In conclusion, then, we may say that solar radiation is the greatest and an inexhaustible supply of energy for our earth. The chlorophyllous plant is a converter of this energy into potential energy; it is from a chemical viewpoint a great reducing mechanism, producing compounds which can combine with oxygen. The transformation of matter involved in this conversion of energy, that is, the chemistry of photosynthesis and metabolism present an exceedingly complex picture. The main reason for this apparent complexity is that photosynthesis is intimately connected with the vital process of the plant and hence subject to the many fine adjustments characteristic of living protoplasm. No analysis of the process of photosynthesis in plants is reliable which does not give due regard to this fact. This need not mean, however, that a photosynthesis attaining the same or analogous results as the plant can never be achieved without the action of living protoplasm. Only, up to the present time no chemical system has been devised which can approach the plant in efficiency or usefulness. An examination of the photosynthetic mechanism of the plant may, therefore, not be without value for discovering the fundamental principles of the transformation of matter by radiant energy.

Chapter 2

The Nature of Photosynthesis as Determined by Observations of Gas Interchange and the Formation of Organic Matter

1. The Gaseous Interchange

The phenomenon of photosynthesis was discovered as a result of investigations on the composition of the atmosphere and the influence of plants on the same. Thus the study of the problems of gaseous interchange or the effect of green plants on the composition of the air surrounding them has been so intimately associated with the development of our conceptions of the phenomenon of photosynthesis, that a consideration of this subject is of primary importance both on account of its direct bearing and because of its historical significance. In fact it can be said that historically considered the subject of plant physiology had its real inception as a recognized branch of experimental science with the study of the interchange of gases by de Saussure.

a. The Path of Gaseous Exchange.

In submerged aquatic plants the carbon dioxide reaches the interior of the plant by means of diffusion of the dissolved gas through the outer walls of the epidermal cells. This is probably also the case in many of the lower land plants. In the leaves of the higher land plants, on the other hand, there are special differentiated organs, the stomata, through which gaseous carbon dioxide passes to the chlorophyll-bearing cells and although the existence of stomata was known for a long time their function in photosynthesis was a subject of great dispute for many years. It had to be determined what portion of the carbon dioxide used by the plant actually passed through the stomata and what portion diffused directly through the outer cuticle of the leaf.

In brief, the stomata are minute mouth-like openings in the surface of the leaf. They are usually on the under side of the leaf though in some species the upper surface also has stomata. There exists considerable variation in the structure of the stomata according to species and habitat of the plant.[1] The cross diameter of the stomata is exceedingly small, 0.0006-0.02 mm. Brown and Escombe[2] found the average area

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[1] Haberlandt, G., *Physiologische Pflanzenanatomie.* Leipzig, 1904, p. 395

[2] Brown and Escombe, Phil. Trans. Roy. Soc. B., **193**, 275 (1900).

of the elliptical cross-section of a perfectly open sunflower stomatum to be 0.0000908 sq. mm. which is equal to the area of a circle 0.0107 mm. in diameter. Their number varies widely, 40 to 300 stomata to the square millimeter according to the species with as high as 700 in some cases. The number of openings in a single leaf thus runs to enormous numbers, a medium sunflower leaf containing about 13 million stomata.

The morphology and details of the functioning of the stomata cannot be entered upon here.[3] Suffice it to state that the cells bordering the openings, the guard-cells, are capable of controlling the size of the opening in response to certain external and internal conditions. It must be borne in mind that the stomata do not serve only for the ingress of carbon dioxide into the leaf, but as well for the egress thereof when the plant is in the dark as well as for the corresponding egress and ingress of oxygen and the escape of water vapor. Thus, for instance, when the stomata close under conditions of extreme heat the rapid loss of water is prevented, but at the same time the conditions are more unfavorable for the absorption of carbon dioxide. In experimental work on photosynthesis it is essential that changes in the size of the stomatal openings of this sort be constantly borne in mind in order to avoid spurious results.

The factors effecting the opening and closing of the stomata are of an extremely complex nature. A very important rôle in these movements of the stomata is played by the starch grains in the guard-cells. The fluctuations in the starch-content of these cells do not run entirely parallel to that of the rest of the leaf, and the behavior of the chloroplasts of the guard-cells is in many respects quite different from that of the chloroplasts of the other cells. The movements of the guard-cells appear to be intimately connected with variations in their osmotic pressure. This in turn is brought about by the hydrolysis and formation of the starch through enzyme action. The influence of various factors on the starch economy of guard-cells has been studied by Iljin[4] who ascribes great importance to certain kations and the hydrogen ion concentration in this phenomenon. Many external factors are of influence on the movements of the stomata. In general, light results in the opening of the stomata and darkness in their closing. This is, however, not a universal rule, for after prolonged darkness the stomata may be wide open. Lloyd[5] found that in blue light stomata open but not so much as normally or as in red light.

Considerable difference of opinion still exists regarding the effect of air enriched in CO_2 on the condition of the stomata. Linsbauer[6] concludes that increasing the CO_2-content of air results in a closing of the stomata,

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[3] Lloyd, F. E., *The Physiology of Stomata.* Pub. No. 82. Carnegie Institution of Washington (1908).

[4] Iljin, W. S., *Biochem. Zeit.*, **132**, 494, 511, 543 (1922). Wiggans, R. G., *Am. J. Botany*, **8**, 30-40 (1921).

[5] Lloyd, *l.c.*, 114.

[6] Linsbauer, K., *Flora*, **109**, 100 (1916).

and the opposite effect is attained with CO_2-free air. Lloyd[7] states: "My conclusion is, therefore, that the presence or absence of CO_2 has no direct influence on stomata, and that, physiologically, they are not at all dependent upon photosynthetic processes within the guard-cells. If this is true, the guard-cell is set off as distinct physiologically from the chlorenchyma-cell. . . . The mere fact that stomata open in the absence of CO_2 shows conclusively that the movement is not directly connected with photosynthetic activity, even if the process takes place normally in the stomata.

"The reduction and increase of starch in the guard-cells, in the absence of carbon dioxide, points rather clearly to the activity of an enzyme, presumably a sort of diastase, as a factor in the mechanism. . . ."

Undoubtedly the causes underlying the opening and closing of the stomata are of a very intricate nature. In view of the fundamental importance of stomata to the photosynthetic process the subject is in great need of thorough investigation on the basis of experimental procedure in which the various factors are subject to careful control. In this connection the findings of Molisch and others (discussed here in the chapter on carbohydrate transformations) of the conversion of starch into soluble sugars in wilting leaves may be of considerable significance.

The old debate as to the path of gaseous exchange has been quite definitely settled. The idea that the CO_2 is absorbed through the cuticle of the leaf, as maintained by Boussingault[8] and Barthélemy,[9] has been replaced by the establishment of the stomata as the main path of gaseous exchange. By stopping-up the stomata, Mangin and Stahl[10] were able to demonstrate the retardation of gaseous exchange. Quantitative relations were first established by Blackman[11] who measured the quantity of carbon dioxide passing in and out of living leaves of which the distribution of the stomata had been determined. The plugging of the stomata with vaseline or water does not completely prevent carbon dioxide diffusion, for Blackman found that more carbon dioxide passes through the surface in which the stomata had been stopped-up with vaseline than through the unvaselined upper surface containing no stomata. Under such artificial conditions there is a slight diffusion of carbon dioxide through the cuticle, but the results seem to indicate that under normal conditions the stomata are the chief path of gaseous exchange in the leaf. Blackman used specially devised glass chambers which were sealed to both sides of the leaves and air was passed through these chambers. By determining the difference in the amount of carbon dioxide in the air before and after passing through the chambers the quantity of carbon dioxide given off by the leaf was

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[7] Lloyd, *l.c.*, 125.

[8] Boussingault, *Agronomic*, **4**, 359 (1868).

[9] Barthélemy, *Ann. Sci. Nat. Bot.*, V Ser., **9**, 287 (1868); **19**, 131 (1874); Compt. rend., **84**, 663 (1877); **85**, 1055 (1877).

[10] Mangin, Compt. rend., **105**, 879 (1887). Stahl, *Bot. Zeitg.*, **52**, 117 (1894). Garreau, *Ann. Sci. Nat. Bot.*, III Ser., **13**, 321 (1850). Merget, Compt. rend., **84**, 376, 957 (1877); **86**, 1492 (1878). Wiesner and Molisch, *Sitzber. K. Akad. Wiss. Wien.*, **79**, I, 368 (1879).

[11] Blackman, *Phil. Trans. Roy. Soc. London, B.*, **186**, 503-562 (1895).

established. These quantities were then correlated to the number of stomata on each surface. A similar procedure was followed in order to establish the path when carbon dioxide is taken up by the leaf in the light. It was concluded that in both cases the carbon dioxide passes primarily through the stomata.

Jörgensen and Stiles [12] have summarized the result obtained by Blackman of the quantities of carbon dioxide emitted from the two surfaces of various leaves in proportion to the number of stomata on each surface.

TABLE 8

AMOUNTS OF CARBON DIOXIDE GIVEN OUT FROM THE TWO SURFACES OF LEAVES.
(From Jörgensen and Stiles.)

Plant	Peculiarity	Stomatic Ratio. $\frac{\text{Upper Surface}}{\text{Lower Surface}}$	CO_2 Respired. $\frac{\text{Upper Surface}}{\text{Lower Surface}}$	
Nerium oleander	Very thick cuticle	$\frac{0}{100}$	$\frac{3}{100}$	$\frac{6}{100}$
Prunus laurocerasus	" " "	$\frac{0}{100}$	$\frac{0}{100}$	$\frac{4}{100}$
Hedera helix	" " "	$\frac{0}{100}$	$\frac{4}{100}$	
Plantanus occidentalis	Thin cuticle	$\frac{0}{100}$	$\frac{3}{100}$	
Ampelopsis hederacea	" "	$\frac{0}{100}$	$\frac{3}{100}$	
Polygonum sacchalinense ..	" "	$\frac{0}{100}$	$\frac{6}{100}$	
Alisma plantago	Aquatic plant. More stomata on upper surface	$\frac{135}{100}$	$\frac{135}{100}$ $\frac{115}{100}$	$\frac{120}{100}$ $\frac{113}{100}$
Iris germanica	Isobilateral leaf	$\frac{100}{100}$	$\frac{105}{100}$	$\frac{110}{100}$
Populus nigra	Stomata on both surfaces, fewer on upper	$\frac{100}{575}$	$\frac{100}{375}$	
Helianthus tuberosus	"	$\frac{100}{240}$	$\frac{100}{273}$	
Tropæolum majus	"	$\frac{100}{200}$	$\frac{100}{265}$	

From Table 8 it can be seen that the quantities of carbon dioxide emitted by the various leaves in most cases is in direct proportion to the stomatic ratio, at least within the experimental error. Similar experiments were carried out by Blackman with illuminated leaves, in which

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[12] Jörgensen and Stiles, *Carbon Assimilation.* London, 1917, p. 53.

he found that the absorption of carbon dioxide by the two leaf surfaces also followed the stomatic ratio.

With some improvements in methods, Brown and Escombe[13] repeated the work of Blackman and determined the amounts of carbon dioxide emitted and absorbed by the two sides of various leaves on which the distribution of the stomata was ascertained by actual counting under the microscope. The results obtained for carbon dioxide evolution are given in Table 9.

TABLE 9

EMISSION OF CARBON DIOXIDE FROM UPPER AND LOWER SURFACE OF LEAVES DURING RESPIRATION. (From Brown and Escombe.)

Plant	Time in Hours	Leaf Area in Sq. Cm.	Stomatic Ratio. $\frac{\text{Upper Surface}}{\text{Lower Surface}}$	CO_2 Emitted. $\frac{\text{Upper Surface}}{\text{Lower Surface}}$
Canna indica	4.75	28.27	$\frac{100}{246}$	$\frac{100}{246}$
“ “	5.00	28.27	$\frac{100}{246}$	$\frac{100}{322}$
“ “	4.23	28.27	$\frac{100}{246}$	$\frac{100}{210}$
Rumex alpinum	5.50	59.44	$\frac{100}{269}$	$\frac{100}{286}$

Similarly the results obtained for the absorption of CO_2 during photosynthesis are given in Table 10.

TABLE 10

ABSORPTION OF CARBON DIOXIDE ON UPPER AND LOWER SURFACE OF LEAVES DURING PHOTOSYNTHESIS. (From Brown and Escombe.)

Plant	Time in Hours	Leaf Area in Sq. Cm.	Stomatic Ratio. $\frac{\text{Upper Surface}}{\text{Lower Surface}}$	CO_2 Fixed. $\frac{\text{Upper Surface}}{\text{Lower Surface}}$
Colchicum speciosum	5.75	59.44	$\frac{100}{119}$	$\frac{100}{72}$
Senecio macrophyllum	4.75	28.27	$\frac{100}{126}$	$\frac{100}{92}$
“ “	4.25	28.27	$\frac{100}{126}$	$\frac{100}{72}$
Rumex alpinum	5.00	59.44	$\frac{100}{269}$	$\frac{100}{144}$
“ “	5.50	59.44	$\frac{100}{269}$	$\frac{100}{130}$

From these experiments it can be seen that in leaves having stomata on both surfaces the ratio of carbon dioxide emitted follows very closely the ratio of the distribution of the stomata on the two surfaces. It seems

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[13] Brown and Escombe, *Proc. Roy. Soc. London, B.*, **76**, 61 (1905).

reasonable to conclude that the path of gaseous exchange under these conditions is primarily through the stomata. If, for instance, in the case of leaves with stomata confined to the lower surface, it were assumed that the carbon dioxide passed through the cuticle this would have to possess a permeability for carbon dioxide 50 to 100 times that of the cuticle on the upper surface. This seems impossible in view of the fact that leaves with thin cuticles gave the same results as those with very heavy cuticles.

Leaves of *Nuphar advena* and *Catalpa bignonioides* which have stomata respectively only on the upper and lower surface show an absorption of carbon dioxide during photosynthesis only on the surfaces containing the stomata.

In the absorption of carbon dioxide during photosynthesis the relations are slightly different. Brown and Escombe found that the intake of carbon dioxide by the lower surface of leaves illuminated on the upper surface is always less than might be expected from the stomatic ratio. The quantity of carbon dioxide taken up by the lower surface is sometimes half the amount expected. Such a difference in the ratios of gaseous exchange to stomatic openings in egress and ingress of carbon dioxide is not surprising. With a constant rate of carbon dioxide formation within the leaf during respiration, the rate at which the gas diffuses out is independent of the degree of opening of the stomata. If the stomata should partially close during the diffusion, the partial pressure of the carbon dioxide in the leaf will increase. This increase in carbon dioxide-pressure will be inversely proportional to the changed linear dimension of the stomata. The increased carbon dioxide-pressure will tend to counterbalance the effect of the diminished aperture, and the quantity of carbon dioxide emitted from the leaf will be only very slightly affected by the changes in size of the stomata. The increase in friction through the smaller openings is probably so slight that it could not be detected by the ordinary methods.

The conditions of absorption of carbon dioxide during photosynthesis are, however, quite different. Given now a constant rate of carbon dioxide fixation within the leaf, the partial pressure of the carbon dioxide in the atmosphere surrounding the leaf is constant. The ingress of carbon dioxide must therefore vary directly with the linear dimensions of the stomata, and the relative rate of carbon dioxide-absorption during photosynthesis will be proportional to the number of stomata per equal area as well as to the degree to which the stomata are opened. In view of the fact that Brown and Escombe in their experiments illuminated the upper surface of the leaves, it is not surprising that they should have found an apparent excess of photosynthesis on this surface. The one sided illumination in all probability brought about the partial opening of the stomata on the illuminated surface and, moreover, the light active in photosynthesis would be largely absorbed by the chloroplasts of the parenchyma into which the stomata of the illuminated surface open. This would result in a steeper diffusion gradient between the atmosphere and the intercellular spaces of the upper surface, a condition which would favor a more rapid absorption of carbon dioxide by the upper surface.

Brown and Escombe [14] then made a very thorough study of the purely physical process by which the carbon dioxide of the atmosphere enters the leaf, and presented the results of their laborious investigations in a masterful fashion. The difficulties in accepting simple diffusion as an explanation of the way in which carbon dioxide gets into the leaf are: (1) the relatively large amount of carbon dioxide absorbed by a leaf during active photosynthesis, about 0.1 cc. CO_2 per sq. cm. of leaf surface per hour; (2) the low partial pressure of carbon dioxide in the atmosphere, 0.031 per cent by volume; (3) the very small portion of the surface of a leaf which represents stomatic openings, 1 to 3 per cent.

Thus, Brown and Escombe state: "As a concrete example we will take the case of a leaf with which we have done a considerable amount of work, that of *Catalpa bignonioides,* in which we have carefully determined both the number of stomata (which here occur only on the lower surface) and the area of the stomatal slits when fully opened. This leaf when placed under favorable conditions for assimilation, can abstract from ordinary air containing three parts CO_2 per 10,000, about .07 cc. of carbon dioxide (measured at 0° and 760 millims. bar.) per sq. cm. of leaf surface per hour. The stomatal slits when fully open have an area of .0000618 square millim., and since there are 145 of them on each square mm. of leaf, the area of the stomatal openings only represents .9 per cent of the total surface of the leaf on which they occur. It follows from this that if we regard the whole of the carbon dioxide as entering the leaf through these openings, diffusion must take place through them at the rate of $\frac{100 \times .07}{.9} = 7.77$ cc. per hour. Now it will be seen later on that the surface of a strong solution of caustic soda, when freely exposed to moderately still air containing the normal amount of carbon dioxide (three parts per 10,000 by volume), absorbs that gas at ordinary temperatures at the rate of only about .120 cc. per sq. cm. per hour, and when the rate of the air current passing over the surface is increased the maximum absorption is found to be .177 cc. per sq. cm. per hour. It follows, therefore, that the absorption of atmospheric carbon dioxide by the whole of the under surface of an assimilating leaf like that of the Catalpa, must proceed at about one-half the rate which the same absorptive surface of leaf would possess if it were covered with a constantly renewed film of a solution of caustic alkali; we may say, in fact, that the coefficient of absorption of the leaf surface under these conditions is about half that of the surface of the alkaline solution. If, however, we assume that the absorption of carbon dioxide in the leaf takes place only through the stomatal openings—which occupy at the

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[14] Brown and Escombe, *Phil. Trans. Roy. Soc., B.*, **193**, 223 (1900). Brown, H. T., *Jour. Chem. Soc.*, **113**, 559 (1918).

outside not more than .9 per cent of this leaf area—we arrive at the somewhat remarkable conclusion that during assimilation the absorption per unit area of these openings must be from 43 to 64 times as fast as the absorption of a unit area of a freely exposed solution of caustic alkali. In other words, under the natural conditions of assimilation the stomatal openings, supposing them alone to be operative, must take in carbon dioxide from the air about 50 times as fast as they would do supposing it were possible to fill them with a constantly renewed solution of caustic alkali."

In order to explain these facts Brown and Escombe carried through an elaborate investigation of the diffusion of gases and liquids under a variety of conditions. They departed somewhat from the conventional method of studying interdiffusivity of gases in which these are at equal pressure so that one gas has to diffuse against an equal and opposite flow of the other, but rather set the conditions so as to study the rate of diffusive flow of carbon dioxide of very low initial pressure down a stationary column of air on its way to an absorbent surface. The absorbent was caustic soda and the surface thereof was 20 to 25 times the cross-section area of the column, thus simulating the conditions extant in the stomata. Their experiments show a variation in the diffusive flow which is inversely proportional to the length of the column and the results may be stated by the following expression:

$$Q = \frac{k(\rho - \rho_1)A \, . \, t}{L}$$

in which Q represents the amount of carbon dioxide flowing down the cylinder towards the absorptive surface, A the area of the cross-section, L the length of the column, t the time, ρ and ρ_1 the partial pressure of carbon dioxide in the outer air and at the surface of the absorbent respectively and k the diffusivity constant of carbon dioxide and air, i.e. the number of cc. of the gas, measured at the temperature of the experiment, which will pass across a section of 1 square cm. in area when the fall of pressure is 1 atmosphere in 1 cm. $\frac{\rho - \rho_1}{L}$ is the concentration gradient of the carbon dioxide and must be constant for unit thickness of any two adjacent layers at right angles to the axis of the cylinder. If the alkaline solution is a perfect absorbent, ρ_1 at the immediate surface of the absorbent is zero and the concentration gradient then becomes $\frac{\rho}{L}$. If the absorption is not perfect ρ_1 will possess a sensible value and then the value for k will be lower than it should be.

Thus Brown and Escombe give the following experimental results:

"The diffusivity, K°, of the atmospheric carbon dioxide at 0° C., in C. G. S. units, is obtained from the equation:

$$K^\circ = \frac{Q_v \,.\, L \,.\, 760}{3600\,\rho\,\,p} \cdot \left(\frac{T^\circ}{T}\right)^2$$

Q_v = the number of cc. of CO_2, *measured at the mean temperature and pressure*, which passes across a *sq. cm.* of the cross-section of the tube *in one hour*.

ρ = the volume of CO_2 contained in unit volume of air.

L = the length of the tube in centims.

T° = the zero of absolute temperature.

T = the mean temperature during the experiment, expressed on the absolute scale.

p = the mean barometric pressure in millims. of mercury."

TABLE 11

RESULTS OF BROWN AND ESCOMBE ON THE RATE OF DIFFUSION OF CARBON DIOXIDE.

Duration of Experiment in Hours	Length of Tube in Cm. Corrected	Diam. Cm.	Total CO_2 Diffused Cc. at 0°, 760 Mm.	CO_2 Diffused per Sq. Cm. per Hour Measured at Temp. and Pressure of Experiment Qvt.	Mean Temperature ° C.	Mean Barometer	Mean CO_2 Content of Air Parts per 10,000	K°
500.9	18.0	2.32	23.89	.01175	7.7	751.2	3.70	.151
501.0	75.0	2.32	6.33	.00311	7.7	751.2	3.70	.167
501.0	18.0	2.32	20.88	.01036	9.1	763.4	3.61	.133
384.0	20.1	2.28	14.91	.00989	13.3	766.3	3.39	.146
383.7	19.9	2.30	15.49	.01260	13.3	766.3	3.39	.185
384.8	36.6	2.23	8.81	.00609	13.3	766.3	3.39	.164
386.4	37.3	2.20	7.91	.00559	13.3	766.3	3.39	.154
						Mean K°......		.157

The mean value for K° of all their experiments was 0.158. Below are given the values obtained by other investigators for the interdiffusivity of carbon dioxide and air with higher partial pressures of the former gas and with both gases at the same pressure.

	K°
Loschmidt[15]	.142
von Obermayer[16]	.132
Waitz[17]	.151–.158
Waitz, recalculated by von Obermayer for dry gases......	.131–.137

Brown and Escombe calculated that their results are but very slightly affected by the presence of water vapor in the air through which the carbon dioxide was passing.

[15] Loschmidt, *Wien. Akad. Ber.*, **61** (2), 62, 367, 468 (1870).

[16] Von Obermayer, *ibid.*, **85** (2), 147, 748; **87** (2), 188; **81**, 1102 (1880).

[17] Waitz, *Ann. Phys. Chem.*, 17, 201 (1882). See also Meyer, O. E., *Kinetic Theory of Gases*, Eng. translation, p. 267 (1899). Stefan, *Wien. Akad. Ber.*, **83** (2), 613 (1881); **79** (2), 161; **78** (2), 161 (1877).

Brown and Escombe having thus determined that, "when a condition of static equilibrium has been established in a diffusing column of gas, vapor, or solute, as the case may be, the amount of diffusion, under like conditions, is proportional to the sectional area of the column, . . ." they proceeded to apply these findings to conditions which simulate those existing in the leaf. They discovered that when a septum with a circular aperture is interposed in the line of flow, diffusion is modified in a remarkable manner. It was found that when the aperture was reduced to a certain point, the carbon dioxide passing through unit area of aperture in a given time showed a marked *increase,* "which could not be satisfactorily accounted for by the mere difference in the gradient of partial pressures of the gas inside and outside the covered disk." The experiments were carried out with great care and the apparatus consisted, in brief, of flasks containing sodium hydroxide to absorb the carbon dioxide. Over the mouths of the flasks were cemented septa with apertures of different diameters. After observing all precautions to maintain all experiments under precise conditions of carbon dioxide-content, temperature, freedom from convection currents, etc., the amount of carbon dioxide absorbed by the sodium hydroxide was determined. Thus it developed that as the size of the restriction decreased the flow *per unit area of aperture* rapidly increased, and as the aperture was diminished below a certain size relative to the cross-section of the column, the amount of carbon dioxide which passed became proportional to the *linear dimension of the apertures.*

The following table is taken from Brown and Escombe's paper and with the exceptions of Nos. 2 and 3 the results show that the rates of diffusion follow very closely the *ratios of the diameters of the openings* as well as the relative increase of carbon dioxide diffused, per area of the aperture per hour. In Nos. 2 and 3 the diameter of the aperture was more than one-half that of the unrestricted opening of the flask in which case other conditions prevail.

TABLE 12

RATE OF DIFFUSION OF CARBON DIOXIDE THROUGH APERTURES.

(Brown and Escombe.)

No.	Diameter of Aperture, mm.	Time of Diffusion in Hours	Total CO_2 Diffused, cc.	CO_2 Diffused per Hour, cc.	CO_2 Diffused per Sq. Cm. of Aperture per Hour	Ratio of Areas of Apertures	Ratio of Diameters of Apertures	Ratio of CO_2 Diffused in Unit Time
1	22.7	—	—	0.23800	.0588	1.00	1.00	1.00
2	12.06	476.5	44.22	0.09280	.0812	.28	.53	.39
3	12.06	477.1	48.57	0.10180	.0891	.28	.53	.42
4	5.86	478.8	26.61	0.05558	.2074	.066	.25	.23
5	6.03	643.1	40.21	0.06252	.2186	.07	.26	.26
6	3.233	863.0	34.41	0.03988	.4855	.023	.14	.16
7	3.216	863.8	34.30	0.03971	.4852	.020	.14	.16
8	2.00	1007.8	24.16	0.02397	.7629	.007	.088	.10
9	2.117	1007.3	26.28	0.02608	.8253	.008	.093	.10

It was also found that the rate of diffusion of water vapor is controlled by the linear dimensions of the apertures. This applies equally when the water is evaporating from a surface of water through an aperture or is absorbed by, e.g., sulfuric acid.

In order to explain their experimental results on the rate of diffusion of carbon dioxide through apertures and the "diameter law" Brown and Escombe pictured "lines of creep" of the carbon dioxide as it passes through the air towards the disc to replace that absorbed. Thus, the simplest case would be a circular disc, capable of absorbing carbon dioxide, freely exposed to the air and the disc surrounded with a rim, in the same plane, three or four times the diameter of the disc. If the air is perfectly still there will be established a steady gradient density of carbon dioxide surrounding the disc. If lines are drawn through all the points of the same carbon dioxide-density above the disc, curved surfaces are formed. These surfaces will be in the form of shells surrounding the disc. If the latter is a perfect absorbent of carbon dioxide each shell will represent a carbon dioxide-density varying from zero at the absorbing surface to the maximum density which will be that of the carbon dioxide in the air. This maximum is at a distance of 5 or 6 diameters from the disc. The gradient of density will thus be a line perpendicular to the shells of equal density. The problem is very similar to that of the lines of force of an electric field.

Stefan and others studied the exact converse case, that of the evaporation from a circular surface. A mathematical analysis of this problem showed that the amount of evaporation is proportional to the linear dimensions of the liquid surface and not to the area. The shells in this case form an orthozonal system of ellipsoids having their foci in the edge of the disc.

Larmor worked out the following formula for the absorption of atmospheric carbon dioxide by a perfectly absorbing circular disc:

$$Q = 2k\rho D,$$

in which Q is the amount absorbed in a given time, k the coefficient of diffusion of carbon dioxide in air, ρ density of atmospheric carbon dioxide, and D the diameter of the disc.

By using discs of very small diameter and perfectly quiet air Brown and Escombe were able to obtain results which showed an absorption depending upon the linear dimensions of the surface according to the formula given above. In Figure 5 are shown Brown and Escombe's conception of diffusion shells. *A* represents the shells in the case of an absorbent disc with rim; the convergent hyperbolic lines of flow, representing the carbon dioxide gradient, terminate in the surface of the disc.

In the case of perforations in a septum which divides two regions of different density the conditions are very different. In the former case, as the experiments show, the absorption, on the basis of linear dimensions, is exceedingly sensitive to any disturbances which affect the hypothetical shells above the disc. In the case of the perforated septum, as shown in *C*, Figure 5, the lines of flow are convergent as they approach the septum, bend around their foci, situated in the edges of the aperture, and form a divergent system on the other side. If the absorbing surface below the septum is a perfect one, there will be formed in the chamber between this surface and the septum (provided the chamber is sufficiently large) a system of shells exactly similar to those on the outside. In these inner shells the gradient of carbon dioxide-density will, of course, decrease away from the septum. In the latter case diffusion on the basis of linear dimensions of the aperture is not affected by movement of the outer air. The inner shells will be protected from the action of air movement (if the aperture is not large) and are quite independent of the outer shells. They will be as effective, therefore, in regulating relative rates of inflow of carbon dioxide according to the linear dimensions of the aperture as the outer shells. The absolute rates of diffusion through an aperture are decidedly influenced by conditions permitting a single or double system of shells.

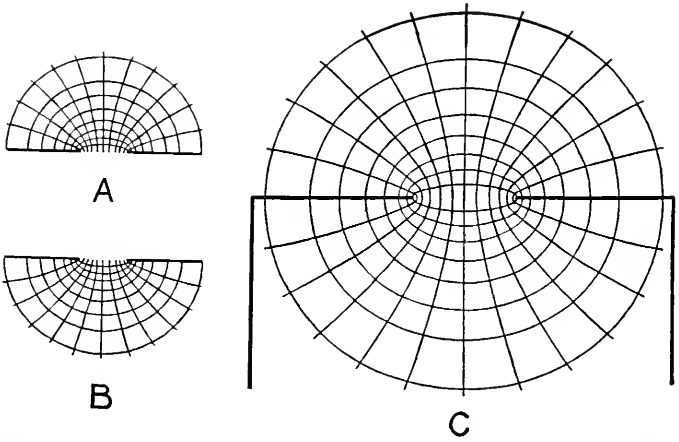

FIG. 5.—Brown and Escombe's diffusion shells or gradients of CO_2 density about a single opening above an absorbing medium.

The experimental observations on the rates of diffusion through small apertures are capable of explanation on the assumption that the converging and diverging lines of flow to and from the aperture "result in a system of shells of equal density, which locally alter the gradient in the immediate neighborhood of the septum."

Brown and Escombe attempted to demonstrate the formation of ellipsoidal shells by allowing a weak solution of methylene blue in 5 per cent gelatine to diffuse into colorless gelatine through an aperture. The formation of a limiting surface of color, ellipsoidal in shape, is clearly shown. Similar arrangements for demonstrating the zones of density gradient in diffusion through apertures are possible by the use of interacting substances such as sulphates and chromates into gels of agar containing barium chloride. The Liesegang phenomena can also be used in such a manner that the reacting substances produce the rhythmical series of zones which follow the contours of surfaces of equal density.

Brown and Escombe's investigations thus established that the effect of interposing a diaphragm pierced by a single circular aperture on the diffusion of a gas was quite significant. The velocity of the flow through unit area of such an aperture varies inversely as the diameter. The original object of their study was to determine the mechanism of gas interchange through the minute apertures in the surface of a leaf and to develop experimental conditions which simulated such a gas interchange. The discovery of the "diameter law" warranted the anticipation that under proper circumstances a thin septum might be pierced by the correct number, size and distribution of apertures so that the septum would cause but little obstruction to the diffusive flow of the gas while the aggregate area of the apertures would represent but a small percentage of the entire area of the septum. That a leaf behaves as such a system had been established by their earlier studies.

Experimental studies with multi-perforated septa showed that these conditions could be realized. Brown and Escombe used celluloid diaphragms fixed over short glass tubes. The latter were provided with a side tube and stop-cock for running in the sodium hydroxide solution to any desired height, as well as another tube for drawing off the solution for analysis. In the multi-perforate septa each hole was 0.38 mm. in diameter and the septum itself was 0.1 mm. in thickness. The factor which was varied in the experiments was the distance the holes were apart, i.e., the number of holes per square centimeter. The experiments were carried out in still air, and the amounts of carbon dioxide which had diffused through the multi-perforate septa were compared with the amounts which would have diffused down the open tube if there had been no obstructing septum present. The latter amount was calculated on the basis of previous results of (1) the diffusion coefficient of carbon dioxide in air, (2) the dimensions of the tube and (3) the mean density of carbon dioxide in the air during the experiment.

In the last column are given the calculated values of the relation of the carbon dioxide diffusion through the septum to the rate of diffusion without the septum. It becomes evident that the multi-perforate septum exerts but a slight effect on the rate of diffusion. The slight increase in No. 1 is due to the fact that in this experiment the air was not perfectly still. In No. 2, for example, 43.2 per cent of the open tube diffusion has taken place although the aggregate area through which the gas could pass was only 2.82 per cent of the cross-section of the tube. In the second portion of the table it will be seen that when the distance between the septum and the absorbing surface is decreased, the actual amount of carbon dioxide absorbed is increased, but the septum diffusion is a smaller percentage of the open tube diffusion. It is, moreover, evident that as the distance between the holes is increased, the efficiency of the holes, i.e., the area of perforations increases. For the optimum efficiency of each perforation, these should be about ten diameters apart. Under these circumstances the rate of diffusion is about forty times the amount it would be if the diffusion were proportional to the area of the cross-section of the tube.

The results are summarized in the following table:

TABLE 13

Diffusion of Carbon Dioxide Through Septa with Holes 0.380 mm. in Diameter. The Distance between the Septa and the Sodium Hydroxide Solution in the Two Experiments was 1 cm. and 4 cm. (Brown and Escombe.)

No.	Area of Tube, Sq. Cm.	No. of Holes per Sq. Cm. Septum	Distance Apart in Diameters	cc. CO_2 Diffused Through Septum per Hour, 0°, 760 mm.	cc. CO_2 Open Tube Diffusion per Hour, 0°, 760 mm.	Percentage Area of Holes in Septum	Percentage of Septum Diffusion of Open Tube Diffusion
(Length of tube = 4 cm.)							
1	9.348	100.00	2.63	0.361	0.346	11.34	104.3
2	9.186	25.00	5.26	0.148	0.342	2.82	43.2
3	9.456	11.11	7.80	0.131	0.352	1.25	37.2
4	9.511	6.25	10.52	0.110	0.353	0.70	31.1
5	9.456	2.77	15.70	0.0683	0.334	0.31	20.4
(Length of tube = 1 cm.)							
6	9.347	100.00	2.63	0.433	0.771	11.34	56.1
7	9.186	25.00	5.26	0.401	0.775	2.82	51.7
8	9.456	11.11	7.80	0.312	0.768	1.25	40.6
9	9.511	6.25	10.52	0.241	0.767	0.70	31.4
10	9.186	4.00	13.10	0.156	0.744	0.45	20.9
11	9.347	2.77	15.70	0.106	0.740	0.31	14.0

Brown and Escombe picture this phenomenon as shown in Figure 6. This is simply a multiplication of the case shown in Figure 5. Assuming perfectly quiet air at some distance from the septum the lines of flux in a diffusive column are approximately parallel. As the stream lines approach the aperture they converge and gradually assume parallelism again on the lower side of the septum. The ellipsoidal shells come closer together at the edges than in the center which would indicate that in these regions the gradient of density is greater and results in greater rate of flow per unit area. The lines of flow, which are at right angles to the gradients of density, thus also converge at the edges of the aperture, and after passing through the opening, again diverge. The lines of flow from adjacent apertures cannot cross each other, as there would then be shells of different density crossing each other, which is impossible. The lines of flow must, therefore, bend around the aperture and again become parallel, the velocity of the flow decreasing at the same time. As is shown in the tables there is an acceleration of flow per unit area as the size of the aperture is diminished. This is caused in the main by the degree of convergence and divergence of the lines of flow. With a smaller aperture the convergence and divergence increase, resulting in an increase in the gradient of density. When the apertures are ten diameters or more apart each perforation behaves independently without interfering with its neighbors and follows the "diameter law."

On this basis Brown and Escombe explain the fact that they were able to block out nearly 90 per cent of the cross-section of a column of diffusing gas leaving 100 circular apertures, and still produce no sensible effect in obstructing the diffusion of carbon dioxide through air. They were able to obtain photographs of the zones of equal density produced by intermittent diffusion of two reacting substances through apertures.

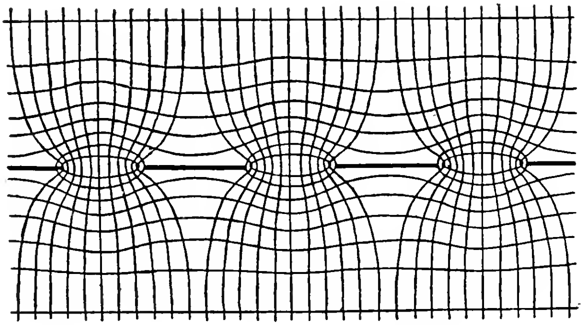

Fig. 6.—Brown and Escombe's conception of the lines of flux in a diffusive column through septa.

Thus this behavior, as would be expected, is characteristic not only for gases but also for substances in solution.

In applying these results to the gaseous exchange of leaves, Brown and Escombe studied an herbaceous plant which has been extensively used for photosynthesis investigations. The sunflower, *Helianthus annuus,* has an arrangement of stomata in the surface of the leaves corresponding to a multi-perforate diaphragm below which is a very large absorptive surface on the sides of the intercellular spaces. In these spaces is ample room for the formation of negative shells as described above. If the stomata are regarded as circular apertures they are about eight diameters apart, permitting nearly full efficiency of each opening. When they are partly closed, full efficiency of each is very probably attained. When the stomata are wide open they form straight-sided tubes about 0.014 mm. in length. This opening corresponds to a circle 0.0107 mm. in diameter and an area 0.0000908 sq. mm. Assuming the following most favorable conditions: wide-open stomata, constant partial pressure of carbon dioxide at the mouth of the stomata by a moving current of air and zero partial pressure of carbon dioxide in the intercellular spaces, by complete absorption, the amount of gas in cc. absorbed by one sq. cm. of leaf per hour will be represented by:

$$Q = \frac{k\rho \,.\, A \,.\, y \,.\, 3600}{L + x}$$

k = diffusion constant of $CO_2 = 0.145$.
ρ = density of CO_2 in outer air in atmospheres = 0.0003.
A = area of stomata = $10^{-7} \times 9.08$ cm.
y = number of stomata per sq. cm. = 33,000.
L = length of stomatic tube = 0.0014 cm.
x = resistance of the column of diffusive flow = $\frac{1}{8}\pi \times$ diameter = 0.00042 cm.
Q = 2.578 cc. CO_2 per hour per sq. cm.

If it is supposed that the air over the leaf is perfectly still Brown and Escombe develop the equation so that the denominator becomes $L + 2x$ and $Q = 2.095$.

A comparison with the actually observed rates of photosynthesis will show that these rates are very much lower than those theoretically possible on the basis of Brown and Escombe's calculations. Probably the chief factor in this discrepancy is the fact that the walls of the intercellular spaces into which the stomata lead are not perfect absorbers of carbon dioxide. In 1850, Graham,[18] in his Bakerian Lecture, pointed out that the "liquid diffusion of carbonic acid is a slow process compared with its gaseous diffusion, quite as much as days are to minutes." The gradient between the carbon dioxide on the outside and inside of the leaf is therefore much smaller than is assumed in the formula. A consideration of the absorptive capacity of the leaf material is taken up in Chapter 5.

The outstanding contribution of Brown and Escombe's studies is the fact that the structure of a leaf with its minute stomata is admirably adapted to the work it has to perform. The surface of a leaf, with the physical properties of a multi-perforate septum, having only 1 to 3 per cent of open area, still permits free gaseous interchange. The stomata can, in fact, be closed to 5 per cent of their maximum and yet permit sufficient carbon dioxide to pass to account for the maximum photosynthesis, provided the absorption is perfect.

This aspect of the photosynthesis problem has received very little attention aside from the very careful studies of Brown and Escombe. It would be highly desirable to have their results verified and extended. Jeffreys[19] has made a mathematical study of the laws of evaporation of water from circular surfaces and from cylinders. The laws of evaporation have a close analogy to those of absorption and from the results of Jeffrey's studies some of Brown and Escombe's conclusions are called

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[18] Graham, *Chemical and Physical Researches*, p. 446.

[19] Jeffreys, H., *Phil. Mag.*, **35**, 270 (1918). Thomas and Ferguson, *ibid.*, **34**, 308 (1917).

in question. This applies more particularly to the conclusions of the latter authors as to the influence of the distance between openings. Brown and Escombe consider that when the small apertures are more than ten diameters apart they influence each other very little, while Jeffreys concludes that the stomata in a leaf must close to a diameter $\frac{1}{50}$ of that of their full aperture before they act independently. There exists thus such a wide divergence between the conclusions of Jeffreys and what has been held for a long time regarding the function of stomata that a reinvestigation of the subject seems highly desirable.

b. The Sources of Carbon Dioxide.

The question has repeatedly arisen whether the atmosphere is the only source of carbon dioxide or is a sufficient supply of this gas for the photosynthetic activity of the plant. From the time of Senebier who maintained that plants absorb the carbon dioxide through the roots this question has been a topic of much controversy. The careful and numerous analyses of Benedict[20] show a very constant per cent of carbon dioxide in the atmosphere, 0.031 per cent. Samples of air collected over the ocean, at different times of the year and on the top of Pike's Peak gave essentially the same results. An appreciable increase in the carbon dioxide-content of the atmosphere is detectable only where there is not perfectly free movement of the air, in cities and industrial centers where the liberation of enormous quantities of carbon dioxide is taking place.

The soil air may vary greatly from the normal, and under circumstances, as for instance when the soil is dunged or in pasture land, the carbon dioxide in the soil may rise considerably. When the air supply is cut off the carbon dioxide-content of the soil may rise to very high percentages.[21] Thus, in India under monsoon conditions Leather found that the carbon dioxide rose to 16-20 per cent.

Lundegårdh[22] has made an extensive study of the carbon dioxide in the soil with a specially devised apparatus. At a depth of 15-20 cm. the carbon dioxide-content of the soil atmosphere ranges from about 2.5 to 0.12 per cent depending upon the type of soil, kind of fertilizer, season, etc. Through the action of the bacteria in the soil large quantities of carbon dioxide are constantly being formed in the soil. Lundegårdh points out that the amount of carbon dioxide formed in the soil approaches that which is absorbed by plants in photosynthesis per unit area. As a consequence of the formation of carbon dioxide in the soil the concentration of this gas immediately above the ground is often considerably higher than that usually reported. Thus, for example, Lundegårdh found in a well fertilized field of beets in October the carbon

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[20] Benedict, Carnegie Institution of Washington, Pub. No. 166 (1912).

[21] Russell, E. J., *Soil Conditions and Plant Growth*, p. 229 (1921).

[22] Lundegårdh, *Der Kreislauf der Kohlensäure* (1924), p. 144. Literature citations.

dioxide-content at the surface 0.0534 to 0.284 volume per cent, above the leaves 0.0401 to 0.0674 and at a height of one meter 0.0375 to 0.0720. From analyses of this nature it has been concluded that the available carbon dioxide for many crop plants may be very much higher than that of the free atmosphere, and since the concentration of carbon dioxide, perhaps more than any other factor, determines the rate of photosynthesis, it is apparent that the production of carbon dioxide by the soil may exert a decided influence on the development of plants.

Boussingault[23] showed definitely that plants grown in an atmosphere lacking carbon dioxide did not increase in carbon content, while Moll[24] was able to demonstrate that plants growing in a carbon dioxide rich humus soil formed no starch in leaves which were kept in an atmosphere free of carbon dioxide. The leaves growing in normal air produced starch as usual. It is conceivable, however, that conditions may exist in which the carbon dioxide absorbed by the roots may contribute to the total amount of carbon dioxide reduced by the plant.[25] Nevertheless, the chief source of carbon dioxide for the plant must be recognized as being the atmosphere.

In aquatic plants the conditions of carbon dioxide-absorption are in some respects different from those obtaining in land plants. The extensive anatomical investigations of aquatic plants have disclosed that in submerged leaves there are generally no stomata, though there appear to be some exceptions to this. In floating leaves the stomata are usually confined to the upper surface. In submerged aquatic plants the ingress of carbon dioxide into the leaf must therefore be accomplished by diffusion through the epidermal cells to the cells containing chloroplasts. In the mosses which are without stomata similar conditions must exist. The mechanism of this diffusion has been more closely studied by Devaux.[26]

Ordinarily the carbon dioxide-content of pure water is, of course, determined by the partial pressure of the carbon dioxide in the atmosphere over the water. Under certain circumstances the carbon dioxide- and oxygen-content of water are greatly influenced by the presence of decaying organic matter on the one hand and by active photosynthesis of aquatic plants on the other.

Of special importance to the carbon dioxide supply of natural waters is the presence of carbonates and bicarbonates. It has been known for a long time that aquatic plants form incrustations of calcium carbonate and that these deposits arise from the conversion of the calcium bicarbonate into the more insoluble carbonate by the withdrawal of carbon dioxide through photosynthetic activity. Draper[27] first showed that

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[23] Boussingault, *Ann. Chim. et Phys.*, (5), **8**, 433 (1876).

[24] Moll, *Arbeit. bot. Inst. Würzburg*, **2**, 105 (1878).

[25] Pollacci, G., *Bull. Soc. Bot. Ital. Genoa*, 208 (1918). Cailletet, Compt. rend., **152**, 1215 (1911). Maquenne, *ibid.*, 1811. Moillard, *ibid.*, **154**, 291 (1912).

[26] Devaux, *Ann. Sci. Nat.*, (7) **9**, 35 (1890).

[27] Draper, *Ann. chim. et phys.*, (3) **11**, 223 (1844).

plants were able to liberate oxygen in the light when grown in solutions of sodium bicarbonate and Hassak[28] carried out quantitative experiments with potassium bicarbonate to demonstrate this fact. In this process the water naturally becomes alkaline.

If a solution of sodium bicarbonate is allowed to stand in contact with air containing carbon dioxide, an equilibrium will be established between the hydrolyzed bicarbonate solution and the carbon dioxide in the air. This equilibrium will depend upon the partial pressure of the carbon dioxide in the air and will consist of a solution containing a high concentration of HCO_3^- and relatively small amounts of normal carbonate. The concentration of HCO_3^- will be higher with increased partial pressure of CO_2 in the air and will be decreased by the addition of normal sodium carbonate or alkali to the solution. These relations have been clearly expressed by Warburg.[29] From the equations of the electrolytic dissociation of carbonic acid:

$$\frac{H^+ \times HCO_3^-}{CO_2} = k_I \left(k_I = \text{the dissociation constant} \times \frac{H_2CO_3}{CO_2} \right)$$

$$\frac{H^+ \times CO_3^-}{HCO_3^-} = k_{II}$$

through division:

$$\frac{(HCO_3^-)^2}{CO_3^{--} \times CO_2} = \frac{k_I}{k_{II}} = K. \qquad (1)$$

If in mixtures of $NaHCO_3$ and Na_2CO_3 the values of HCO_3^- and CO_3^{--} are known we can calculate the CO_2 concentration in each case if K is known. If $NaHCO_3$ and Na_2CO_3 represent the total concentration of bicarbonate and carbonate in moles per liter, α the dissociation of the bicarbonate $\left(\frac{HCO_3^-}{NaHCO_3}\right)$ and β the dissociation of the carbonate $\left(\frac{CO_3^{--}}{Na_2CO_3}\right)$, we get from (1)

$$\frac{\alpha^2(NaHCO_3)^2}{\beta Na_2CO_3 \times CO_2} = K$$

$$\frac{(NaHCO_3)^2}{Na_2CO_3 \times CO_2} = K\frac{\beta}{\alpha^2} = K^1. \qquad (2)$$

In solutions which are not too dilute, i.e. where the hydrolysis can be neglected this equation agrees with that of McCoy[30] and Seyler and

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[28] Hassak, *Unters. bot. Inst. Tübingen*, 2, 465-477 (1888). Pringsheim, N., *Jahrb. wiss. Bot.*, 19, 138 (1888).

[29] Warburg, *Biochem. Zeit.*, 100, 238 (1919).

[30] McCoy, *Amer. Chem. Jour.*, 29, 437 (1903).

Lloyd.[31] K^1 is constant when the total Na-content is kept constant. When the latter is varied $\frac{\beta}{\alpha^2}$ changes, and with it K^1.

If c designates the total Na-content in milli-equivalents per liter, Warburg gives the empirical formula

$$K^1 = 8739 - 1671 \log c. \tag{3}$$

as holding when c = 100 to 1000 at 25°.

Although there are no determinations of K^1 for temperatures other than 25°, Warburg calculated the CO_2-concentration at other temperatures. This was done by calculating the change of K^1 with temperature from the heat of decomposition of the bicarbonate. Unfortunately the latter are not very accurate. In the dissociation of 2 moles of $NaHCO_3$ (aq.) in 1 mole CO_2 (aq.) and 1 mole Na_2CO_3 (aq.) 2028 calories are absorbed. On the further supposition that the degree of dissociation of the salts does not change markedly with temperature there is obtained for absolute temperature T

$$\log K^1_{(T)} = \frac{\left(\frac{2028}{2 \times T} - \frac{2028}{2 \times 298}\right) + 2.3 \log K^1{}_{(298)}}{2.3}. \tag{4}$$

By means of equations (2) (3) and (4) Warburg has calculated the CO_2-concentration at three different temperatures of various mixtures of Na_2CO_3 and $NaHCO_3$. These must, of course, be regarded in the light of the assumptions made in the equations.

In mixtures of this sort many aquatic plants can carry on photosynthetic activity for longer or shorter periods depending upon conditions of light intensity, temperature and the nature of the plant itself. In general, the length of time during which plants can survive in the mixtures depends upon the hydrogen-ion concentration, i.e. the time decreases with increasing alkalinity. Great care must be exercised in the use of these solutions and preliminary experiments must be carried out to determine whether the plants can live in the solutions without injury.[32] The presence of a living plant in anyone of these carbonate mixtures disturbs the equilibrium either by the liberation of carbon dioxide from the plant in the dark or by the absorption of carbon dioxide from the solution in the light. In the latter case when carbon dioxide is removed from the solution the equilibrium is shifted, so that bicarbonate is decomposed into carbonate and carbon dioxide, and the concentration of all

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[31] Seyler and Lloyd, *Jour. Chem. Soc.*, **111**, 138 (1917). See also Kendall, *J. Am. Chem. Soc.*, **38**, 1480 (1916). Auerbach and Pick, *Arb. Reichsgesundh.*, **38**, 274 (1911). For dilute solutions McCoy has shown that in the equation $NaHCO_3 + HOH \rightleftarrows NaOH + H_2CO_3$ the amount of NaOH is about 1/20 of the amount calculated for a normal hydrolysis, because of the secondary reaction: $NaOH + NaHCO_3 \rightleftarrows H_2O + Na_2CO_3$.

[32] Harder, R., *Jahrb. wiss. Bot.*, **60**, 538 (1921).

TABLE 14

Carbon Dioxide-concentration of Different Carbonate Mixtures as Calculated by Warburg.

No. of Mixture	Composition of the Mixture		Mille-Moles Na per Liter	5°		10°		25°	
	cc. 0.1 Molar Na_2CO_3	cc. 0.1 Molar $NaHCO_3$		K'	Moles CO_2 per Liter	K'	Moles CO_2 per Liter	K'	Moles CO_2 per Liter
1	85	15	185	6400	0.41×10^{-6}	6000	0.44×10^{-6}	5000	0.53×10^{-6}
2	80	20	180	6400	0.78×10^{-6}	6000	0.83×10^{-6}	5000	1.00×10^{-6}
3	75	25	175	6400	1.30×10^{-6}	6000	1.40×10^{-6}	5000	1.70×10^{-6}
4	70	30	170	6400	2.00×10^{-6}	6000	2.10×10^{-6}	5000	2.60×10^{-6}
5	60	40	160	6500	4.10×10^{-6}	6100	4.40×10^{-6}	5100	5.30×10^{-6}
6	50	50	150	6500	7.70×10^{-6}	6100	8.20×10^{-6}	5100	9.80×10^{-6}
7	35	65	135	6700	18×10^{-6}	6200	19×10^{-6}	5200	23×10^{-6}
8	25	75	125	6700	33×10^{-6}	6200	36×10^{-6}	5200	43×10^{-6}
9	15	85	115	6800	71×10^{-6}	6300	76×10^{-6}	5300	91×10^{-6}
10	10	90	110	6800	120×10^{-6}	6300	129×10^{-6}	5300	153×10^{-6}
11	5	95	105	6900	260×10^{-6}	6500	274×10^{-6}	5400	330×10^{-6}

components changes. However, if the quantity of carbon dioxide taken up by the plant is small in comparison to the total amount of the carbonate salts present, the changes of concentration are negligible and the carbonate mixtures play the rôle of buffers. Thus, for example, taking Warburg's figures for 25° for the carbonate mixture No. 9

$C_{NaHCO_3} = 0.085$ moles per liter.
$C_{Na_2CO_3} = 0.015$ " " "
$C_{CO_2} = 91 \times 10^{-6}$ " " "
$K^1 = 5.3 \times 10^3$ " " "

If from 10 cc. of this mixture 0.2 cc. CO_2 are removed, there will be taken 0.9×10^{-3} moles CO_2 per liter. Thereby the same amount of Na_2CO_3 molecules will be formed and twice the number of $NaHCO_3$ molecules will disappear. So that from equation (2)

$$C_{CO_2} = \frac{(0.085 - 1.8 \times 10^{-3})^2}{(0.015 + 0.9 \times 10^{-3}) \times 5.3 \times 10^3} = 82 \times 10^{-6},$$

i.e. C_{CO_2} decreases from 91×10^{-6} to 82×10^{-6} or about 10 per cent.

Nathanson [33] has tried to show that it is not the accumulation of OH ions but only the reduction of free CO_2 which is responsible for the decrease in the rate of photosynthesis in carbonate mixtures as these become more alkaline. This is based upon the fact that certain aquatic plants which are capable of storing small quantities of carbon dioxide can utilize this for photosynthesis in relatively strongly alkaline solutions. In view of the complexity of such a system the evidence is not altogether convincing, however.

Large bodies of water and particularly the sea are solutions of carbonates which must be viewed in the light of the equilibrium between atmospheric carbon dioxide and carbonates. The water, being in constant contact with the atmosphere, an equilibrium has been established between solution and gas so that the carbon dioxide pressure in the air is about equal to that in the water. The conditions under which plants carry on photosynthesis in the sea and in the atmosphere are therefore about the same as far as the carbon dioxide supply is concerned. Although the total carbon dioxide-content of sea-water, i.e. both free and combined CO_2, is about 50 times greater than that of the atmosphere, the larger portion of this is not available to the plant.

It appears that no other gas can take the place of carbon dioxide in the photosynthetic process. The effect of carbon monoxide in low concentrations is like other indifferent gases, e.g. nitrogen, and plants do not survive when confined in an atmosphere containing carbon monoxide but no carbon dioxide.[34] Higher concentrations of carbon monoxide are

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[33] Nathanson, *Stoffwechsel der Pflanzen*, p. 166. (Leipzig, 1910.)

[34] Boussingault, *Agronomie*, **4**, 300 (1868). Stutzer, *Ber. chem. Ges.*, **9**, 1570 (1876).

toxic to phanerogams.[35] Bottomley and Jackson,[36] however, report that this gas can to some extent replace carbon monoxide in photosynthesis. Boussingault also tried hydrocarbons without success.

c. The Evolution of Oxygen.

As has already been pointed out, one of the first facts which was observed in connection with the phenomenon of photosynthesis was that in the light carbon dioxide was taken up by the plant and oxygen was emitted. Thus the emission of oxygen as well as the absorption of carbon dioxide have been utilized as a means of studying the phenomenon. While a variety of qualitative methods, based upon the evolution of oxygen have been devised, relatively little work has been done on the laws governing this phase of the phenomenon. The methods which have been used in studying the emission of oxygen are described in Chapter 4.

That gas is emitted in the process of photosynthesis can be observed most easily in submerged aquatic plants. These absorb the dissolved carbon dioxide and emit the oxygen in form of minute bubbles. Leaves of land plants are not always suited to this demonstration, because the gas is not allowed to escape from the minute stomata on account of the capillary surface formed by the water. Leaves covered with a waxy surface are therefore more suitable. The demonstration of the evolution of oxygen in a striking manner is made possible by the use of the leuco compound of methylene blue or indigo carmine. An aquatic plant is allowed to remain over night in water the surface of which is covered with a layer of paraffin oil. When the oxygen has been removed from the water by the respiratory activity of the plant, a small quantity of the leuco dye is added to the water and the plant is illuminated. The evolution of oxygen from the illuminated plant is made evident by the formation of the bright colored dye close to the plant.

It should be stated at once that the gas which is thus emitted is not pure oxygen, but contains from 25-85 per cent of this gas with admixtures of nitrogen. Since it is impossible to obtain an exact analysis of the gas as it is emitted from the leaves of land plants, the results obtained with aquatic plants give more reliable data on this point. In land plants the path of oxygen escape is through the stomata, while in aquatic plants under normal conditions the oxygen escapes from the whole leaf surface by diffusion into the surrounding medium. The diffusion coefficient of oxygen is slightly higher than that of carbon dioxide. According to Carlson[37] $k_{CO_2} = 1.378$ and $k_{O_2} = 1.607$ (per sq. cm. per 24 hrs. at 16°) giving a ratio between the coefficients of oxygen and carbon dioxide of 1.166.

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[35] Richards and MacDougal, Bull. Torrey. Bot. Club, **31**, 57 (1904).

[36] Bottomley and Jackson, Proc. Roy. Soc., **72**, 130 (1903). Krascheninnikoff, *Rev. Gen. Bot.*, 21, 177 (1909).

[37] Carlson, *J. Am. Chem. Soc.*, **33**, 1027 (1911).

A practical method of determining the rate of oxygen evolution is the counting of the bubbles which escape from the cut end of a submerged aquatic plant. In this case the gas passes through the gas-filled intercellular spaces of the stem thus taking the path of least resistance. The determination of rates of escape of the gases by counting the bubbles is the basis of a method of comparative measurement of the rate of photosynthesis which has been in use for a long time. The description and necessary precautions of this method are given in Chapter 4. The rate at which these bubbles escape varies with the factors which affect photosynthesis, more particularly the light intensity. It is evident that a direct proportionality between the rate of the escaping bubbles and photosynthesis can exist only if the gas is pure oxygen or contains only negligible traces of other gases, or if the per cent of oxygen in the gas bubbles is constant and independent of their rate of escape. That these conditions ordinarily are not met has been demonstrated by Kniep.[38] He showed that the oxygen-content of the liberated gas varies as much as 30 per cent.

Below are given Kniep's analyses of the gas escaping from an illuminated plant of *Helcodea canadensis.* The percentages of oxygen and nitrogen are calculated for the total volume of gas after removal of the carbon dioxide. The rate is figured on the basis of the time required for 20 bubbles to escape from the plant.

TABLE 15

COMPOSITION OF GAS ESCAPING FROM AN AQUATIC PLANT WITH DIFFERENT RATES OF PHOTOSYNTHESIS. (Percentages of O_2 and N_2 calculated for total volume after removal of CO_2.)

Rate: 20 Bubbles in Seconds	Per Cent O_2	Per Cent CO_2	Per Cent N_2
7.0	49.6	0.9	50.4
5.2	54.4	0.6	46.6
14.7	36.6	1.2	63.4

The oxygen-content of the emitted gas increases with increased rate of bubble formation and *vice versa.* From this it follows, that with changing light intensity the rate of gas escape increases or decreases more slowly than the true rate of photosynthesis (as determined by the oxygen-content of the gas). A certain amount of the oxygen formed will, of course, escape by diffusion into the surrounding water. This fact, however, hardly affects the foregoing conclusion, because the diffusion out will certainly not be less when the intercellular spaces have a high oxygen-content than when they have a low one.

The explanation of this phenomenon is probably to be sought in the difference of the solubility of the gases rather than in the difference of their diffusion coefficients as is maintained by Kniep. The plant obtains

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[38] Kniep, *Jahrb. wiss. Bot.*, 56, 460 (1915).

its carbon dioxide from the surrounding water in which the gas is dissolved. The water is in equilibrium with oxygen and nitrogen of the atmosphere, the solubility of these two gases in water being relatively low as compared with carbon dioxide. The carbon dioxide reaches the centers of photosynthetic activity, the chloroplasts, by diffusion. Here, through photosynthesis, the carbon dioxide is reduced, and we may assume with a fair degree of safety, an equal volume of oxygen is formed. This oxygen can migrate either an exceeding small distance to the intercellular spaces, or in the other direction, out of the plant into the surrounding water. What actually happens is that the oxygen escapes from the plant through the intercellular spaces. If it were simply a matter of differences in diffusion coefficients the oxygen would never appear as bubbles of the gas. Kniep erroneously assumes that carbon dioxide has a very much higher diffusion coefficient than oxygen. Actually the reverse is the case and, strictly speaking, the diffusion coefficient is independent of solubility. However, carbon dioxide is much more soluble in water than oxygen and the water is already saturated with the latter gas at ordinary pressures and temperatures. The oxygen which is formed in photosynthesis produces a slight increase in pressure, partially dissolves and diffuses out, but mainly escapes into the intercellular spaces. Assuming the water is under atmospheric pressure, the gas will escape from the intercellular spaces when the pressure therein has attained a value slightly higher than atmospheric pressure and sufficient to overcome the resistance of the capillary surfaces in the intercellular canals. As this pressure increases more oxygen is dissolved in the water bounding the intercellular spaces and escapes by diffusion. Therefore, all of the oxygen produced in photosynthesis does not escape as a gas, although on account of the low solubility of oxygen in water the quantity removed by diffusion is very small compared to the gaseous emission.

The higher the rate of oxygen formation the faster the gas will escape from the intercellular spaces. But the water contains dissolved, not only oxygen, but also nitrogen and carbon dioxide. Therefore these gases are also present in the intercellular spaces. The bubbles which escape from these spaces contain these gases also. As has been stated, the higher the rate of oxygen formation, the faster the gas will escape, sweeping out with it the nitrogen and carbon dioxide. As the pressure of these latter gases is reduced in the intercellular spaces the equilibrium with the water bounding the spaces is disturbed and some nitrogen and carbon dioxide pass from the dissolved state into the gaseous form. When the rate of photosynthesis is high and the escaping gas stream correspondingly high, the oxygen sweeps out some of the nitrogen and carbon dioxide faster than it can be replaced. Thus, per unit time, with a high rate of oxygen formation, much nitrogen is carried out. This represents the condition in which the oxygen-content of the escaping gas is high during rapid photosynthesis. Conversely when the rate of photosynthesis is low, oxygen formation is low, the escaping gas stream is slow, and the nitrogen and carbon dioxide which have been swept out are replaced in the intercellular spaces from the surrounding water. Under these conditions the gas which escapes from the plant contains a higher percentage of nitrogen and carbon dioxide than when the photosynthetic rate is high.

Kniep's results also show that when the rate of the escaping gas stream is high the *absolute* amount of nitrogen evolved in unit time is higher than during a low rate of gas emission. This is in spite of the fact that the *percentage* of nitrogen is lower during a high rate than during a low one. This would indicate that during high rate of gas emission the partial pressure of nitrogen in the intercellular spaces is sufficiently reduced to cause a relatively rapid escape of this gas from the surrounding water into the intercellular spaces.

In all probability oxygen is the only gas which is emitted by the plant during photosynthesis. Pollacci[38] reported the presence of small quantities of hydrogen and some hydrocarbon in the emitted gas, but this single observation has never been substantiated.

Carbon monoxide[39] has been found in plants as a product of respiratory activity but has not been found in the gas emitted during photosynthesis.

Most of the analyses of gases emitted during photosynthesis are based upon relatively small samples of gas; it might prove interesting if large quantities of the gas were analyzed for traces of other gases.[40]

d. The Photosynthetic Quotient.

In the study of the respiration of both animals and plants the determination of the ratio of the volume of oxygen absorbed to that of carbon dioxide expired has been a most valuable means of determining the nature of the oxidation processes. Similarly in photosynthesis the ratio of the volume of carbon dioxide absorbed to that of oxygen emitted has been extensively studied in attempting to establish the nature of the reduction process. The much quoted early work of de Saussure, Boussingault and some others, while it established that this ratio was close to unity, is nevertheless, in the light of modern methods, not sufficiently accurate for our present needs. There are a number of factors in the determination of the ratio which exert a profound influence on the values obtained, but which are extremely difficult to regulate. Probably the most significant of these is respiration. The oxidative processes comprising respiration, in which carbon dioxide is formed, it is generally assumed, are going on simultaneously with photosynthesis during the

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[38] Pollacci, *Atti dell' R. Inst. Bot. Pavia*, 7, 97 (1902).
[39] Langdon, G. C., *Science*, 49, 573 (1919).
[40] Boussingault, *Agronomie*, etc., 3, 271 (1864).

periods of illumination. It has been an exceedingly difficult problem to separate these two reactions, proceeding, as it were, in opposite directions. It needs no further analysis to realize that even slight variations in the respiratory ratio would affect profoundly the results of the photosynthetic ratio. It must be borne in mind that respiration is an exceedingly complex process, the resultant of a series of reactions, some of which lead to the synthesis of material necessary for the life of the plant, others to end or waste products. Many of the latter are rich in oxygen and are broken down only under certain conditions. So that the absorption of oxygen and liberation of carbon dioxide may be separated by many intermediate reactions.[41]

Unfortunately there exists in the literature a great deal of confusion as to the manner of designating the photosynthetic and respiratory quotients. The former has been written $\frac{O}{CO_2}$, $\frac{O_2}{CO_2}$, and $\frac{CO_2}{O_2}$. In these pages the photosynthetic quotient will be designated by $\frac{CO_2}{O_2}$, i.e. $\frac{\text{vol. } CO_2 \text{ absorbed}}{\text{vol. } O_2 \text{ emitted}}$ and similarly the respiratory quotient $\frac{O_2}{CO_2}$. This will necessitate that in some of the quoted works the symbols must be specially noted because they are given as they stand in the original publications. Bonnier and Mangin[42] endeavored to separate the photosynthetic and respiratory processes and made a number of determinations of the photosynthetic quotient under special conditions. In their publication there are a number of misprints and arithmetical errors, which, however, it is difficult to correct on account of the lack of original experimental data. By means of the following four methods it was attempted to separate the gaseous exchange due to photosynthesis and respiration. These methods have been extensively used for this purpose, with little further critical examination.

1.—Comparison of the gas interchange in the dark and in light. The rate of respiration is determined in the dark, and in the light the gas interchange due to photosynthesis plus respiration is measured. From this data it is possible to obtain the values for the changes in the composition of the gas surrounding the plant due to photosynthesis alone. Thus, if in a given time c is the volume of carbon dioxide emitted in the dark, o is the volume of oxygen absorbed in the dark $\frac{o}{c} = \frac{O_2}{CO_2} = r$, the respiratory quotient, and if in the same time c_1 is volume of carbon dioxide absorbed in the light, o_1 is volume of oxygen emitted in the light, the total carbon dioxide absorbed in photosynthesis is $c + c_1$ and the total oxygen produced, $o + o_1$, and the true photosynthetic quotient is

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[41] Spoehr, H. A., Carnegie Institution of Washington Pub. No. **287**, 1-23 (1919).
[42] Bonnier and Mangin, *Ann. Sci. nat. Bot.*, (7) **3**, 1-44 (1886).

$$\frac{c + c_1}{o + o_1} = \frac{CO_2}{O_2} = P.$$

That is, the photosynthetic quotient is determined from the difference in the carbon dioxide and oxygen-content of the air surrounding the plant in the two determinations. In employing this method there are a number of corrections which must be applied. Great care must be exercised to avoid fluctuations in temperature in the two experiments. It is a question whether the rate of respiration is the same in the light and in the dark. Under the experimental conditions of Bonnier and Mangin [43] there were a number of disturbing influences which unquestionably affected their results.

They also attempted to separate the two processes of respiration and photosynthesis by comparing the rate of respiration of plant parts that contained chlorophyll with other portions that contained no pigment. It is apparent now, however, that such procedure is not permissible.

2—Inhibition of Photosynthesis by means of narcotics. Bonnier and Mangin, based upon the old observations of Bernard [44] with chloroform, found that photosynthetic activity is much more sensitive to the action of ether than respiration. It was attempted to determine the photosynthetic quotient by determining the gas interchange in parallel experiments with and without ether. The method is not exact, because it is very difficult to establish the exact dosage of the narcotic which completely inhibits photosynthesis and does not affect respiration (see the effect of narcotics on photosynthesis discussed later). It is very doubtful whether the rate of respiration in an anesthetized plant is the same in the light and in the dark.[45]

3—Suppression of photosynthesis by removal of carbon dioxide. The method depends upon the comparison of the gas interchange of leaves in two similar vessels. One of these contains a solution of barium hydroxide in order to absorb the carbon dioxide of the atmosphere and that liberated in respiration, the other contains an equal volume of water. From the difference between the oxygen-content and carbon dioxide-content of the two vessels at the end of the experiment the quantities of oxygen evolved and carbon dioxide absorbed during photosynthesis can be calculated. This method is open to serious error on account of the fact that the rate of carbon dioxide emission (respiration) in the two vessels is not the same. Spoehr and McGee [46] have shown that the rate of carbon dioxide emission of leaves is greatly influenced by the partial pressure of carbon dioxide in the surrounding atmosphere. They showed that when the carbon dioxide-content of the air surrounding a leaf is changed from a

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[43] Bonnier and Mangin, Compt. rend., **96**, 1075 (1883), **99**, 160 (1884). *Ann. Sci. nat. Bot.*, (6) **18**, 293 (1884).

[44] Bernard, *Leçons sur les Phénomènes de la Vie*, **1**, 278. Paris (1878).

[45] Usher and Priestley, Proc. Roy. Soc., *B* **77**, 369 (1906), **78**, 318 (1906).

[46] Spoehr and McGee, *Amer. J. Bot.*, **11**, 493 (1924).

higher to a lower concentration, the leaf shows a primary increased rate of carbon dioxide emission. Conversely, when the carbon dioxide-content of the air changes from a lower to a higher concentration the leaf shows a reduced rate of carbon dioxide emission. In the method just described, the leaf in the vessel containing barium hydroxide would therefore emit carbon dioxide at a higher rate than the leaf in the vessel containing only water. This would result in an apparently higher rate of respiration than is actually the case in the vessel containing a higher concentration of carbon dioxide. There are a number of modifications of this method, but the principle and errors involved are the same.

4—Comparison of leaves with different chlorophyll-content. Hereby the gaseous interchange of leaves of the same plant which are unequally green is measured, on the assumption that the two varieties differ only as to photosynthetic rates. One difficulty in this method is to obtain leaves of the same age and yet differing in their chlorophyll-content. Willstätter and Stoll[47] have shown that there is no direct proportionality between chlorophyll-content and photosynthetic activity. Also Plester[48] has shown that the light-green or *aurea* varieties, with low chlorophyll-content have a low respiratory activity as compared with the normal varieties.

By means of these methods Bonnier and Mangin determined the photosynthetic quotient of a large number of plants. Following their notation, $\frac{\text{vol. O}}{\text{vol. CO}_2,}$ the values obtained for the photosynthetic quotient were usually greater than 1, and ranged between 1.1 and 1.3.

In the 40 years since the publication of the work of Bonnier and Mangin a number of publications have appeared on the subject of the photosynthetic quotient notably by H. Jumelle[49] and Th. Schloessing fils.[50] With the exception of Willstätter and Stoll, whose work will be considered later, no contributions of note have been made to the problem of measuring separately the respiratory and photosynthetic activities. Maquenne and Demoussy,[51] employing essentially the first method of Bonnier and Mangin, made an extensive investigation of the quotients of gas interchange. Their results of the respiratory and photosynthetic quotients are given in Table 16. They used a closed chamber and the composition of the gas was determined after exposure to light and darkness.

In 29 of the 34 plants studied the photosynthetic quotient was equal to or very slightly less than the respiratory quotient. After making certain corrections Maquenne and Demoussy conclude that the volume of oxygen emitted is equal to the volume of carbon dioxide decomposed.

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[47] Willstätter and Stoll, *Untersuchungen ueber die Kohlensaeureassilation*, p. 86. Berlin (1918).

[48] Plester, *Beiträge zur Biol. d. Pflanzen*, **11**, 249 (1912).

[49] Jumelle, Compt. rend., **112**, 888 (1891); **113**, 920 (1891); *Rev. gén. bot.*, **4**, 49 (1892).

[50] Schloessing, Compt. rend., **115**, 881, 1017 (1892); **117**, 756, 813 (1893).

[51] Maquenne and Demoussy, *Echanges Gaseux des Plantes Vertes avec l'Atmosphère.* Paris, 1913.

TABLE 16

RESPIRATION AND PHOTOSYNTHETIC QUOTIENT DETERMINED BY MAQUENNE AND DEMOUSSY.

Species	Respiratory Quotient $\frac{CO_2}{O}$	Photosynthetic Quotient $\frac{O}{CO_2}$
Ailanthus	1.08	1.02
Aspidistra	0.94	1.00
Aneuba	1.11	1.10
Begonia	1.11	1.03
Cherry-laurel	1.03	0.97
Chrysanthemum	1.02	1.01
Dahlia	1.07	1.07
Geranium	1.02?	1.05?
Grape-vine	1.01	0.99
Ivy	1.08	1.00
Kidney bean (young)	1.12	1.12
“ “ (average)	1.07	1.07
Lilac	1.07	1.03
Lily(?)	1.07	1.00
Mahonia (autumn)	0.95	0.99
Pea	1.07	1.04
Pear	1.10	1.08
Poppy	1.09	1.09
Privet	1.03	1.02
Rhubarb	1.02	1.00
Ricinus	1.03	1.03
Rose	1.02	1.10
Rose Laurel	1.05	1.01
Spindle-tree	1.08	1.02
Sorrel	1.04	1.04
Tobacco	1.03	1.04
Turnip	1.11	1.06
Wheat	1.03	1.02

It is probably evident from the foregoing that the chief difficulty in obtaining an exact determination of the photosynthetic quotient is occasioned by the fact that an error in the determination of the respiratory rate also appears in that of the photosynthetic quotient. The separation of the two processes is exceedingly difficult so that no matter whether the respiration is determined in the dark, with narcotics in the light, or with leaves poor in chlorophyll, every inexactness of the respiratory quotient affects the value of the photosynthetic quotient. Willstätter and Stoll[52] have endeavored to surmount this difficulty by determining the photosynthetic quotient under conditions in which the photosynthetic activity is very much greater than respiration, so that small errors in the determination of the latter would have but a very slight effect on the accuracy of the photosynthetic quotient. They made another departure in that instead of using a closed system, a stream of air was passed over the leaves. This is the only method by which the leaves are exposed to constant conditions

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[52] Willstätter and Stoll, *l.c.*, 325.

of photosynthesis. Of course the differences in the composition of the gas to be analyzed are very much smaller than in the closed chamber method, but these differences can be determined with modern methods of gas analysis. For this purpose Willstätter and Stoll employed conditions of maximal photosynthetic activity, so that respiration was 1/20 to 1/30 of photosynthesis. Thus slight variations in respiration, as occasioned, for instance, by the differences in respiration in light and in the dark, had but an insignificant effect on the determination of the photosynthetic quotient. Carbon dioxide-concentrations of 5-6.5 volume per cent and light intensity of about 45,000 lux were used. Under these conditions the photosynthetic quotient $\frac{CO_2}{O_2}$ at 10 to 35° was found to be constant and exactly 1. This applied to a variety of plants including *Ilex aquifolium, Sambucus niger, Pelargonium zonale*, and *Aesculus hippocastanum*. Even under conditions of photosynthetic inhibition through the accumulation of products (see below) the value of 1 was maintained.

The importance which attaches to the value of the photosynthetic quotient can be readily seen from the following considerations. Just as from the value of the respiratory quotient definite information can be gained as to the nature of the material which is oxidized, the photosynthetic quotient indicates the nature of the material formed in the decomposition of carbon dioxide. It has been found that the respiratory quotient $\frac{\text{vol. } CO_2 \text{ emitted}}{\text{vol. } O_2 \text{ absorbed,}}$ of animals on a diet of 100 per cent fat and 0 per cent carbohydrate is 0.707, and when on a diet of 0 per cent fat and 100 per cent carbohydrate is 1.00. Quotients intermediary between 0.707 and 1.00 indicate that mixtures of the two materials are being used. This principle has become one of the most useful tools in the hands of the animal physiologist and has been elaborated to include a large variety of substances and conditions.

TABLE 17

CALCULATED PHOTOSYNTHETIC QUOTIENTS FOR DIFFERENT PRIMARY PRODUCTS OF CARBON DIOXIDE REDUCTION.

Compound	Formic Acid	Oxalic Acid	Glyoxalic Acid	Glycollic Acid	Malic Acid	Glycol-aldehyde	Formal-dehyde
Photosynthetic quotient $\frac{CO_2}{O_2}$	2.0	4.0	2.0	1.33	1.33	1.0	1.0

Similarly the photosynthetic quotient may yield some information relative to the first product formed in photosynthesis. Between carbonic acid and carbohydrates there are a number of possible reduction products. A great deal of speculation has centered about the question as to which is the first substance formed in the process of photosynthesis. In the previous table are given a number of the possible reduction products together with the photosynthetic quotient which corresponds to their formation calculated from the carbon dioxide absorbed and oxygen split off.

When the photosynthetic quotient is exactly 1 it is evident that the carbon dioxide has been reduced to carbon, $CO_2 \rightarrow C + O_2$, or in the hydrated form carbonic acid has been reduced to a carbohydrate. Since the best evidence indicates that the photosynthetic quotient is 1, this would signify that carbonic acid is reduced to the formaldehyde stage. However, the evidence of the value of the photosynthetic quotient does not throw any light on the question as to the exact compound which is formed. It may be formaldehyde, glycolaldehyde, or any other carbohydrate of the general formula $C_nH_{2n}O_n$, so that the results of the gaseous exchange during photosynthesis can contribute little toward settling this much debated question. The evidence of the photosynthetic quotient does contribute to determining whether the first products formed in photosynthesis are compounds other than those possessing an empirical formula $C_nH_{2n}O_n$. The old theory of Liebig[53] that organic acids are the precursors of carbohydrates in photosynthesis still finds adherents who have modified and elaborated the original theory. These views will be discussed in another chapter. Suffice it to point out here, that it is now clearly established that the hydroxy-acids which play so important a rôle in the Liebig theory are products of a modified respiratory activity and that a photosynthetic quotient of 1 excludes them from being the first direct products of carbon dioxide reduction. Similarly the theory that fats are the first products of photosynthesis finds no support in the values of the photosynthetic quotient, for these substances would demand a quotient, $\frac{CO_2 \text{ absorbed}}{O_2 \text{ emitted}}$ in the neighborhood of 0.7.

One other point regarding the photosynthetic quotient deserves consideration. It has been known for a long time that the fleshy plants or succulents exhibit respiratory and photosynthetic quotients which differ greatly from the values obtained with thin leaf species. These plants ordinarily show a very low photosynthetic ratio.

The structural arrangements of the succulents are such that the plant loses relatively little water through transpiration. The ratio of the surface to the volume is low and there are relatively few stomata. This results in inhibiting the gaseous exchange. A striking feature of the metabolism of these plants is the accumulation of organic acids at night or in the dark and the decomposition of these acids during periods of illumination. This phenomenon has been investigated very thoroughly recently by Richards[54] who also discusses the older literature. The accumulation

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[53] Liebig, *Die Chemie in ihrer Anwendung auf Agrikultur und Physiologie*, I, 52 (1862).

[54] Richards, Carnegie Inst. Washington, Pub. No. 209 (1915). Hempel, Compt. rend. du Laboratoire de Carlsberg, **13**, 1-129 (1917).

of organic acids results from the incomplete oxidation of sugars. In the light these acids disappear, partly on account of a greater oxygen-supply due to photosynthesis, and also due to the direct photolysis of the acids as described by Spoehr.[55] There are therefore several complications which arise in determining the photosynthetic quotient. The organic acids (e.g. malic acid in the cacti) break down in the light with the liberation of carbon dioxide. Thus it happens, that, as Richards records, carbon dioxide is given off by the plant when exposed to diffuse light or direct sunlight. The oxygen also varies greatly under these circumstances; in diffuse light there is an absorption of oxygen while in intense illumination oxygen is emitted. It must also be realized that in the interior of the plant the breaking down of the acids serves as a source of carbon dioxide, which in the light is used in photosynthesis. When this oxygen escapes from the plant it results in a greater volume of oxygen being emitted than carbon dioxide is absorbed, i.e., $\frac{CO_2 \text{ absorbed}}{O_2 \text{ emitted}} < 1$; in some cases this ratio has been found to be less than 0.5. With long continued exposure to light the ratio increases and slowly approaches unity. These processes have the general significance that in plants which are protected against great water loss the gaseous exchange is slow and carbon dioxide is dealt with most economically.

Kostytschew[56] has brought out the very interesting fact that the $\frac{CO_2}{O_2}$ ratio during illumination varies with time. The initial carbon dioxide content in his experiments was about 6 per cent, i.e. considerably above normal air. He found that during photosynthesis the leaves absorb initially a great deal more carbon dioxide than oxygen is given off; about one third the absorbed carbon dioxide is fixed without oxygen emission. After a short time these conditions are reversed, more oxygen is given off than carbon dioxide is absorbed, and finally the $\frac{CO_2}{O_2}$ ratio attains a constant value of 1. Kostytschew found that these relations maintain for aquatic plants as well as for leaves of land plants. These observations are found in accord with those of Spoehr and McGee[57] on the absorptive capacity of leaf material for carbon dioxide. They are especially important in their bearing on the methods of measuring photosynthetic activity. If this behavior is universal it would follow that photosynthetic activity should be measured by the amount of carbon dioxide absorbed rather than by the amount of oxygen emitted. It is also of direct bearing on the theory of a primary chemical reaction of carbon dioxide with an absorbing substance as the first step in photosynthesis.

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[55] Spoehr, *Biochem. Zeit.*, **57**, 95 (1913).
[56] Kostytschew, *Ber. bot. Ges.*, **39**, 319 (1921).
[57] Spoehr and McGee, *Amer. Jour. Bot.*, **11**, 493 (1924).

2. Factors Which Influence the Rate of Photosynthesis

a. The Principle of Limiting Factors.

In endeavoring to understand the photosynthetic process it is essential to bear in mind that we are dealing with a complex system. It is complex because there are a number of factors involved, each one is necessary for the successful and continuous operation of the process. It is complex because each of these factors influences the process more or less independently. Add to this that we are dealing with a series of chemical reactions of different types including photochemical and catalytic reactions, and it must be evident that to express these relationships in quantitative terms becomes an exceedingly difficult task. And finally the fact must never be lost sight of that photosynthesis is a function of the *living plant*. Although a great deal of work has been done to determine the influence of the various factors involved in photosynthesis it is evident now that no one of these factors can be studied without at the same time taking into consideration all of the others.

The factors which are primarily concerned in determining the rate of photosynthesis in any chlorophyllous organ are as follows:

1—The partial pressure of carbon dioxide in the air or water surrounding the plant.
2—The intensity and frequency of the light used.
3—Temperature, more particularly that of the chloroplasts.
4—The amount and composition of the chlorophyll.
5—The amount of water available.
6—Certain internal factors.

It has been customary for physiologists to endeavor to determine three cardinal points in studying quantitatively the influence of various factors or conditions on any physiological phenomenon. These cardinal points are: the minimum, below which the phenomenon ceases, the optimum, at which the phenomenon takes place to the highest degree, and the maximum, above which it ceases. Many attempts which were made to establish the cardinal points of the various factors operative in photosynthesis led to contradictory results. It was F. F. Blackman[58] who in 1905 called attention to the fact that in a phenomenon such as photosynthesis where there are several factors at work simultaneously, the focusing of attention on a single factor with disregard to the influence of the others leads to erroneous results. A study of the "inter-relation of conditioning factors" led Blackman to formulate his principle of limiting factors which he stated in the following axiom: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor." Any of the factors mentioned above can

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[58] Blackman, *Ann. of Bot.*, **19**, 281 (1905).

act as the limiting one and thus determine the rate of photosynthetic activity.

There has been some debate and misunderstanding regarding this theory. Below is given Blackman's original illustration. "Suppose a leaf in a glass chamber to have enough light falling upon it to give energy equal to decomposing 5 cc. of carbon dioxide per hour. Then, as one gradually increases the carbon dioxide in the air current through the chamber from the amount (or pressure) that causes 1 cc. to diffuse into the leaf through its stomata up to five times that pressure, so steadily the assimilation will increase from 1 cc. to fivefold. After that, further increase of carbon dioxide will produce no augmentation of the assimilation, but will give continually an effect of 5 cc. of carbon dioxide—the light being now the limiting factor. The curve obtained will be of the form A B C. (Figure 7.)

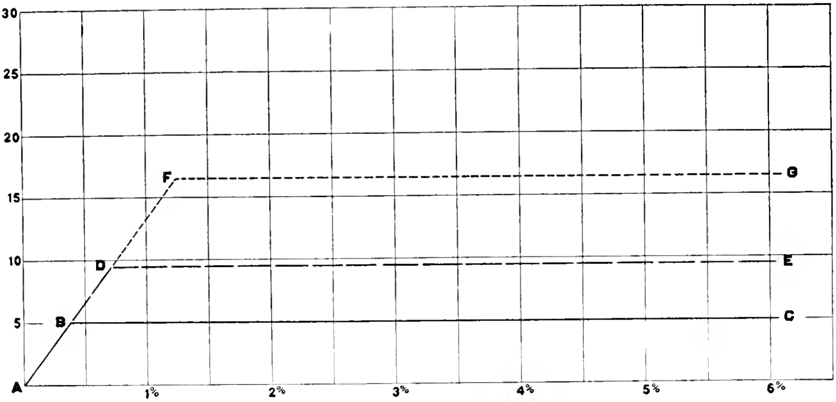

FIG. 7.—Blackman's conception of the effect of limiting factors on the rate of photosynthesis.

"Ultimately, if the supply of carbon dioxide in the air current be increased up to 30, 50, 70 per cent, the carbon dioxide will have a general depressing effect on the whole vitality, and before suspension of all function a diminution of assimilation undoubtedly occurs; this is, however, quite a separate process. Now, secondly, suppose light falling on the leaf to be sufficient for the decomposition of 10 cc. of carbon dioxide per hour, then twice the external pressure of carbon dioxide will be required to reach the limit and the angle of the curve, which will now be A B D E. With still stronger light we should get A B D F G. Those who would be prepared to admit that a curve like A B C shows an optimum, only with a very long drawn-out top, would have to further admit that for *each intensity of light falling on a leaf there is a different optimum amount of carbon dioxide.* This is not to be entertained.

"The light energy available fixes an upper limit to the carbon dioxide that can be decomposed, and when that amount is attained, which even for direct sunlight could be provided with a current of air containing less than 1 per cent, if the current were sufficiently fast, the limit of effect of carbon dioxide is reached: any more provided is wasted, and has no further effect till many times that concentration is reached and a general depressing effect comes in. . . ."

Similarly, any of the other factors can act as the limiting one. It is evident that in all experimental work all of these factors must be taken into account and that it is essential to determine that no other factor beside the one under consideration is acting as the limiting one.

The principle of limiting factors has its analog in the step-reactions of chemistry. Here if a reaction is made up of a number of steps, the rate of the total reaction will be determined by the rate of that step which is proceeding at the lowest rate.

Also, as early as 1843, in his book, "Chemistry in its application to Agriculture and Physiology," Liebig had formulated his well-known Law of Minimum which stated: "by the deficiency or absence of *one* necessary constituent, all the others being present, the soil is rendered barren for all those crops to the life of which *that one* constituent is indispensable." At the time, of course, Liebig was not familiar with all the factors which affect the growth of plants and it required a great deal of experimental work by many investigators before this principle found an approximate mathematical expression. It is of interest here on account of the fact that the problem of crop yield is very similar to that of photosynthetic activity and has been dealt with in much the same way. Liebig's law of minimum has undergone many modifications and the facts which this law endeavored to express have finally been put into mathematical form though there still exists some controversy regarding it. It can be dealt with here but very briefly.[59]

There are five soil factors which influence the growth of plants: (1) Water supply, (2) Air supply, (3) Temperature, (4) Supply of mineral nutrients, (5) Injurious factors. The effect of these factors may be determined by the total amount of dry matter formed by the plant under definite conditions. The effect of the fourth factor, the supply of mineral nutrients can be most easily determined. When then, the weights of dry matter formed under conditions in which but one essential factor is varied, are determined, there are obtained smooth curves which are amenable to mathematical expression. Mitscherlich has done this on the basis of many crop experiments. From these results it is evident that in the law of minimum we are dealing with a logarithmic function. The crop yields obtained with a single varying factor can be expressed in curves which are asymptotic to a maximum. This maximum would be attained if all conditions were ideal and there is a shortage in yield corresponding to the

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[59] Russell, E. J., *Soil Conditions and Plant Growth.* London, 1921. Pfeiffer, T., *Der Vegetationsversuch.* Berlin, 1918.

deficiency of any essential factor. The exact mathematical formula for these curves is still a matter of controversy.[60] The important fact is that they are logarithmic curves.

In the problem of crop yield and the manner in which this is affected by various factors, there has been obtained, through a great mass of experimental data, a fairly accurate mathematical expression of the influence of the deficiency of one of these factors. This expression is undoubtedly an outgrowth of the original law of minimum, though in its course of development it has undergone considerable change.

In the principle of limiting factors the photosynthetic activity, as first formulated by Blackman, we have a state of affairs which is in many respects analogous to that of crop yield. Blackman's theory has been one of the most helpful conceptions which has been contributed to the study of the problem of photosynthesis. Within the last few years there has been some agitation regarding the general applicability of Blackman's theory.[61] Some of the writers have gone so far as to maintain that the principle is entirely erroneous. It would seem, however, that these are rather developmental phases such as occur in the evolution of any fundamental theory. In the more valid objections which have been raised, the conflict centers about the graphic or mathematical expression to be given to the theory rather than to the more general underlying principles. With more refined methods and extensive data it is not surprising that some modifications should come to light which may have escaped the original propounders with their rather limited experimental methods and data.

Benecke [62] has given a very good elucidation of the principle of limiting factors. He points out that Blackman's theory is usually stated in such a manner as to predicate that only one of the various factors is limiting, while probably Jost's [63] position is more tenable. The latter states that it may happen that all factors but one have so high an intensity or concentration, that this single factor alone may determine the rate of photosynthesis. Benecke shows that the curve representing the rate of photosynthesis, when only one factor is varied, does not, when the intensity of this factor is augmented, exhibit a straight line increase and then a sharp turn when some other factor prevents the further increase in the photosynthetic rate. But rather, the curve is a logarithmic one as, for example, is shown by Warburg's curve of the effect of light intensity. Fig. 8. At first this is practically directly proportional to the light intensity, then it gradually bends to the horizontal. Here the photosynthetic rate is no longer directly proportional to the intensity of a single factor. This is the region where other factors interact and more than one factor limits. Finally, the curve is almost parallel to the abscissa, and here some other factor besides the one which was varied becomes the limiting one. As

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[60] Baule, *Landw. Jahrb.*, **54**, 495 (1920).
[61] Hooker, *Science*, **46**, 197 (1917). Crocker, *Bot. Gaz.*, **65**, 287 (1918).
[62] Benecke, *Zeit. f. Bot.*, **13**, 424 (1921).
[63] Jost, *Bot. Zeitg.*, **64**, 72 (1906).

will be shown under the discussion of the effect of light intensity according to Harder's experiments, there is a portion of the curve where more than one factor is limiting.

Benecke considers that any factor is in "absolute minimum" only when that factor is entirely absent, e.g., light is in "absolute minimum" when the plant is in darkness. If we start with very weak light and then gradually increase the intensity, the light factor is in "relative minimum" the moment of first illumination. Other factors can at the same time be in "relative minimum." The difficulty of obtaining strictly mathematical results lies in the experimental complexities involved, in the fact that it is almost impossible to alter only one factor without at the same time affecting some other factor. Even if corrections can be applied to such factors as temperature on bicarbonate dissociation or solubility of carbon dioxide, it is impossible to correct for the effect of external agencies on internal conditions, as e.g., the action of light on chlorophyll and certain stimulatory effects.

The principles involved in the theories of limiting factors can be more clearly elucidated in the discussions of the influence of various individual factors in the following sections.

b. The Influence of Light.

Investigations on the influence of light on photosynthesis have been directed in two main courses. One of these has been chiefly concerned with the effect of different intensities of illumination on photosynthetic activity and the other with the influence of different frequencies or wave lengths. Some of the earlier work on both of these phases of the problem is contradictory and confusing. This is due, first of all, to a lack of realization on the part of the older workers of the interrelation of the various factors affecting photosynthesis, which was first clearly recognized in Blackman's theory of limiting factors, and secondly, to technical difficulties in the measurement of light intensity. With a clearer understanding of the influence of other factors involved in the photosynthetic process, that of light too was somewhat clarified. Also, the recent development in our conceptions of the nature of radiant energy, the quantum theory, the laws of photochemical action have contributed much to better experimental procedure. It may be expected that the mastery of these newer conceptions of the nature and action of light by experimenters in this field will be of great service in gaining a better understanding of the rôle which light plays in photosynthesis. Already the two lines of investigation mentioned, that of the effect of different intensities and that of the influence of different frequencies have been brought closer by the approach from the viewpoint of the quantum theory.

In the dark a chlorophyllous plant absorbs oxygen and gives off carbon dioxide in the process of respiration. Even illumination of very low intensity is apparently capable of inducing a reduction of carbon dioxide by the chloroplasts. However, under these circumstances photosynthesis does not become evident by an evolution of oxygen, but only by an apparently lower rate of respiration, that is, the amounts of carbon dioxide given off and of oxygen absorbed are less. There is no evidence that feeble illumination affects the rate of respiration itself, so that it has been concluded that the lower rate of carbon dioxide evolution and of oxygen absorption are due to the fact that in the very low light intensity a portion of the carbon dioxide evolved in the process of respiration, is taken up by the chloroplasts and a small amount of oxygen is evolved which in turn is immediately utilized in respiration again. This results in a diminished gas interchange of the plant with the atmosphere. With increasing illumination a point is finally reached where the rate of photosynthesis just balances that of respiration and there is no gas interchange whatsoever. A discussion of this state, the so-called compensation-point, will be taken up later. Exact determinations of the minimum intensity of light which is necessary for carbon dioxide reduction have not yet been made.[64] Most of the measurements consider the point of oxygen evolution, that is, where photosynthesis overbalances respiration. But this, of course, is not the lowest intensity of light necessary for photosynthesis, but merely an intensity where the rate of photosynthesis is greater than that of respiration.

Early experiments showed that photosynthetic activity increases proportionately to the intensity of light and that there is a limit to the increase above which increase in the light intensity results in no augmentation of the photosynthetic rate. Moreover, every plant has its own specific requirements and the capacity to use light as well as the efficiency varies widely in different species. The underlying principles for these phenomena have been brought to light by more recent investigations.

Some reference to the influence of light on the rate of photosynthesis has already been made under the discussion of the effect of carbon dioxide. It has repeatedly been stated that none of the factors affecting the rate of photosynthesis can be studied alone or without regard to the influence of other factors. From a consideration of the principles which have been given expression in the theory of limiting factors, it is evident that changes of light intensity have an effect on the rate of photosynthesis only when light is the limiting factor or "in minimum." Considering first Blackman's conclusions in formulating this conception we can summarize, that where carbon dioxide supply and temperature are in excess the rate of photosynthesis is proportional to the intensity of illumination. This, of course, assumes that other factors affecting photosynthesis, as chlorophyll-content and water supply are constant. Blackman and Matthaei[65] used sunlight and perforated screens to reduce the light in-

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[64] For Moonlight: Boussingault, J. B., *Ann. d. sci. nat.* (Ser. V), **10**, 335 (1869). Ursprung, A., *Ber. bot. Ges.*, **35**, 62 (1917). Midnight-sun: Curtel, G., *Rev. gen. bot.*, **2**, 7 (1902). Kostytschew, *Ber. bot. Ges.*, **39**, 334 (1921).

[65] Blackman and Matthaei, Proc. Roy. Soc. London, B **76**, 402 (1905). Blackman and Smith, ibid., B **83**, 389 (1911).

tensity. Exact values for the light intensities were not obtained. But they were able to determine what proportion of direct sunlight was required to give the maximum possible photosynthesis at a definite temperature. Thus the photosynthetic rates determined at 29.5° at mid-day of August 8 and 16 respectively were:

Cherry-laurel:	0.28	sunlight	gives	0.0116	g. CO_2	per	50	sq. cm.	per	hour.
Helianthus:	0.62	"	"	0.0224	"	"	"	"	"	"

These values then represent the minimum light intensity which is sufficient for a maximum rate of photosynthesis at that temperature and assuming there is no other factor limiting. *Helianthus* is therefore capable of a much higher rate of photosynthesis than cherry-laurel at the same temperature, and neither of the plants is capable of utilizing for photosynthesis the whole of the solar radiation. The highest photosynthetic rate observed by Blackman and Matthaei equaled about 2900 cc. CO_2 per square meter per hour for *Helianthus* at 29.5°. According to the Blackman view, in order to determine whether photosynthesis as observed at any given time is limited by light intensity, temperature or some other factor, it is necessary only to alter the intensity of each factor separately.

It has just been stated that the two different species of leaves, cherry-laurel and *Helianthus,* exhibit different maxima of photosynthetic activity at the same temperature. This raises the question whether different types of leaves have different specific photosynthetic characteristics. Blackman and Matthaei have also investigated this question using different plants as *Helianthus, Tropaeolum, Bomarea* and *Aponogeton.* They conclude that "leaves in general have the same coefficient of economy in the photosynthetic process." While at 29.5° *Helianthus* can assimilate twice as much carbon dioxide as the cherry-laurel, at low temperatures, they have similar photosynthetic maxima. Blackman and Matthaei harmonize the latter statement with their assumption of "equal coefficients of economy" by pointing out that *Helianthus* requires twice as much light to attain the double assimilation at 29.5°. "The fundamental existing specific differences would seem to lie in their different coefficients of acceleration of activity with increase in temperature." For a rise of 10° the increase with cherry-laurel is 2.1 and with *Helianthus* it is about 2.5.

The question of the specific photosynthetic value of leaves requires further investigation before Blackman and Matthaei's dictum can be entirely accepted. There are two points which suggest themselves at once. The first of these is that the differences of acceleration due to temperature of photosynthetic activity are probably related to enzyme action or what we here include under internal factors, which, under the experimental conditions, were governing the rate of photosynthetic activity. Of this we know as yet very little. Secondly, it must be kept in mind that leaves differ greatly in their photosynthetic activity according to their chlorophyll-content, but that there is probably no direct relationship between the chlorophyll-content and photosynthetic activity. Willstätter has shown that the effect of the internal factor is increased by raising the temperature. However, an increase in the activity of the internal factor is of influence on photosynthesis only in leaves with a sufficiently high chlorophyll-content. The latter question is discussed in another section of this book.

It is a matter of common observation that some plants thrive in the shade, protected from the direct rays of the sun, while others do best under conditions of direct insolation. Undoubtedly the causes which underlie such an adaptation to environmental conditions are complicated. Yet under these different conditions of illumination the respective plants are capable of carrying on their photosynthetic activity with a total net gain to each in dry matter. Boysen-Jensen[66] has made a study of the photosynthetic activity of some typical "shade" and "light" plants with special reference to their gain in dry matter, i.e. "the amount of dry matter produced in unit time, calculated in per cent of dry matter in the plant in question at the beginning of the experiment." As an example of a light plant *Sinapis alba* is taken, and as a shade plant *Oxalis acetosella*. In Boysen-Jensen's experiments the former were grown in "full daylight" and the latter in "a light intensity as small as possible." His own conclusions are as follows: "In *Sinapis* the intensity of CO_2 assimilation is very great, rising to at least 6 mg. CO_2 per 50 cm.^{2} per hour at 20°. Also the respiration in the leaves is great, about 0.8 mg. CO_2 per 50 cm.^{2} per hour at 20°. The point of equilibrium between CO_2 assimilation and respiration lies at a light intensity of 1.0 (Bunsen units × 100). The development of a *Sinapis* plant is very quick. In four weeks the dry matter content rises from 0.5 g. to 38 g. per 100 plants. In favorable conditions the daily per cent production of dry matter can be estimated as about 15.

"In *Oxalis* the maximal intensity of CO_2 assimilation is very small, about 0.8 mg. CO_2 per 50 cm.^{2} per hour at 20°. Also the respiration of the leaves is small, about 0.1-0.2 mg. CO_2 per 50 cm.^{2} per hour at 20°. The point of equilibrium between CO_2 assimilation and respiration lies at a light intensity of 0.2. The daily per cent production of dry matter is 2.1."

From these experiments it is apparent that, as nearly as two such plants can be compared, the one growing in the sunlight does more photosynthetic work than the other, a conclusion which is not surprising. In both cases respiration took about 13 per cent of the material synthesized. Boysen-Jensen also calculated from Combes'[67] data the per cent production of dry matter of a number of rapidly growing plants. The results are given in Table 18.

Of course, the foregoing results contribute very little to the question of the efficiency of different plants in utilizing light for photosynthetic work. This question is discussed in the section on energy relations. Some

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[66] Boysen-Jensen, *Bot. Tidsskrift*, **36**, 219 (1918). Johansson. *Svens K. Bot. Tidsskrift*, **17**, 215 (1923).

[67] Combes, *Ann. d. Sci. nat.*, IX Ser. Bot., **11**, 75 (1910). See also Weis, F., Compt. rend., **137**, 801 (1903). Weber, *Arb. Bot. Inst. Würzburg*, **2**, 346 (1879).

TABLE 18

Per Cent Production of Dry Matter of Different Plants. (Boysen-Jensen.)

Plant	Number of Days	Per Cent Production of Dry Matter
Triticum vulgare	35	6.5
" "	77	5.9
Raphanus satious	19	16.8
Pisum sativum	17	5.9
Tropæolum majus	11	11.2
" "	30	13.4
Salsola kali	24	10.1

results of Warburg and Negelein[68] are of direct bearing on this question and of interest in this relation. They studied the photosynthetic activity of the unicellular alga *Chlorella* and observed great variations in the efficiency of these plants. They found that when the plants are raised under conditions of high light intensity, the amount of radiant energy utilized is low. That is, plants raised under these conditions convert but a small fraction of the absorbed radiant energy into chemical energy. While plants grown under low light intensity convert a larger portion of the absorbed energy into chemical energy. In other words, Warburg and Negelein consider that they can at will produce "light" and "shade" plants and that the photosynthetic efficiency of the two types of plants is different. It would be interesting to determine whether shade plants such as the *Oxalis* used by Boysen-Jensen, while they may in total produce a smaller amount of dry matter, do not utilize a greater proportion of the light absorbed than plants growing in the direct sunlight.

In a previous section some discussion has already been devoted to the interaction of the different factors which influence photosynthesis. According to the original theory of Blackman on "limiting factors" "the rate of photosynthesis is determined by the intensity of the weakest factor." If the intensity of this latter factor is increased, the rate of photosynthesis increases until some other factor becomes relatively the weakest or limiting one. Further increase in the intensity of the first factor does not result in an augmented photosynthetic rate because this factor is no longer the limiting one. These conditions have been described graphically under the sections devoted to the "Principle of Limiting Factors" and the "Partial Pressure of Carbon Dioxide," where the recent investigations of Warburg and of Harder are also discussed. The latter has followed the rate of photosynthesis when one factor only is changed as well as when two factors (light intensity and CO_2-concentration) are altered.

Harder's results appear to necessitate a modification of Blackman's original conception of the influence of various factors, in the sense that the effect of the factor which is varied is not the same throughout the

[68] Warburg and Negelein, *Zeit. physik. Chem.*, **102**, 246 (1922).

course of the curve but depends upon the intensity of the other factors. When the other factors, carbon dioxide-concentration, temperature, etc., are relatively high and light intensity is low, the increase in the rate of photosynthesis is about proportional to the increase in light intensity. As the latter factor continues to be augmented the effect on the rate of photosynthesis becomes less, so that the curve flattens, and the influence of other factors becomes noticeable. Such curves of the effect of light intensity have been obtained by Boysen-Jensen,[69], Warburg [70] and Harder [71] and are reproduced in Figure 8.

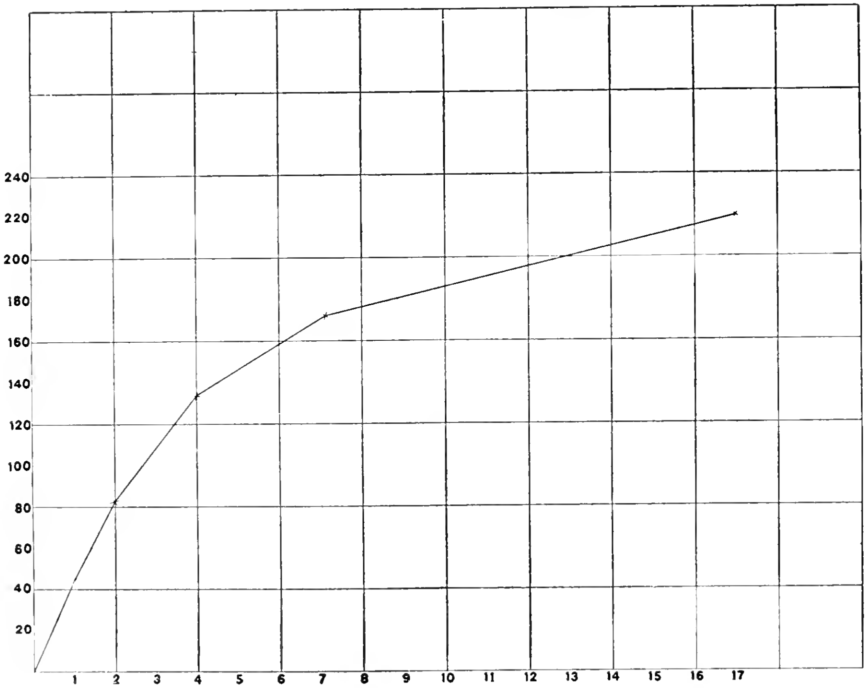

FIG. 8.—The influence of light intensity on the rate of photosynthesis of *Chlorella*. The ordinate represents the rate of photosynthesis, the abscissa the illumination intensity. (From Warburg.)

Harder measured the photosynthetic activity of *Fontinalis* at different light intensities and varying carbon dioxide-concentrations. The light intensity of the highest was 216 times that of the lowest intensity, and the carbon dioxide-concentration was varied from 0.01 to 0.32 per cent. The results are shown in Table 19 and represent true photosynthetic rates.

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[69] Boysen-Jensen, P., *Bot. Tidsskrift*, **36**, 220 (1918).
[70] Warburg, O., *Biochem. Zeit.*, **100**, 255 (1919).
[71] Harder, R., *Jahrb. wiss. Bot.*, **60**, 531 (1921).

TABLE 19

RATE OF PHOTOSYNTHESIS OF "FONTINALIS" AT SIX DIFFERENT LIGHT INTENSITIES AND FOUR DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE. (From Harder.)

$KHCO_3$	Light Intensity (in Meter Candles)					
	167	667	2000	6000	18,000	36,000
0.01 per cent...	0.12	0.40 0.42 } 0.41	0.75	0.90	1.06	1.07
0.04 per cent...	0.26	0.94 0.88 } 0.91	2.24	3.45	4.70	—
0.16 per cent...	—	1.086 1.12 } 1.10	3.45	6.40	11.83 11.47 } 11.35 10.75	—
0.32 per cent...	—	1.23	4.80 4.60 } 4.70	8.60	14.10 15.83 } 15.20 15.70	16.645

If the table is read from left to right it becomes evident that each increase in light intensity results in an increased photosynthetic rate; also if the table is read down it can be seen that each increase in carbon dioxide concentration produces an increase in the rate of photosynthesis. Harder therefore maintains that the rate of photosynthesis under all circumstances is dependent upon the light intensity as well as upon the concentration of carbon dioxide. He takes the stand that Blackman's conception of the principle of limiting factors as well as Liebig's law of minimum have no application to the photosynthetic process.

There is apparently little doubt that conditions exist in which the intensity of two or possibly more factors determines the rate of photosynthesis. It is here that the difficulty with Blackman's theory arises. Blackman and Smith[72] state: "When several factors are possibly controlling a function, a small increase or decrease of the factor that is limiting, and that factor only, will bring about an alteration of the magnitude of the functional activity." Contrary to this dictum Harder's experiments show that changes in both carbon dioxide-concentration and light intensity are able to effect changes in the photosynthetic rate. The intensity of one factor influences the effect produced by changes in intensity of some second factor. Or, what amounts to about the same, the effect produced by changes in intensity of this second factor is not the same for all intensities of the first factor. Thus, for example, the increase in rate of photosynthesis produced by augmented light intensity is dependent upon the concentration of carbon dioxide; the effect produced by augmented light intensity is greater the higher the concentration of carbon dioxide.

When determining the influence of a single factor on the rate of photosynthesis, all other factors being kept constant, the amount by which an increase in this single factor augments the photosynthetic rate depends, according to Harder, in every case on the concentration of the factors kept constant. The higher the concentration of these factors, the higher is

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[72] Blackman and Smith, Proc. Roy. Soc. London, B **83**, 397 (1911).

each point on the curve of the photosynthetic rate. Moreover, a change in that factor which in comparison to the others is in minimum concentration (or intensity) has the greatest effect on the rate of photosynthesis. Thus with high light intensity and low carbon dioxide-concentration, an increase in the concentration of the latter produces the greatest change and vice versa. Theoretically it is inconceivable that only one factor can determine the photosynthetic rate, though Harder concedes that practically it may occur that a single factor is so greatly in minimum concentration compared to the others that it appears as though the concentration of this one alone is determining the rate of photosynthesis. Harder also points out (inconsidering primarily the influence of the two factors, carbon dioxide-concentration and light intensity) that in every photosynthetic rate curve there are two portions to be differentiated: the one in which light intensity is the determining factor, the other in which carbon dioxide-concentration plays this rôle. Between these two portions of the curve there is a point at which the two factors are of equal influence. This point represents the most favorable relative combination of the two factors, and Harder concludes that the product of the two intensities at this point is lower than for any other combination of the two factors producing the same photosynthetic rate.

The interaction of the various factors is undoubtedly complex. Harder's chief point is that the augmenting effect on the rate of photosynthesis of a single factor is not directly proportional to the intensity of this factor at all concentrations of the other factors. The higher the intensity of light the greater is the augmenting effect of an increase in carbon dioxide-concentration, and conversely the higher the carbon dioxide-concentration, the greater is the augmenting effect of an increase in light intensity. Finally, an important point in Harder's conclusions is this: that the augmenting effect in the photosynthetic rate just described pertains to *absolute rates*. *Relatively* the augmenting effect of an increase in carbon dioxide-concentration is greatest with low light intensity as is also the relative effect of light greatest with low carbon dioxide-concentration.

Lundegårdh [73] has formulated a theory which is essentially the same as that of Harder. To this Lundegårdh has given the name Relativity Law of Photosynthetic Factors. According to this theory the rate of photosynthesis is determined by both the light intensity and the carbon dioxide-concentration. The more either one of these factors is relatively in minimum concentration, the greater will be the resulting augmentation in the photosynthetic rate when this factor is increased. Under natural conditions sunlight is available in excess, while the relatively minimum factors are carbon dioxide and chlorophyll content.

Recently Miss M. Henrici [74] obtained curves of the rate of photosynthesis with increasing light intensity which show two maxima. At low illumination intensities the rate rises sharply, with increasing intensity

[73] Lundegårdh, *Der Kreislauf der Kohlensäure in der Natur*. Jena (1924), p. 74.
[74] Henrici, *Verhand. Naturf. Ges. Basel*, **32**, 107 (1921).

drops again and attains a second and principal maximum. Thereafter increased light intensity has no influence on the rate, so that the curve becomes almost parallel to the abscissa on which the light intensities are plotted. With the exception of the two maxima the curves bear a strong resemblance to the original curves of Blackman in that they are in no sense logarithmic. Miss Henrici's experiments, however, were apparently carried out with atmospheric air, so that the limiting influence of the carbon dioxide concentration is clearly evident. These investigations are also discussed under the influence of temperature on photosynthesis.

Turning now to the effect on photosynthesis of light of different wave-lengths, this subject has for over a century engaged the attention of plant physiologists and has resulted in a most voluminous literature. The results, however, are by no means commensurate with the efforts expended. The problem of the effect of different wave-lengths involves experimental difficulties which at first glance are not apparent and in many cases have entirely escaped the notice of the experimenter. These difficulties are concerned with obtaining a satisfactory source of light, the measurement of energy in the different portions of the spectrum, methods of obtaining light of sufficient intensity of various wave-lengths either through filters or a prism, the relative absorption by the leaf of light of different frequency, temperature effects, as well as devising reliable methods of measuring photosynthetic rates. The most common error is the disregard of the intensity or more properly, the energy of the different wave-lengths employed. Colored glasses and light filters rarely yield monochromatic light; reliable data on their transmission can be gained only from spectrographs. In using prisms consideration must be given to the degree of dispersion of the different spectral regions.

A perusal of the many different investigations which have been undertaken to determine the effect on photosynthesis and plant growth in general of the various portions of the spectrum lead to the conclusion that the cause for the disappointing outcome of many of these elaborate and costly experiments lies in a disregard of some of the fundamental principles of the physics of light. There is a wealth of information originating in physical and chemical laboratories which has direct application to these problems and cannot be disregarded if intelligible results are to be hoped for. The question of the source of light used in such experiments is of paramount importance. While it seems desirable often to adhere to natural conditions of illumination by using sunlight, for experimental work, in spite of the high intensity, this source of light is not very satisfactory. The cause is that sunlight, at the surface of the earth, varies both in intensity and composition from hour to hour and from day to day. The high intensity of the infra-red rays in sunlight also introduces experimental difficulties. Artificial sources of light have many advantages furthermore, from the viewpoint of regulating intensity, constancy and composition of the light. Only it is necessary that some study be devoted to the physical problems involved, so that the experimenter is thoroughly familiar with the tools he is using. The great industrial development in illumination during recent years and the wealth of exact physical information which has accompanied it has made available tremendously valuable facilities for experimental work in this field. There are also a number of authentic compilations of data in book form relative to these matters.[75]

In Table 20 is given the relative distribution of energy in the visible spectra of a number of common sources of light with wave-length 0.59 μ equal to 100. It is evident from this that differences in photosynthesis are to be expected when different sources of light are employed.

TABLE 20

RELATIVE DISTRIBUTION OF ENERGY IN THE VISIBLE SPECTRA OF DIFFERENT SOURCES OF LIGHT. (From Luckiesh, "Color and Its Application." 1921.)

Wave-length μ	Black Body at 5000° Abs. Noon Sunlight	Blue Sky	Carbon Incandescent Lamp 3.1 w.p.m.h.c.	Tungsten Incandescent Lamp (Vacuum) 1.25 w.p.m.h.c. 7.9 Lumens per Watt	Tungsten Incandescent Lamp (Gas) 0.5 w.p.m.h.c. 22 Lumens per Watt	D.C. Arc (Open)
0.41	72.0	177.0	4.0	—	16.5	—
0.43	79.0	185.0	7.0	—	22.5	21.8
0.45	84.3	187.0	12.0	16.7	30.0	29.0
0.47	91.0	180.0	18.0	23.5	38.0	37.0
0.49	92.5	162.0	25.5	32.7	47.0	45.5
0.51	96.0	146.0	34.5	42.6	56.5	55.0
0.53	98.0	132.0	47.0	54.9	67.0	65.5
0.55	99.0	120.0	62.0	68.6	78.0	76.0
0.57	100.0	108.0	79.0	83.4	88.0	88.0
0.59	100.0	100.0	100.0	100.0	100.0	100.0
0.61	100.0	93.0	123.0	117.0	111.0	113.5
0.63	98.5	87.0	148.0	136.0	121.5	127.0
0.65	97.1	82.0	176.0	157.0	131.0	142.0
0.67	95.5	77.0	204.0	179.0	140.0	156.0
0.69	93.5	72.5	234.0	202.0	147.5	170.0

The first to observe a difference in photosynthetic activity in light of different color was Senebier.[76] This Swiss scientist devised the double walled bell jars, capable of being filled with solutions of different color, which have found extensive use in plant-physiology. Senebier found that plants growing under identical conditions under white, red, and blue light formed most oxygen in the white, next in the red, and least in the blue

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[75] Sheppard, S. E., *Photochemistry*. Longmans, Green Co., 1914. Plotnikow, J., *Photochemische Versuchstechnik*. Leipzig, 1912. *Idem., Allgemeine Photochemie*. Berlin and Leipzig, 1920. Luckiesh, M., *Ultraviolet Radiation*. Van Nostrand Co., 1922. *Idem., Artificial Light. Its Influence Upon Civilization*, 1920. *Idem., Color and Its Application*, 1921. Ellis and Wells, *The Chemical Action of Ultraviolet Rays*. Chemical Catalog Co., 1925. Hübl, A., *Die Lichtfilter*. Halle, 1921.

[76] Senebier, Jean, "Memoires physico-chimiques sur l'influence de la lumière solaire pour modifier les êtres des trois règnes de la nature et surtout ceux du règne végétale," Genève, 1782. German translation by F. H. Jacobäer, Leipzig, 1785, Vol. I, p. 153.

light. Draper,[77] using a prismatic spectrum, concluded that the plant reduced carbon dioxide most readily in yellow light. A number of investigators endeavored to establish the region in the spectrum of maximum photosynthesis. Most of these concurred that maximum activity is in the red portion of the spectrum though they differed as to the precise frequency. Most of these investigations contributed some data of a qualitative rather than of a precise quantitative nature, also a number of interesting demonstration experiments and much experience in the experimental difficulties entailed.

Against the conclusion that photosynthetic activity exhibits a single maximum in the red-yellow portion of the spectrum were the results published by Timiriazeff, Englemann and later by Kohl. Timiriazeff used the solar spectrum and determined the rate of photosynthesis by means of gas analyses. In practical agreement with these were the results of Englemann who employed his very sensitive bacteria method in a microspectrum. These investigators maintained that besides the photosynthetic maximum in the red there was a second maximum in the blue. We shall not enter into the lengthy discussions arising from these different results. The experimental methods employed contained numerous errors and the variant results can in part be ascribed to errors due to dispersion and to the unequal absorption of the different wave-lengths in the screens and prisms.

In most of the older investigations not sufficient attention was devoted to the distribution of energy in the various spectral regions studied. The impetus for most of these researches was the desire to determine whether the regions in the spectrum of maximum or high photosynthetic activity correspond to the absorption bands of chlorophyll. While it is true that there can be no photochemical action without absorption of light, it does not follow that absorption of light results in photochemical action.

Within recent time several very thorough investigations have appeared which elucidate most of the problems with which the older investigators struggled with only partial success. The earlier experiments therefore have largely only an historical interest and we shall confine our discussion to the more recent publications. A new attempt to study this subject was made by the investigations of Kniep and Minder.[78]

These investigators started with the realization that it was essential to determine accurately the energy of each region of the spectrum they were using and the relation of the different wave-lengths to each other and to the source of light. When sunlight is used as a source of light it must be borne in mind that the intensity of different portions of the

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[77] Draper, J. W., "Scientific Memoirs," New York, 1878, p. 184. *Phil. Mag.*, **25**, 169 (1844). Doubeny, C., *Phil. Trans. Roy. Soc. London*, **126**, 149 (1836). Lommel, E., *Pog. Ann.*, **143**, 568 (1871). Sachs, J., *Bot. Zeitg.*, **22**, 353-358, 361-367, 369-372 (1864). Pfeffer, W., *Arbeit. bot. Inst. Würzburg*, **1**, 1-76 (1871). Timiriazeff, C., *Ann. Chim. Phys.*, Ser. V, **12**, 355 (1877). Reinke, J., *Bot. Zeitg.*, **42**, 1, 16, 33, 49 (1884). Richter, A., *Rev. gen. Bot.*, **14**, 151-169, 211-215 (1902). Englemann, T. W., *Bot. Zeitg.*, **40**, 419 (1882); **42**, 81, 97 (1884). Kohl, F. G., *Ber. Bot. Ges.*, **15**, 111 (1897); **24**, 39 (1906).

[78] Kniep and Minder. *Zeit. f. Bot.*, **1**, 619 (1909).

spectrum may vary independently of one another. Kniep and Minder, working in Naples, consider that the intensity of sunlight about the noon hours is relatively constant, and checked each determination with direct measurement of the energy falling on the leaf. For measuring the intensity of radiation they used a Rubens thermopile with 20 elements which permitted the determination of less than 0.0000001° and was calibrated to a scale of gram calories per unit time. As a source of light sunlight between 11 A.M. and 2.30 P.M. was used; the light was filtered through water to absorb the heat rays.

Filters of colored glass were used to produce different spectral regions: (1) a red glass filter passing wave-lengths in μ μ, with the coefficient of transmission D from infra-red to about 620 μ μ, and (2) a blue filter.

1.	μ μ	644	578	546	509					
	D	0.846	0.00056	0.000057	0.000					
2.	μ μ	546	509	480	436	405	384	361	340	332
	D	0.00	0.0109	0.177	0.455	0.395	0.267	0.078	0.010	0.000

Green light was produced by the use of a filter made by mixing a solution of potassium chromate and ammoniacal copper hydroxide. This solution allowed light of wave-lengths 512 to 524 μ μ to pass through, though no quantitative data are given. From the data on the coefficients of transmission and from the curve of distribution of energy in the sun's spectrum it was possible to construct curves of the distribution of energy in the various regions passing through the light filters.

The plant employed was *Elodea canadensis* and for determining the rate of photosynthesis the bubble counting method was used (see chapter on Methods of Measuring Photosynthetic activity). Unfortunately this method is subject to many errors, as was later recognized by the authors who subsequently made a careful analysis of the sources of error involved. As a consequence they had to reduce the intensity of radiation by a series of screens which to a measure diminished the value of their careful determinations of radiation distribution. The conclusion is drawn that red and blue light of the same intensity produce about the same rate of photosynthesis. The light intensity in these experiments was very low and probably was the factor of relatively minimum intensity, though little regard was apparently paid to the other factors. The real value of Kniep and Minder's investigations is the demonstration of the fact that there is no sense in considering the photosynthetic activity in different colored light without at the same time determining the energy relations of the light employed. The maximum photosynthesis in the red-yellow portion of the spectrum, observed by earlier investigators could thus be ascribed to the higher intensity of these wave-lengths in the spectra used.

By throwing a spectrum on a leaf Timiriazeff[79] endeavored to demonstrate the capacity of different wave-lengths for photosynthetic work. The method has been used by several investigators and consists essentially in allowing the light to act for a certain length of time, extracting the chlorophyll, and treating the leaf with an iodine starch reagent. The starch formed in photosynthesis thus becomes blue to black and the depth of color serves as a rough measure of the rate of photosynthesis. By means of the iodine reagent the starch is thus "developed" producing an effect not entirely dissimilar to the development of a photographic plate.

Ursprung[80] has used the method with prism and grating spectra and a variety of light sources. Timiriazeff had obtained hardly any photosynthetic effect with blue and violet rays. Ursprung found the lower limit of starch formation at about 759 μ μ. There was no starch formation in the infra-red. In the violet he found starch formation up to 330 μ μ. With the use of quartz apparatus no photosynthetic action could be detected in the ultra-violet. Considering the region of maximum activity as that in which starch formation is first detected, the region 687-656 μ μ would receive this value. This applies for light from the sun, a carbon arc or filament lamp with prism and grating spectra. There appears to be little doubt that all light between wave-lengths 760-330 μ μ is capable of producing starch if sufficient time of illumination is allowed; in the normal solar spectrum starch formation in the blue requires longer time than in the red. The exact spectral limits of photosynthesis have not been determined and it is, in fact, probable that considerable variation exists in different plants in this regard. In general, the method of measuring the rate of photosynthesis by the appearance of starch in the leaf cannot be considered as being very accurate. The formation of starch is itself not dependent upon the presence of light, as is indicated by the fact that starch can be formed by the leaf from glucose, sucrose and other substances in the dark. Between the actual reduction of carbon-dioxide by light and the appearance of starch in the leaf there are a number of steps all of which may be influenced by light and other factors.

A somewhat different line of approach was followed by Wurmser.[81] This author has made a careful study of the effect of light on chlorophyll solutions and the decoloration of such solutions in different wave-lengths. The rate of decomposition of an acetone solution of chlorophyll by light of different wave-lengths was utilized by Wurmser as a measure of the incident light. He used the green alga *Ulva lactuca* and the method of Osterhout and Haas for determining the rate of photosynthesis. The latter method, which is described in another chapter of this book, is based upon the fact that certain marine algae, when exposed to light convert some of the dissolved bicarbonates into carbonates and thus increase the hydroxyl ion concentration of the solution, the rate of increase of the

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[79] Timiriazeff, *Proc. Roy. Soc. London*, B. **72**, 424 (1903).

[80] Ursprung, *Ber. bot. Ges.*, **35**, 44 (1917).

[81] Wurmser, "Récherches sur l'assimilation chlorophyllienne," Paris, 1921.

latter serving as a measure of the rate of photosynthesis. For light filters Wurmser used solutions of potassium dichromate, transparent up to about 560 μ μ, cuprous chloride transmitting from 560 μ μ to 460 μ μ and ammoniacal copper sulphate, transmitting from about 540 μ μ to 450 μ μ. Wurmser's results, which are expressed in arbitrary values are summarized as follows:

	Red Light	Green Light	Violet Light
Photosynthesis = v	100	24	80
Enegry absorbed = Pa	100	6	34
Ratio = v : Pa	1.0	4.00	2.35

This is indeed a surprising conclusion, while the highest value for photosynthesis was found to be in the red and next in the blue light, the highest value in proportion to the energy absorbed was in the green. From these observations Wurmser formulates a hypothesis of the mechanism of photosynthesis. This assumes that the photochemical reaction takes place on the surface of the "pigment." A more exact statement would probably be that the photochemical reaction takes place on the surface of the chloroplasts, the chlorophyll acting as a photochemical sensitizer. The photochemical reaction is but the first step in a series of reactions in the subsequent steps of which the activity of the protoplasm plays an important rôle. The rate of photosynthesis thus depends not only upon the energy absorbed but upon the activity of the protoplasm as well. Views quite analogous to this have recently been expressed by a number of investigators of the problem and will be discussed in greater detail in another chapter. Wurmser's results do not permit, however, of an accurate calculation of the photosynthetic efficiency in light of different wavelengths especially because his method of measuring light intensity has no direct physical basis.

Probably the most successful investigations of the effect of different wave-lengths on photosynthetic activity have been carried out by Warburg and Negelein.[82] These are described in greater detail in the chapter on Energy Relations of Photosynthesis. Warburg and Negelein conclude that the efficiency of the photosynthetic process becomes less with decreasing wave-length. There is apparently no relation between photosynthetic efficiency and the absorption bands of chlorophyll. Thus the yield in the red, a region of high absorption is greater than in the green, a region of low absorption; while the yield in the green is greater than in the blue, the region of highest absorption. For the reduction of one molecule of carbon dioxide with red (660 μ μ) or yellow (578 μ μ) light there are required about four quanta, with blue light (436 μ μ) about five quanta. There are undoubtedly several factors which affect these results. Probably the most important of these is the concentration of the chlorophyll and carotin in the plants. That these vary with the conditions of previous culture of the plants is a matter of common experience. The influence

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[82] Warburg and Negelein, *Zeit. physik. Chem.*, **106**, 191 (1923).

of light as a cultural condition has already been alluded to; its effect on plants under natural conditions has been a subject of long investigation from which some important conclusions have already been drawn.

Englemann[83] first offered the hypothesis that the color of certain marine plants was complementary to the color of the light to which they were exposed. It is a familiar fact that water has a higher absorption for the longer wave-lengths of light than for the shorter wave-lengths. As a consequence the deeper the water, the greater is the relative proportion of blue light. Thus, if 1000 represents the light intensity at a depth of one meter the distribution of the different wave-lengths is about as follows:[84]

Depth in Meters	Red	Orange Yellow	Green	Blue	Blue Violet
5	3.7	2.5	230	450	866
50	0.0021	—	—	—	—
100	—	0.001	—	—	—
1000	—	—	0.0003	0.001	0.003
1500	—	—	—	—	0.0001

Shelford and Gail[85] found that the brown algal zone is at a depth of 5 to 20 meters where the shorter wave-lengths have an intensity of about 10 per cent of full sunlight and the longer, red wave-lengths about 1 per cent. The red algal zone lies between 10 and 30 meters in depth where the blue light is 2 to 10 per cent of full sunlight and the red light is 0.032 to 1 per cent.

Gaidukow[86] reported that he was able to alter the color of certain marine algae by growing them under colored light and thus obtained plants with a color complementary to the color of the light in which they were grown. These and other similar observations gave rise to the theory of complementary chromatic adaptation. The theory and some of the ramifications and deductions which have been drawn therefrom have been attacked from different sides, a discussion of which would lead too far afield.[87] Some of the principles involved are, however, of direct bearing on the photosynthesis problem. In its broader significance, of course, the question resolves itself into the old one of why vegetation is green.

This question has been subjected to an interesting theoretical analysis by Stahl. The theory is really an extension to land plants of Englemann's theory of the distribution of marine plants. Stahl[88] points out

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[83] Englemann, *Bot. Zeitg.*, **42**, 81 (1884).

[84] Monaco, The Prince of, *Sci. Monthly*, **13**, 177 (1921).

[85] Shelford, V. E., and Gail, F. W., Pub. Puget Sound Biological Station, 1920, 141. Gail, F. W., *ibid.*, 177-193. Geitler, I., *Int. Rev. d. ges. Hydrobiol. Hydro.*, **10**, 681 (1922). Pascher, *Bot. Arch.*, **3**, 311 (1923).

[86] Gaidukow, *Ber. bot. Ges.*, **21**, 484, 517 (1903); **24**, 1, 23 (1906).

[87] Baresch, K., *Jahrb. wiss. Bot.*, **52**, 145 (1913); *Ber. bot. Ges.*, **37**, 25 (1919); *ibid.*, **39**, 93 (1921); *Zeit. f. Bot.*, **13**, 65 (1921). Magnus, W., and Schindler, B., *Ber. bot. Ges.*, **30**, 314 (1912). Pringsheim, E., *Cohn's Beitr. z. Biol. d. Pflanzen*, **12**, 49 (1914). Tobler, F., *Die Naturwissenschaften*, **1**, 845 (1913). Savageau, C., *Compt. rend. Soc. biol.*, **64**, 95 (1908).

[88] Stahl, "Laubfarbe und Himmelslicht," Jena, 1906.

that the chlorophyll apparatus is adapted to the conditions of illumination to which the plant is exposed. Thus a plant receives not only the direct rays of the sun, but also diffuse light, which in composition has been considerably altered by reflection and dispersion. While the direct rays of sunlight are altered by absorption of the blue-violet rays in passing through the atmosphere, in diffuse light the blue-violet rays are relatively in excess. In the direct rays of the sun there is therefore a relative preponderance of red-yellow light while in diffuse light there is a preponderance of blue light. This is based upon the well-known theory of Lord Rayleigh. Stahl sees in the absorption of light by chlorophyll a direct adaptation to this condition. The pigments of the leaf are of two different colored components capable of absorbing the different spectral components of the light incident on them. Stahl considers that in the chlorophyll, the blue-green pigment serves to absorb the red-yellow rays of direct sunlight and the yellow pigment in leaves absorbs the diffuse reflected blue rays. That the plant does not absorb the green and infra-red rays he explains on the ground that in diffuse light these rays are of such low intensity that the plant can make no use of them, while in direct sunlight these rays are of such high intensity that their absorption would result in an increase of temperature harmful to the leaf. Stahl considers that the plant absorbs and utilizes those rays which are the most constant components of the light to which it is exposed, at the same time avoiding the absorption of rays which have little value for photochemical reactions. These views are still largely speculative. It appears to be true that light over a wide range of frequency is capable of inducing photosynthesis. Interesting in this relation, it may parenthetically be stated, is the fact, observed by Stern,[89] that the chlorophyll in the cell fluoresces. Chlorophyllous cells illuminated with yellow, green or blue light fluoresce red light, 630-650 μ μ and 660-690 μ μ, that is, light which is in the red absorption band of chlorophyll.

In view of the fact that marine plants growing at some distance below the surface are subjected to illumination of much more restricted spectral regions than land plants, the theory of complementary adaptation has been studied primarily in relation to marine life. It is also in these plants that differences in color, due to pigments besides chlorophyll, are most striking. According to the Engelmann theory the production of these pigments is the reaction to a particular factor in the environment, the frequency of the light in which the plants are growing; and these pigments play an important rôle in the photosynthetic process of the plants. On the basis of experimental work Richter [90] comes to a very different conclusion. Working with a variety of marine algae of different color and with light of various intensities and frequencies he concludes that it is not the wave-length of the light which determines the color of the algae but the intensity of the light. While Gaidukow, already cited, stressed particularly the de-

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[89] Stern, *Ber. bot. Ges.*, **38**, 28 (1920).
[90] Richter, *Ber. bot. Ges.*, **30**, 280 (1912).

velopment of color in marine plants grown under light of different color, Richter emphasizes that only the photosynthetic activity of the variously colored plants under the different conditions of illumination is of real biological significance. Richter therefore takes the stand that the distribution of the different colored algae is a matter of light intensity, quite analogous to "light" and "shade" land plants. The vertical zonal distribution is a matter of light intensity or "Lichtgenuss" in the sense of Wiesner, and the other pigments in the plants play no essential rôle in photosynthesis. Thus Richter concludes that only the chlorophyll proper plays a rôle in photosynthesis.

Regarding the phenomenon of zonal distribution of aquatic plants primarily from the viewpoint of photosynthesis, an aspect of much interest is whether the blue and red complementary pigments, phycocyanin and phycoerythrin play a rôle in the photosynthetic process. It is evident from the work of Richter, just cited, that consideration must be given the intensity of the light as well as the wave-length in interpreting the complementary color adaptation phenomenon. Also from what has already been said it is evident that attention must be paid to the intensity and wave length of the light not only during the course of the determinations of photosynthetic rates but as well for a period prior to making such determinations. Considerable information on these questions has been gained through an investigation by Harder.[91] He used a blue-green alga, *Phormidium foveolarum* which in red light becomes green and in blue light takes on a purple color. Boresch [92] has shown that the purple form contains phycoerythrin and that the green form contains the blue pigment phycocyanin. By means of light filters, Harder obtained blue and red light the intensity of which he measured with a Rubens thermopile. The rate of photosynthesis was determined from the oxygen content of the water by Winkler's method.

Harder upholds the conclusions of Englemann of complementary adaptation; the purple varieties of the alga, containing the red pigment, phycoerythrin, have a higher rate of photosynthesis in blue light than in red, while the blue-green varieties containing the blue pigment, phycocyanin, show higher rate of photosynthesis in red light than in blue. These results apply also when equal intensities of the different colored lights are used; the rate of photosynthesis is relatively and absolutely higher in those wave-lengths which are complementary to the color of the plant.

That the intensity of light in which plants have been growing, and to which they have in a sense become adjusted, is a very important factor in the rate of photosynthesis has been recognized for some time. This is, of course, the basis of the behavior of "light" and "shade" plants and has already been touched upon. It is, in fact, possible to produce artificially with the same species, as far as their light requirements for photo-

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[91] Harder, *Zeit. f. Bot.*, **15**, 305 (1923).

[92] Boresch, *Arch. f. Prostistenkunde*, **44**, 1 (1921).

synthesis are concerned, both "light" and "shade" varieties.[93] By cultivating the same alga under different intensities of light, Harder found that the plants grown under light of low intensity can utilize lower intensities of light for their photosynthetic work than plants grown under high light intensity, while plants grown in light of high intensity attain a higher rate of photosynthesis in light of high intensity than those grown in light of low intensity. The color of the plant, i.e., the presence of phycocyanin or phycoerythrin seems to be of little significance herein. This adaptation or accommodation to light of different intensity is not recognizable by any outward appearance of the plant, but is a more important factor than color. For this reason it is extremely important that it be taken into consideration in all determinations of the rate of photosynthesis. Just what the nature is of these accommodations to light of different intensities it is difficult to tell. Presumably it is associated with the chlorophyll-content and possibly also with internal plasmatic factors.

There exists, it is evident, a contradiction between the conclusions of Richter and those of Harder. The former regards that the color of the plant or the presence of pigments besides chlorophyll is of no consequence to photosynthesis. Harder considers that this is an extreme view. There is no doubt that the light to which the plants are accommodated is an important factor. This is evident from the following extreme conditions: plants which have been raised in weak red light (blue-green "shade" plants) show relatively higher photosynthesis in weak blue light than in strong red light, and plants raised in strong blue light (red "light" plants) show relatively higher photosynthesis in strong red light than in blue light of low intensity. That is, the typical behavior of "light" and "shade" plants is maintained even when the light is not complementary to the color of the plants. This would be quite in agreement with Richter's claims. However, Harder points out, that when the light intensity is duly taken into consideration, the factor of wave-length is always discernible, in the sense that the plants show relatively higher photosynthesis in those wave-lengths which are complementary to the color of the plant. This action is often covered by results of differences of intensity. Harder comes to this conclusion from the following facts. Photosynthesis of different colored plants in light of different wave-lengths, *but of the same intensity*, is highest in those wave-lengths which are complementary to the color of the plant. Also, when plants are accommodated to light of different wave-lengths but of *the same intensity*, photosynthesis is highest in those wave-lengths which are complementary to the color of the plant, no matter what intensities of other wave-lengths are used.

Harder's conclusion that "light" plants show greater photosynthesis in light of high intensity than "shade" plants and the latter greater photosynthesis in light of low intensity than "light" plants, is in apparent contradiction to the conclusions of Warburg and Negelein, already referred

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[93] Lubimenko, W., *Ann. sci. nat.* (9), 7, 321 (1908). Rosé, *ibid.* (9), 17, 1 (1913).

to. It will be recalled that these investigators found that the unicellular alga used by them, when cultivated in light of high intensity, converted but a small amount of the light absorbed in the process of photosynthesis, while the plants cultivated in light of low intensity converted a large portion of the absorbed energy in the photosynthetic process. It is essential to bear in mind that Warburg and Negelein worked with light of low intensity only, they did not expose their "light" plants to illumination of high intensity. Thus their results as far as they go are really in agreement with those of Harder. Finally, it should be pointed out that the results of Harder and those of Warburg and Negelein are not strictly comparable. The latter investigators were concerned with photosynthetic efficiency, namely, the proportion of radiant energy, absorbed which is converted into chemical energy. This is a strictly quantitative and physical method of measurement. Harder was satisfied with the relative amounts of oxygen liberated by the two types of plants under different conditions of illumination; there is no measure of the ratio of energy absorbed to that converted. Thus there is no information on the efficiency of the two types of plants under the different conditions. The investigations of Harder and those of Warburg and Negelein were carried out from different viewpoints and had not the same goal in view.

Aquatic plants lend themselves better to experimentation of the nature just discussed than land plants. Particularly on account of errors due to temperature changes within the leaf and variations in the stomatal openings which give rise to alterations in the gaseous exchange and water loss; thus, experimentations with land plants are associated with many difficulties. While the principles underlying the reactions involved in photosynthesis are probably the same in aquatic and land plants, it is in the latter that greatest interest centers. Lubimenko[94] has worked with a number of land plants in relation to their behavior in light of different wave-lengths. His results are difficult to interpret on the basis of other findings. Thus, he concludes that while the reduction of carbon dioxide is greater in red light than in blue or violet the increase of dry substance is greater in blue light than in red.

Concerning the rôle of ultraviolet rays in the photosynthesis process, it may be stated that it is highly improbable that these are of significance under natural conditions. The intensity of ultraviolet light at sea-level is exceedingly low. The solar spectrum ends very abruptly near wavelength 300 μ μ; even at great heights above sea-level there is no appreciable energy of wave-lengths shorter than 290 μμ.[95] Moreover photosynthesis is carried on normally by plants under glass which is opaque to ultraviolet light. Whether it is possible for photosynthesis to take place in ultraviolet light is as yet uncertain. Bonnier and Mangin's[96] ex-

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[94] Lubimenko, *Zentr. Biochem. Biophys.*, **10**, 803 (1910); *Rev. gen. bot.*, **23**, 1 (1911); *Compt. rend.*, **145**, 1191 (1907); **177**, 606 (1923).

[95] Luckiesh, "Ultraviolet Radiation," 1922, p. 24.

[96] Bonnier and Mangin, *Compt. rend.*, **102**, 123 (1886).

periments would indicate that this was the case, yet there is considerable doubt as to whether their filters excluded all other light of lower frequency. The problem is made difficult on account of the harmful action of ultraviolet light on plants and the complications arising from the effect of these rays on enzymes, starch and other components of the plant.[97]

Similarly, there is no evidence that the infra-red rays play a part in the reduction of carbon dioxide, although it appears that chlorophyll absorbs some infra-red rays. On the other hand active photosynthesis is caused in purple bacteria by the infra-red rays (800-900 μ μ).[98]

The composition of the light is changed as it penetrates successive layers of chlorophyll so that the chloroplasts in the center of a leaf are exposed to light of different composition from those near the surface. This is one factor which may account for the varying behavior of different species of leaves. Thus the red rays (700-660 μ μ) cause very active photosynthesis, but are also largely absorbed by the outer layers of the leaf and can thus act upon fewer chloroplasts than the yellow rays which penetrate more deeply. It is conceivable therefore that the maximum carbon dioxide reduction in relation to the amount of light of different wavelengths absorbed will vary in relation to the thickness of the leaf, and in thick leaves maximum photosynthesis will be shifted toward the blue. The reflection as well as the absorption of light by a leaf are both of considerable importance in determinations of photosynthesis. Undoubtedly some leaves in nature, due to their glossy surface, reflect a large proportion of the light incident on them.

The absorption spectra of alcoholic solutions of chlorophyll show a slight displacement toward the blue compared with spectra of chlorophyllous leaves. Although there has been considerable controversy as to whether a leaf is capable of carrying on photosynthesis in light filtered through a chlorophyll solution or through another leaf, there appears to be little doubt that this is possible if the intensity of the light is sufficiently high as was shown by Ursprung[99] with direct sunlight.

There is some evidence that there is a difference of effect on photosynthesis between continuous and intermittent light. Warburg[100] has tested this by means of rotating sectors which in one revolution cut off one half the light so that the period of illumination was equal to that of darkness. Comparison between the effects of continuous and intermittent illumination were made on the basis of equal periods of illumina-

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[97] Kluyver, A. J., *Oestreich. Bot. Zeit.*, **63**, 49 (1913); *Sitzber. Wien. Akad.*, **120**, I, 1137 (1911). Bovie, W. T., *Bot. Gaz.*, **59**, 149 (1915); **61**, 1 (1916). Maquenne and Demoussy, *Compt. rend.*, **149**, 756 (1909). Stoklasa, *Centralbl. f. Bakt.*, **31**, II, 477 (1912). Raybaud, L., *Rev. gén. bot.*, **25**, 38 (1913). Ursprung and Blum, *Ber. bot. Ges.*, **35**, 385 (1917).

[98] Englemann, *Bot. Zeitg.*, **40**, 419 (1882); **41**, 1 (1883); **42**, 81 (1884). Pfeffer, W., *Arb. bot. Inst. Würzburg.*, **1**, 41 (1871). Ursprung, *Ber. bot. Ges.*, **35**, 55 (1917). Warburg and Negelein, *Zeit. physik. Chem.*, **106**, 191 (1923).

[99] Ursprung, *Ber. bot. Ges.*, **35**, 64 (1917).

[100] Warburg, *Biochem. Zeit.*, **100**, 260 (1919).

tion and not simply on the basis of equal time. With high illumination intensity equal quantities of radiant energy reduced more carbon dioxide when the light was intermittent than when it was continuous. The excess of photosynthesis with intermittent illumination was about 100 per cent when the alternations of the rotating sector were 8000 per minute, and only about 10 per cent when the alternations were 4 per minute. With low light intensity there is no difference between intermittent and continuous illumination. It is important that in a period of time which is long in comparison with the length of time occupied by a single flash of light in the intermittent illumination, the amount of carbon dioxide reduced is the same whether the light is continuous or intermittent.

There are two possible explanations for the effect of intermittent illumination. Either photosynthesis continues at an undiminished rate during the periods of darkness, which would be in accord with an older conception of Tswett,[101] or photosynthesis is interrupted during the periods of darkness and is doubled during the periods of illumination. It would seem that the latter is the more probable explanation. Thus, during the dark periods carbon dioxide would have an opportunity to enter the centers of photosynthetic activity and synthesized material move away, both of which would tend to increase the rate of photosynthesis. When the chloroplast is then again illuminated there are available higher concentrations of dissociable material. With light of low intensity, in which there is little difference between equal periods of illumination of the continuous and intermittent type, the reduction of the concentration of carbon dioxide in a given length of time of illumination is less; the rate of replacement is therefore also less and the influence of the periods of darkness is less pronounced. This is virtually the same explanation that Willstätter and Stoll[102] give for the results of Brown and Escombe[103] with intermittent light.

c. Partial Pressure of Carbon Dioxide.

The early observations on the influence of various concentrations of carbon dioxide were naturally of a qualitative nature.[104] Thus it has been known for a long time that leaves which ordinarily produce only soluble carbohydrates but no starch can be induced to form the latter by exposing them to an atmosphere enriched in carbon dioxide. Moreover Kreusler[105] and others clearly demonstrated that an increase in the partial pressure of the carbon dioxide resulted in increased photosynthetic activity. The object of these investigators was to establish the optimal

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[101] Tswett, *Zeit. physik. Chem.*, **76**, 413 (1911).

[102] Willstätter and Stoll, "Untersuchungen ü. die Ass. der Kohlens," Berlin, 1918, p. 240.

[103] Brown and Escombe, *Proc. Roy. Soc. London*, **76** B, 86 (1905).

[104] Godlewski, *Flora*, **56**, 378 (1873); **60**, 215 (1877). Schimper, *Bot. Zeitg.*, **43**, 737 (1885).

[105] Kreusler, *Landw. Jahrb.*, **14**, 951 (1885).

percentage of carbon dioxide which the plant was capable of utilizing. The results of different workers varied greatly; in some cases 4 per cent carbon dioxide produced injurious effects, in others 10 per cent produced optimal activity. As is now evident the cause for these contradictions lay primarily in the fact that the other factors which influence photosynthetic activity were not taken into consideration, that is, the various investigators were using different light intensities, temperatures, plants with unknown chlorophyll-content, etc., and while focussing their attention on the influence of carbon dioxide-concentration, became entangled in complications resulting from the interaction of these other factors. Treboux,[106] in his study of the effects of a variety of substances on photosynthesis, established a direct proportionality between this activity and carbon dioxide-concentration and concluded that with low light intensity the carbon dioxide optimum shifts with the light intensity.

It was Blackman who first broke away from the conception of a single optimum factor and developed the theory of the interaction of various factors. Blackman and Smith[107] studied the effect of varying concentrations of carbon dioxide on the photosynthetic activity of the aquatic plants, *Fontinalis antipyretica* and *Elodea*. They obtained curves which are of the nature of those shown in the diagram in Fig. 7. Of these they state: "In the weaker solutions of CO_2 the curve shows steadily increasing assimilation proportional to the increase of CO_2-supply. Here the light and temperature are in excess, but, at a certain point, sharply defined, increase of CO_2 is no longer followed by further increase of assimilation but the value of the assimilation remains at a fixed level. . . . This part of the curve is due undoubtedly to the limiting action of either illumination or the temperature. . . .

"Had a more intense light and higher temperature been fixed upon, then the ascending part of the curve would have been prolonged further and a fixed (but higher) level only attained with a greater concentration of CO_2. With less light the limiting value would have been arrived at sooner."

The outstanding feature of Blackman and Smith's conclusions on the relation of the rate of photosynthesis to the environmental factors, CO_2-supply, temperature and light intensity is that photosynthesis "in every combination of these factors is determined by one or the other of them acting as a limiting factor.

"The identification of the particular limiting factor in any definite case is carried out by applying experimentally the following general principle. *When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and that alone, will be found to bring about an increase of the magnitude of the function.*"

Blackman and Smith also give an interpretation of the results of

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[106] Treboux, *Flora.*, **92**, 63 (1903).
[107] Blackman and Smith, *Proc. Roy. Soc.*, **83** B, 389 (1911).

Treboux (cited above) and of Pantanelli[108] on the basis of limiting factors; these seem to lend considerable substantiation to the theory. It is necessary to bear in mind that Blackman and Smith's[109] experiments were the first in which a conscious effort was made to test out this conception of the influence of environmental factors. The method used for determining photosynthetic activity was new and the results from which the final curves were plotted were taken from experiments ranging over three years.

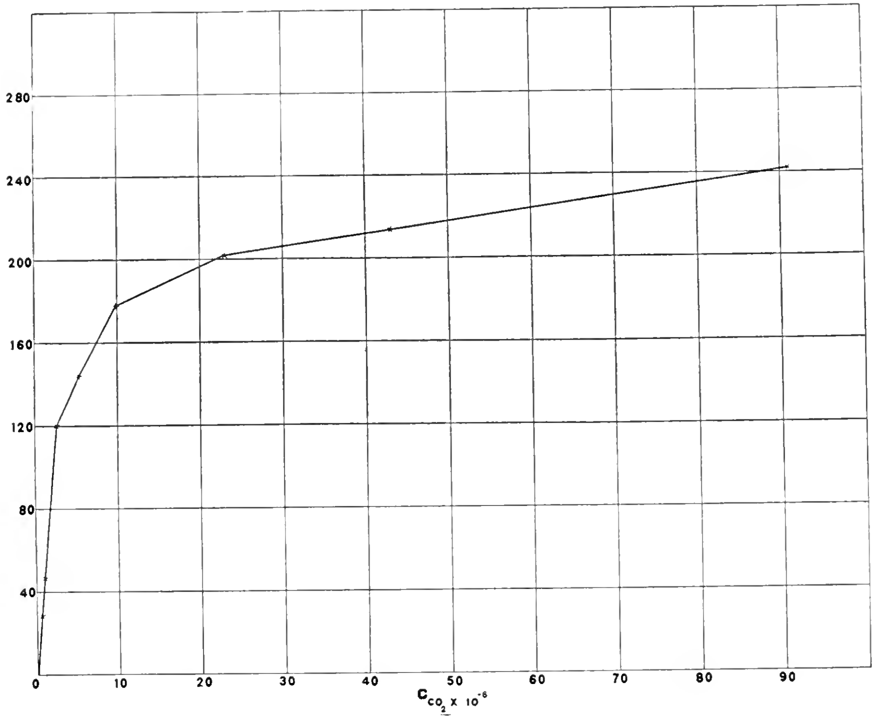

Fig. 9.—The rate of photosynthesis at different concentrations of carbon dioxide. The ordinate represents the rate of photosynthesis, the abscissa the concentration of carbon dioxide. (Constructed from values given by Warburg.)

Within recent years conceptions differing from those of Blackman have been advanced by a number of investigators. These modifications of Blackman's views are largely the result of careful experimentation with a variety of different plants and improved means of measuring photosynthetic rates.

In 1919 Warburg[110] devised a method of determining the rate of

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[108] Pantanelli, *Jahrb. f. wiss. Bot.*, **39**, 167 (1903).
[109] Blackman and Smith, *Proc. Roy. Soc.*, **83** B, 374 (1911).
[110] Warburg, *Biochem. Zeit.*, **100**, 230 (1919).

photosynthetic activity of the unicellular green alga *Chlorella.* This method makes use of the principle of the Haldane-Barcroft method of blood gas analysis and permits rapid and accurate determinations to be made. By using solutions of sodium carbonate and sodium bicarbonate the concentration of carbon dioxide in the water could be controlled with great accuracy. The source of light was a metal filament lamp of 1500 watts current consumption which at 15 cm. distance exceeded the light intensity of direct sunlight. The concentrations of carbon dioxide in solution ranged from that which is in equilibrium with air containing $\frac{1}{20}$ to 10 times the normal amount. The temperature was 25°. It is thus quite certain that carbon dioxide was at first the limiting factor. In Figure 9 is given the curve which Warburg obtained under these conditions.

Warburg's curve indicates that at low concentrations of carbon dioxide the rate of photosynthesis is closely proportional to the carbon dioxide-concentration. Above a concentration of about 2×10^{-6} moles per liter progressive increase in the carbon dioxide-concentration results in a continuously smaller increase in the rate of photosynthesis until finally the latter seems to be independent of carbon dioxide-concentration. Warburg interprets the form of the curve on the basis that the rate of photosynthesis is proportional to the concentration of carbon dioxide and to the concentration of a second substance which reacts with carbon dioxide. Thus if A represents the total amount of this absorbing substance in a cell, x and A — x respectively the amounts which are in the free and combined condition and C_{CO_2} the concentration of carbon dioxide, then in any steady state $\frac{C_{CO_2} \cdot x}{A - x}$ would be constant.

Warburg's conception of the photosynthetic process thus involves that of an absorbing substance for carbon dioxide, a conception the necessity of which has been demonstrated in a number of different ways and of which there is further discussion in another part of this chapter. An important assumption in the idea that carbon dioxide first goes into combination is that the rate of photosynthesis, even at the lowest carbon dioxide-concentrations is governed by the rate of a chemical reaction and not only by diffusion. It would seem that Warburg's experimental conditions have certain advantages over previous ones for determining photosynthetic rates, because the use of the unicellular organism reduces the element of diffusion to a minimum. In higher plants possessing an internal atmosphere it is evident that these conditions are more complex. This was already found by Blackman, who showed that the Bryophyte, *Fontinalis*, has a photosynthetic rate about half that of the Phanerogam, *Elodea.* The fact that carbon dioxide diffuses more rapidly as a gas than in solution may account for this difference. In the higher plants, which are of a more complex structure, under conditions of low carbon dioxide supply, there would rarely be equilibrium between the carbon dioxide-concentration surrounding the plant and that of the centers of photosynthetic activity. In the case of the higher plants there would thus be introduced the factor of diffusion in determining the rate of photosynthesis for any one condition of carbon dioxide-concentration and consequently be more complex than under the conditions studied by Warburg.

There is no rational basis upon which the photosynthetic activity of two plants of widely different structure can be compared. Such measures as area, fresh weight, chlorophyll-content and number of chloroplasts, which have occasionally been used, are either not universally applicable or can be determined only for certain types of plants. A quantitative comparison of two such widely different plants as a unicellular alga and the leaf of a sunflower is impossible as yet.

Harder[111] has made a rather extensive study of the principle of limiting factors. He was particularly concerned with the form of the curves of photosynthetic activity under varying external conditions and subjects Blackman's theory to rather severe criticism. In order to test this theory Harder sets the following conditions:

1. The course of the rate of photosynthesis must be followed when all factors but one are maintained constant. This single factor must be gradually increased. Under these conditions it will be disclosed whether the photosynthetic rate curve rises in a straight line and then abruptly becomes horizontal or whether the transition to the horizontal is gradual.
2. The course of the rate of photosynthesis must be followed when more than one factor is increased. Under these conditions it can be determined whether the rate of photosynthesis is actually affected only by the one factor which is the limiting one, or whether the other factors which are changed, also influence the rate.

As experimental material *Fontinalis antipyretica*, *Cinclidotus aqucatiles*, and two species of *Cladophora* were employed; the rates of photosynthesis were determined by analysis of the water for oxygen, and metal filament lamps used as the source of light.

Harder found that with all of the plants used, when carbon dioxide-concentration or light intensity alone are altered, the photosynthetic rate curve does not exhibit an abrupt change, but changes gradually from the almost vertical to a line approaching the horizontal. The form of the curves varies with different plants; an example is given in Figs. 10 and 11.

So Harder concludes that when only one factor is changed the curve representing the rate of photosynthesis does not exhibit an abrupt turn: it is rather a logarithmic curve. In other words the point at which one factor ceases to be the limiting one and some other factor becomes such is not a sharply defined one, but the transition is a gradual one. Harder furthermore, attempted to apply the mathematical formula which Baule[112]

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[111] Harder, R., *Jahrb. wiss. Bot.*, **60**, 531 (1921).

[112] Baule, *Landw. Jahrb.*, **51**, 361 (1918).

had developed for the effect of nutrients on crop yield, to the effect of carbon dioxide on the rate of photosynthesis. He found, however, that the calculated values are not in accord with the experimental results.

In the second set of Harder's experiments he endeavored to determine the effect on the rate of photosynthesis of altering two factors. He argues that according to Blackman's view, only that factor which is limiting should affect the photosynthetic rate. The experimental results do not support this conclusion. A series of experiments with six different light intensities and four concentrations of potassium bicarbonate showed that an increase in either light intensity or carbon dioxide-concentration resulted in an augmented photosynthetic rate. The latter is therefore dependent upon both these factors under all circumstances. Harder's results are reproduced in Figures 10 and 11.

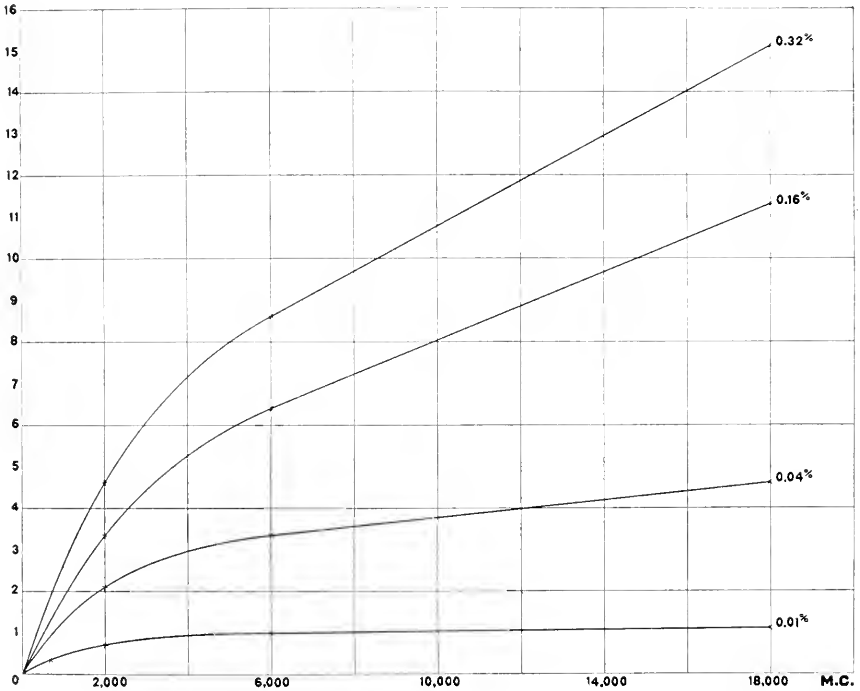

FIG. 10.—Effect of change in light intensity on the rate of photosynthesis of *Fontinalis* with different concentrations of carbon dioxide. (From Harder.)

From these curves it appears that with either factor held constant an increase in the other factor results in a rise in the rate of photosynthesis. The effect of the factor which is varied is not the same throughout the course of the curve but depends upon the intensity of the other factor. Harder therefore concludes that a variation in the intensity of that factor which is relatively to the greatest degree in minimum concentration or intensity produces the greatest change in the photosynthetic rate. This is a rather awkward statement. It is Harder's conception that the relation of the photosynthetic rate to the two factors, carbon dioxide-concentration and light intensity, is a complicated one and that these two factors are mutually interdependent. The augmenting effect of an increase of one of these factors is not the same at all concentrations or conditions of intensity of the other factor. In fact, the augmenting effect of the first factor will be higher the greater the intensity of the second factor. In other words, the higher the light intensity, the greater will be the augmenting effect of an increase in carbon dioxide-concentration and the higher the existing carbon dioxide-concentration, the greater will be the rise in the photosynthetic rate caused by an increase of the light intensity.

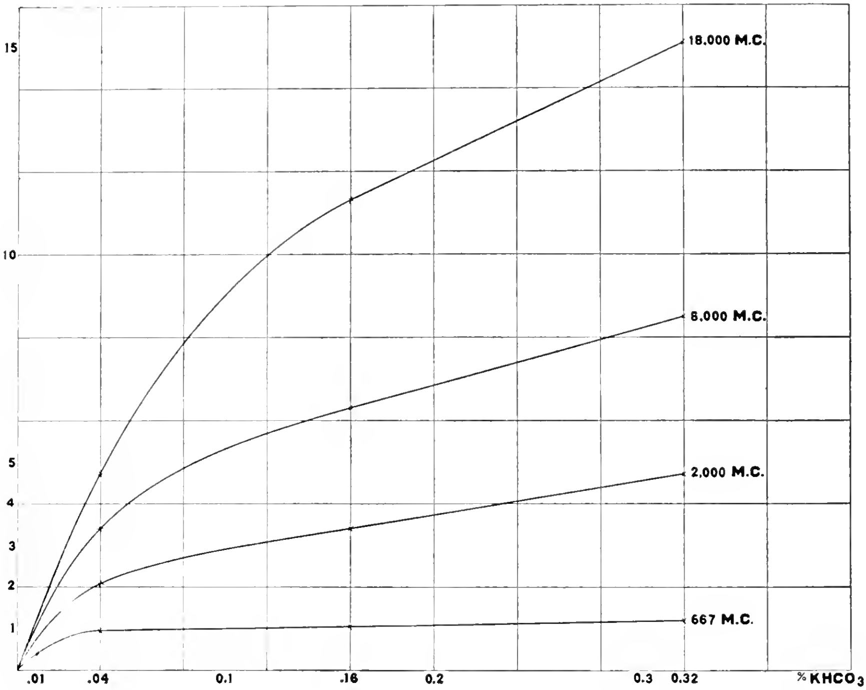

Fig. 11.—Effect of change in concentration of carbon dioxide on the rate of photosynthesis with different intensities of light. (From Harder.)

From what has already been said it is evident that in any endeavor to determine the effect of carbon dioxide-concentration on photosynthesis, in any quantitative sense whatsoever, it is essential that the other factors be accurately controlled. For this reason many of the older investigations, in which the influence of the other factors was not recognized or in which these are described in uncertain terms, can contribute but little.

The determination of the influence of varying concentrations of carbon dioxide on the photosynthetic activity of land plants is associated with many more difficulties than is the case with aquatic plants. These difficulties are largely due to the more complex structure of land plants; the opening and closing of the stomata are occasioned by water supply of the leaf, temperature, light and carbon dioxide-concentration and are of profound influence on the gaseous exchange of the leaf, and hence, of course, also on the rate of photosynthesis. It is therefore not surprising that some of the earlier work is difficult to interpret.

Brown and Escombe[113] found a direct proportionality between carbon dioxide-concentration and rate of photosynthesis. However, the number of experiments was small and there was some indication of pathological effects. They also reported an increase in the chlorophyll-content in plants intermittently exposed to higher concentrations of carbon dioxide. This has also been found to be the case by Lundegårdh. An increase in chlorophyll-content would of itself tend to increase the photosynthetic activity.

Lundegårdh,[114] working with leaves of land plants, has arrived at very similar conclusions to those of Warburg and Harder. Lundegårdh's curves are very much like those of Harder. In Table 21 are given the results of experiments on the photosynthetic rate with varying carbon dioxide-concentrations.

TABLE 21

Photosynthesis with Varying CO_2-Concentrations and Light Intensities $\frac{1}{40}$, $\frac{1}{20}$, and $\frac{1}{4}$ that of Direct Sunlight, in Terms of CO_2 Fixed.
(From Lundegårdh.)

Plant	CO_2 Concentration Per Cent	Photosynthesis per 50 Sq. cm. per Hour $\frac{1}{40}$ mg.	$\frac{1}{20}$ mg.	$\frac{1}{4}$ mg.
Oxalis acetosella	0.03	0.45	1.1	2.3
	0.06	0.9	2.3	3.7
	0.09	1.3	2.5	4.9
	0.12	1.6	2.7	6.3
	0.24	1.6	—	—
Stellaria memorum	0.03	0.7	1.0	1.5
	0.06	0.9	1.7	2.3
	0.09	1.0	2.4	3.0
	0.12	1.3	4.0	3.9
	0.24	1.9	—	5.3

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[113] Brown and Escombe, *Proc. Roy. Soc. London*, B **70**, 397 (1902). Kreusler, *Landw. Jahrb.*, **16**, 711 (1887); **17**, 161 (1888); **19**, 649 (1890). Giltay, E., *Ann. Jard. Buitenzorg.*, **15**, 43 (1898).

[114] Lundegårdh, H., *Svensk. bot. Tidsskrift.*, **15**, 59 (1921); *Biol. Centralbl.*, **42**, 337 (1922). "Der Kreislauf der Kohlensaeure in der Natur." Jena, 1924, p. 74.

Lundegårdh's results would indicate that with light intensity $\frac{1}{40}$ that of direct sunlight an increase in carbon dioxide-concentration above the normal (0.03 per cent) results in an increased photosynthetic rate. Also, with increasing carbon dioxide-concentration the augmentation of the photosynthetic rate is high at first and gradually becomes less. With higher light intensities, i.e., illumination approximating conditions existing in nature, the photosynthetic rates are raised considerably by an increase in carbon dioxide-concentration. In absolute measure these latter increases are high, though relatively they are not as high as with low illumination. This point is taken up under the discussion of the effect of light intensity.

From what has been said of the effect on photosynthetic activity of increasing the carbon dioxide concentration, it may be concluded that the normal carbon dioxide-concentration of 0.03 per cent in the atmosphere represents a condition in which this factor is ordinarily to be expressed as the "limiting one" in terms of the Blackman conception or as being "in minimum" in the terms of Harder and Lundegårdh. From the practical viewpoint of agriculture the question of photosynthetic rate is naturally a very important one and the problem of how to increase this rate has occupied plant physiologists for a long time, for on the rate of the photosynthetic process depends more or less directly the amount of the plant product, the crop. The question then arises, can an increase in crop yield be attained by raising the carbon dioxide-concentration in the atmosphere surrounding the plants?

The question has received much attention especially during the past few years. A full discussion of all the aspects and developments of the problem would reach beyond the purpose of this monograph. The problem has been widely discussed in Germany where flue gases from the blast furnaces have been used as a source of carbon dioxide.[115] Many technical problems have arisen which still require much study. It is becoming evident that increasing growth and dry material is not simply a matter of increasing the photosynthetic rate. There are questions of water relations, and migration of the material formed which play a very important rôle. Different species exhibit very different behavior toward air enriched in carbon dioxide. Finally, growth, the formation of new tissue, and the laying down of storage material, are in part only dependent upon the photosynthetic process. There are many other factors which also come into play. As a result of the neglect of due consideration of this interrelation of various factors in the development of plants, many contradictory observations have been made and erroneous conclusions drawn. This is probably inevitable when it is attempted to apply intricate

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[115] Reinau, "Kohlensäure und Pflanzen," Halle, 1920. Bornemann, "Kohlensäure und Pflanzenwachstum," Berlin, 1923. Classen, H., *Chem. Zeitg.*, **44**, 585 (1920). Cerighelli, R., *Ann. Sci. Agron.*, **38**, 68 (1921). Fischer, H., "Pflanzenbau und Kohlensaeure," Stuttgart, 1921. Wagner, H., Die Kohlensaeure als Wachstumsfaktor. Die Umschau., 27, 758 (1923). Lundegårdh, "Der Kreislauf der Kohlensaeure in der Natur.," Jena., 1924. Jess, *Jour. agr. pract.*, N. S. **35**, 250 (1921).

scientific principles to a large scale industrial undertaking. In some cases the experiments were carried out in glasshouses during the winter with no measurements of the intensity and duration of the light. Under these conditions it is not surprising that an atmosphere enriched in carbon dioxide produced only a very slight increase or even deleterious effects. Thus, for instance, Tjebbes and Uphof[116] who carried out experiments in greenhouses in December found that air enriched with carbon dioxide produced an effect only by the aid of electric light. Similarly, when total growth or crop yield are taken as a criterion, careful attention must be given the questions relating to soil, such as mineral nutrients, aeration, etc.

Among the first to study the effect of enriching the atmosphere with carbon dioxide was Demoussy.[117] He used glass chambers of about one cubic meter. The control plants were exposed to an atmosphere of normal carbon dioxide-content while others were in an atmosphere of 0.15-0.18 per cent. After two months the plants in the enriched atmosphere were cut and weighed; they were 122-262 per cent or an average of 157.6 per cent in excess of the control plants. The increased yields of the plants grown in the enriched atmosphere were not, however, directly proportional to the carbon dioxide-concentration; also different plants vary greatly in the increases shown.

TABLE 22

EFFECT OF INCREASED CARBON DIOXIDE-CONCENTRATION ON FRESH-WEIGHT YIELD.
(Results of Lundegårdh.)

	CO_2—House	Control House	Excess
Cucumbers—shoots	1.807 Kg.	0.890 Kg.	0.917 Kg. = 103%
—fruits	25.879 "	15.187 "	10.692 " = 74
Tomatoes—shoots	2.700 "	1.310 "	1.390 " = 124
—fruits	1.210 "	1.120 "	0.090 " = 8
Bean—fruits	7.080 "	3.343 "	3.737 " = 112
Total	38.676 "	21.850 "	16.826 " = 77%
Mean CO_2—content	0.065%	0.043%	0.22% = 51%

The recent results obtained by Lundegårdh[118] in Sweden give a good idea of the effect of carbon dioxide fertilization. The experiments were carried out in glasshouses, the carbon dioxide was continuously injected and the plants grew for ten weeks. Carbon dioxide determinations were made daily. In Table 22 are given some of Lundegårdh's results in terms of the fresh weight of the different plants. It would have been of greater value if the results had also been calculated on the basis of dry weight.

Similar experiments have been carried out on a larger scale in the open field.[119] Here the carbon dioxide, taken from cylinders or blast-

[116] Tjebbes and Uphof, *Landw. Jahrb.*, **56**, 313 (1921).
[117] Demoussy, *Compt. rend.*, **139**, 883 (1904).
[118] Lundegårdh, "Der Kreislauf der Kohlensäure in der Natur.," p. 127.
[119] Lundegårdh, l. c., p. 135. Bornemann, l. c., p. 85.

furnace exhausts, is piped in or on the ground. In general an increased yield has been obtained. But the results are naturally dependent upon climatic conditions and soil fertilization, and it is difficult to calculate any regular or reliable ratio between increased yield and the carbon dioxide factor.

High concentrations of carbon dioxide exert a narcotic effect on photosynthesis. In pure carbon dioxide photosynthesis quickly ceases. Different species exhibit a wide variation in the amount of carbon dioxide they can withstand.[120] Mosses and the lower plants are especially resistant to the effects of asphyxiating gases. It is highly probable that the effect of high concentrations of carbon dioxide is due to a toxic influence on the protoplasm. Higher concentrations of this gas, 15-25 per cent, retard and finally completely inhibit growth.[121] Darwin [122] has also found that in an atmosphere rich in carbon dioxide the stomata tend to close. The effect of high concentrations of carbon dioxide on photosynthesis is undoubtedly of a complex nature in which other functions of the plant play an important rôle.

d. The Influence of Temperature.

The wide range of temperature under which plants are found to thrive in nature indicates that the photosynthetic process can be carried on at these temperatures, or at least that the photosynthetic apparatus is not permanently injured at the extreme temperatures noted. Thus McGee [123] found that joints of a prickly pear (*Opuntia*) reach a temperature of 55° in the open without injury. On the other hand the marine algæ of the polar regions must be exposed to temperatures of about zero degrees for long periods; while Jumelle [124] reports that *Picea excelsa* still showed photosynthesis at — 35° and *Juniperus* at — 30 to — 40°, while *Physcira cilaris* and *Cladonia rangifernia* ceased at — 25° and *Primastri* at — 37°. Miss Henrici [125] found the threshold temperature of certain alpine phanerogams at — 16° and for some lichens at — 20°. Ewart [126] has shown that recovery of the capacity to do photosynthetic work after exposure to extreme temperatures depends very much upon the length of time of exposure. Similar results were obtained by Wurmser [127] and Jacquot who found that marine algæ, subjected to temperatures of 36 to 45° for from 1 to 15 minutes had a lower rate of photosynthesis when returned to the normal temperature of 16°, the de-

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[120] Ewart, *Journ. Linnéan Soc.*, **31**, 404 (1896).

[121] Chapin, *Flora.*, **91**, 348 (1902). Brown and Escombe, *Proc. Roy. Soc.*, **70** B, 397 (1902).

[122] Darwin, *Phil. Trans. Roy. Soc.*, **190**, 531 (1898).

[123] McGee, Carnegie Inst. of Washington, *Year Book*, **20**, 47 (1921).

[124] Jumelle, *Compt. rend.*, **112**, 1462 (1891).

[125] Henrici, *Verh. Natur. f. Ges. Basel.*, **32**, 107 (1921).

[126] Ewart, *Journ. Linnéan Soc.*, **31**, 368 (1896).

[127] Wurmser, *Bull. Soc. chim. Biol.*, **5**, 305 (1923).

gree of depression depending upon the temperature and length of time the plants were exposed to the higher temperatures.

The methods of studying photosynthesis in the field have not been perfected so that reliable results can be obtained from plants growing under natural conditions. As has been repeatedly emphasized the interaction of so many factors makes it impractical to determine all of these simultaneously, and even if this were possible, to attain a rational interpretation of the results of photosynthesis determinations under constantly varying conditions leads only to spurious results and false conclusions. So that, highly desirable as it undoubtedly is to obtain determinations of the influence of various external conditions, as existing in the field, on the photosynthetic activity of different plants, we are, nevertheless, confined to laboratory experimentation in order to attain results approaching scientific accuracy.

One of the most difficult problems in the determination of the influence of temperature on photosynthesis is the ascertaining of the temperature of the plant itself. In aquatic plants the problem is somewhat simplified because with plants of small volume their temperature during illumination probably does not differ greatly from that of the surrounding water. But with land plants the problem is much more difficult. An illuminated leaf absorbs radiant energy, only a small portion of which is converted into chemical energy; the greater proportion of the absorbed energy is converted into heat which results in the evaporation of water from the leaf, in alterations in the size of the stomatal openings and in other more obscure internal changes. In working with land plants it is therefore essential to know the internal temperature of the leaf and to obtain this, several methods have been employed.[128]

Brown and Escombe [129] recognized that a certain amount of the radiant energy absorbed by a leaf is dissipated through the evaporation of water and they endeavored to calculate this amount. The following physical and chemical changes taking place within the leaf are of influence on the thermal relations of the leaf and its environments: 1—the evaporation of water, 2—photosynthesis, 3—respiration. Of these 1 and 2 are endothermic while 3 is exothermic. The actual determination of each of these factors and the calculation of their energy relations is associated with considerable difficulty. It is, first of all, necessary to know the thermal emissivity of the leaf. This term includes the gain or loss of heat due to radiation, convection and conduction of a unit area of leaf in unit time with unit difference of temperature between the leaf and its surroundings. The simplest condition is that of a leaf in the dark in an atmosphere saturated with water-vapor. Owing to the oxidative processes taking place within the leaf there is a tendency of the leaf to rise in temperature. This thermal disturbance due to respiration is,

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[128] Shreve, Edith, *Plant World*, **22**, 100 (1919). Miller and Saunders, *Jour. Agric. Res.*, **24**, 15 (1923).

[129] Brown and Escombe, *Proc. Roy. Soc. London*, B **76**, 69 (1905).

however, exceedingly small. Brown and Escombe have calculated that the heat of respiration of a *Helianthus annuus* leaf is 0.000582 calory per square centimeter of leaf lamina per minute when the leaf is respiring 0.7 cc. CO_2 per sq. decimeter per hour. Brown and Wilson[130] have determined the thermal emissivity of such a leaf per square centimeter for still air and for a temperature excess in the leaf of 1° C. as being 0.015 callory per sq. cm. per minute. This amount must be doubled for the two sides of the leaf so that the rate of cooling becomes 0.030 calory per sq. cm. per minute for 1° excess. Thus, $\frac{0.000582}{0.030} = 0.019°$ will represent the maximal excess temperature which the leaf will attain above its surroundings under still-air conditions when there is no transpiration, and respiration remains constant at 0.7 cc. CO_2 per sq. decimeter per hour.

Such conditions are, of course, purely theoretical. Any rise of temperature within the leaf will increase the partial pressure of the water-vapor in the intercellular spaces of the leaf; this will diffuse from the leaf into the surrounding atmosphere and even this small theoretical excess temperature of 0.019° will not be reached. Moreover, in experimental work we can rarely deal with still-air conditions nor with fully saturated atmosphere. Also, the loss of heat due to transpiration is normally of a much greater magnitude than the energy liberated in respiration.

If the slight exothermic disturbance due to respiration is neglected, Brown and Escombe consider that: "the amount of water, Q, lost by unit-area of leaf surface in unit-time is a measure of the energy flowing into the leaf from its surroundings, and if we know the temperature difference between the leaf and its surroundings, i.e. the temperature gradient $\Theta - \Theta_n$ we can determine the rate of interchange of energy between the leaf and its surroundings in absolute units for a temperature difference of 1° C., that is to say, the coefficient of thermal emissivity." This method has been applied to leaves in still and moving air by Brown and Wilson.

Brown and Escombe regard that the following data are required to determine the thermal relations of a leaf to its surroundings when it is exposed to direct solar radiation:

1. The total amount of radiant energy incident on the leaf per unit time and area.
2. The amount of this energy absorbed by the leaf.
3. A measure of the internal work due to (a) evaporation of water, (b) photosynthesis.
4. The influence of air currents on the thermal emissivity of the leaf.

The first, 1, can be determined by direct measurement of the intensity of solar radiation; 2 is the absorption coefficient of the leaf. A measure

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[130] Brown and Wilson, *Proc. Roy. Soc.*, B **76**, 122 (1905).

of the evaporation of water, 3, is the water lost by transpiration per unit area and unit time Q; 592.6 calories is the latent heat of vaporization of one gram of water at 20°. The internal work from evaporation of water thus becomes 592.6 Q calories. The energy of photosynthesis can be calculated from the quantity of carbon dioxide absorbed. The heat of combustion of a hexose carbohydrate is 3760 calories per gram, and one cc. of carbon dioxide corresponds to 0.001336 grams hexose N.T.P., so that the conversion of one cc. of carbon dioxide into hexose sugar corresponds to the absorption of $0.001336 \times 3760 = 5.02$ calories. With the volume of carbon dioxide absorbed in photosynthesis per square centimeter per minute designated by c, the energy absorbed in photosynthesis is expressed by 5.02 c per square cm. per minute. Alterations in external conditions affect mainly the thermal emissivity of the leaf. Thus, the emissivity of a leaf in still air is doubled by an air current of 44.2 meters per minute. This and other influences which affect the emissivity of the leaf make Brown and Escombe's approach to this problem exceedingly complex and indicate how, under natural conditions, the thermal relations of a leaf with its surroundings must change from moment to moment. Brown and Escombe's method involves a number of determinations which are exceedingly difficult to make so that it has found little application in further investigations.

One example of Brown and Escombe's results will indicate the difference between the energy used in photosynthesis and that of transpiration:

Energy used for photosynthesis	0.66
" " " transpiration	48.39
Total energy expended in internal work	49.05
Solar energy transmitted by leaf	31.40
Energy loss by "thermal transmission"	19.55
	100.00

External conditions such as temperature and humidity greatly influence the results so that wide variations in the various items are obtained. We shall enter into these more fully in the consideration of the Energy Relations of Photosynthesis. We wish here to emphasize that the temperature of the leaf and its surroundings greatly affect the performance of the leaf as to its photosynthetic function. In plants well adapted for transpiration the superfluous or excessive radiant energy absorbed by the leaf can be readily dissipated through the evaporation of water. Transpiration rates of 500 to 1000 cc. of water per sq. meter per hour are not unusual; this would correspond to the dissipation of about 0.5 to 1.0 calory per sq. cm. per minute. With light intensity of about 1 calory per sq. cm. per minute incident on the leaf it can readily be seen that the plant may be kept at the temperature of the surroundings or absorb heat therefrom. In plants specially adapted to resist transpiration as in the succulents, thermal emission of absorbed radiant energy becomes highly important. Under natural conditions the problem becomes much more complex with the introduction of such factors as the angle of incidence of the light, etc.

It may here also be mentioned, that since the photosynthetic process is endothermic, it has been suggested that photosynthetic activity would reduce the transpiration from a leaf to a certain degree. A plant in an atmosphere freed of carbon dioxide and so incapable of photosynthesis, would thus show a higher rate of transpiration than when the plant was photosynthetically active. Claims of having observed such a phenomenon have been affirmed and denied from several sources.[131] In view of the fact that in land plants but a small proportion of the absorbed energy is converted into chemical energy, the "cooling effect" of photosynthesis would be relatively slight and it is possible that the phenomenon, when observed, was actually due to some other cause. In this connection the observation of Detlefsen [132] and of Puriewitsch [133] are of interest. Both found that the amount of radiant energy absorbed was greater when leaves were in an atmosphere containing carbon dioxide than in one free of this gas. The photosynthetically active leaf absorbs 1.7 to 11.7 per cent more energy than the leaf in a carbon dioxide free atmosphere.

Blackman and Matthaei [134] have made careful determinations of the internal temperature of leaves exposed to bright illumination. They used small thermocouples of copper and constantan connected with a galvanometer. One junction was inserted in the mid-rib of the leaf, and the other junction was placed in a water bath. The internal temperatures of the leaves were measured by bringing the temperature of the water bath to a point where the galvanometer deflection was zero. The temperature of the two couples was then the same and the temperature of the water bath was taken as that of the leaf. The leaves used for the determination of the photosynthetic rates were in a special frame submerged in a water bath. The temperature of the bath varied only slightly, while "the leaf temperature oscillated up and down with the varying fine shades of natural illumination through a range of 9°."

Miss Matthaei has made a study of the influence of temperature on photosynthesis by determining the amount of carbon dioxide absorbed. The smoothed curve which she constructed from a number of separate determinations is shown in Figure 12. This gives the rates of photosynthesis from — 6° to 43° under optimal conditions of light and carbon dioxide supply.

Miss Matthaei's work was carried out with the viewpoint of Black-

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[131] Déhérain, *Ann. sci. nat.* (VI), **4**, 177 (1876). Jumelle, *Rev. gén. bot.*, **1**, 37 (1889); **2**, 417 (1890); **3**, 241 (1891). Burgerstein, "Transpiration der Pflanzen," 46.

[132] Detlefsen, *Arb. bot. Inst. Würzburg.*, **3**, 534 (1888).

[133] Puriewitsch, *Jahr. wiss. Bot.*, **53**, 210 (1914).

[134] Blackman and Matthaei, *Proc. Roy. Soc. London*, B **76**, 402 (1905); *Phil. Trans. Roy. Soc. London*, B **197**, 75 (1904).

man's theory of limiting factors. She found that increasing the temperature produces an increase in the rate of photosynthesis only up to a certain point. Here some other factor, either light intensity or carbon dioxide-concentration determines the rate and a further increase in temperature has very little effect on photosynthesis. However, when there is an excess of light and carbon dioxide, it is apparent that the rate of photosynthesis increases rapidly with increasing temperature and it is concluded that "corresponding to each temperature there is a certain definite amount of assimilation which may be termed 'maximal assimilation' for that temperature. This cannot be exceeded and will not be reached unless both light and carbon dioxide supply are adequate."

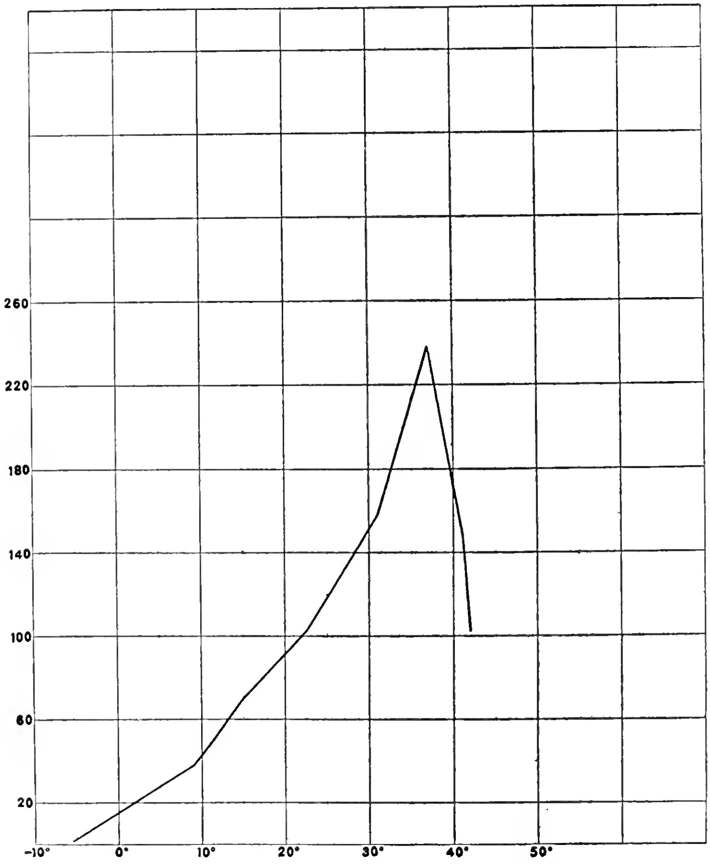

FIG. 12.—Influence of temperature on the rate of photosynthesis. (From Matthaei.)

Most of the older work on the influence of temperature on photosynthesis is of limited value either because the effect of other factors as light and carbon dioxide-concentration, were not fully recognized or on account of inadequate methods of measuring the temperature and the photosynthetic rate.[135]

A second, very important element of the effect of temperature on photosynthesis is that this is greatly influenced by time. At high temperatures a leaf can maintain a maximal rate of photosynthesis only for a short time. The higher the temperature the shorter is the time the maximal rate can be maintained and the steeper is the slope of its decline. As a consequence the rate of photosynthesis at higher temperatures is not the same during two successive hours and the maximum value

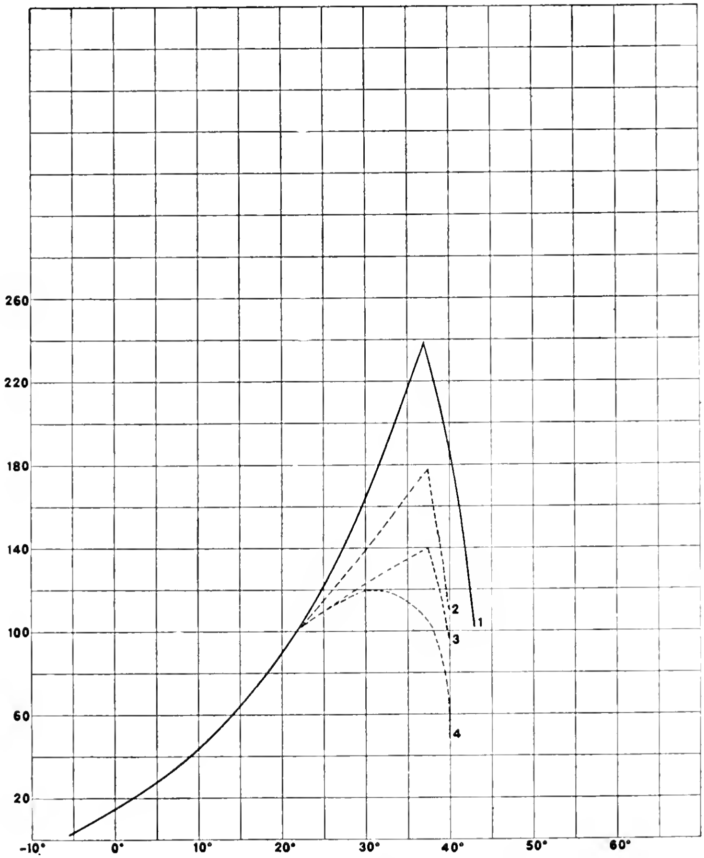

FIG. 13.—Influence of temperature and time on the rate of photosynthesis. (From Duclaux.)

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[135] Kreusler, U., *Landw. Jahrb.*, 16, 711 (1887).

observed will depend upon the time that has elapsed between the beginning of the experiment and the determination of the rate. This effect is known as the "time factor" and is discussed in more detail in a later section of this book.

In Figure 13 is shown schematically the manner in which the time factor affects the rate of photosynthesis. At lower temperatures the rate of photosynthesis is fairly constant for successive periods of time. Above about 24° there is a constant falling off of the rate with time. The exact temperature at which this begins very probably varies with different leaves. In Figure 13 the broken lines 2, 3, 4, indicate rate of photosynthesis after the second, third and fourth hours. The result thus is a shifting of the maximal rate to lower temperatures.

As a result of theoretical considerations developed by Blackman[136] and others the influence of time on the optimal rate of photosynthesis has been interpreted on the basis that there are two opposed reactions involved. This principle of superposition of two curves has been employed for a variety of reactions.[137] From Miss Matthaei's results it can be determined that the temperature coefficient, Q_{10}, for the rate of photosynthesis of leaves of the cherry laurel is 2.1 for temperatures between 5° and 25°. That is, between these temperatures the rate of photosynthesis follows closely what is frequently called the van't Hoff rule. Above and below these temperatures it does not follow this rule and, as has been observed in other reactions of living organisms, when the limits of temperatures are approached there is a great variation in the rates from that expected from the van't Hoff rule.

The type of curves obtained from a study of the effect of temperature on photosynthetic rate bears a strong similarity to the curves of the effect of temperature on the rate of catalysis of a number of different chemical reactions. The interpretation of these latter reactions have been used to explain the results obtained with photosynthesis. More particularly have the results of Tammann[138] and of Duclaux[139] on the effect of temperature on fermentation been taken as types of reactions which find application to the photosynthesis problem. These workers have shown that the relation of temperature to the rate of a fermentation reaction depends upon two different factors, the temperature and the concentration of the ferment. The latter are thermolabile substances, i.e., they are destroyed or inactivated at ordinary temperature and the rate of this destruction increases with temperature. As the temperature of the fermenting mixture is increased, the rate of the fermentation reaction is accelerated at the same time; however, the ferment is also being destroyed, as this cannot endure high temperatures for any length of time. The latter reaction reduces the concentration of the ferment which re-

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[136] Blackman, *Ann. of Bot.*, **19**, 282 (1905). Kanitz, "Temperatur und Lebensvorgänge," Berlin, 1915, p. 16. Jost, *Biol. Zentralb.*, **26**, 225 (1906).

[137] Bredig, G., *Ergeb. Physiol.*, **1**, I, 198 (1902).

[138] Tammann, *Zeits. physiol. chem.*, **16**, 317 (1892).

[139] Duclaux, "Traité de microbiologie," **2**, 193, Paris, 1899.

sults in the fermentation reaction attaining a maximal rate at a certain temperature but with a continuation of this temperature decreasing and finally falling to zero when all the ferment has been destroyed.

Duclaux has pictured the phenomenon as shown in Figure 14. In this the curve OA represents the rate of reaction with increasing temperature, DB is the rate of destruction of the ferment with temperature and OMC the observed rate of the fermentation reaction with increasing temperature. This general view has been applied to the observed maximal rates of photosynthesis at different temperatures. Thus OA would represent the rate of photosynthesis with increasing temperature, DB would be the inactivation of the chloroplasts due to higher tempera-

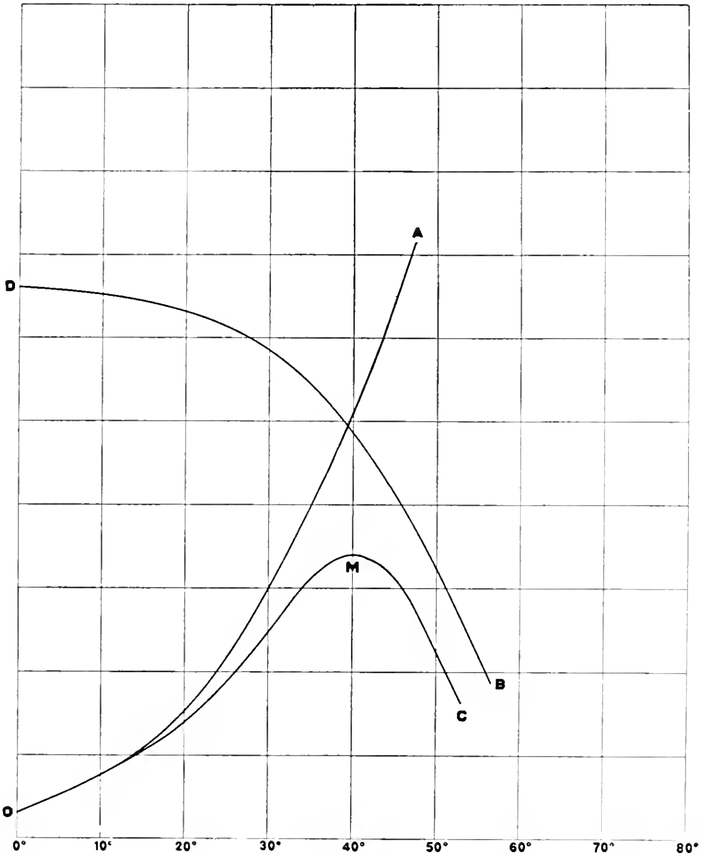

Fig. 14.—Influence of temperature and time on the rate of photosynthesis. (Blackman.)

ture and OMC the rate of photosynthesis at the different temperatures observed after longer periods of time.

This interpretation of the influence of temperature and time on the rate of photosynthesis can probably not be taken to picture the kinetics of the reaction in every detail. As a matter of fact we know very little about the inactivation of the chloroplasts, either as to the rate thereof or of the causes which bring this about. As will be shown later the activity of the chloroplasts can be diminished not only by high temperatures but as well by high light intensity and by the accumulation of the products of photosynthesis. The importance of this is emphasized by the fact that in order to obtain uniform results on the rate of photosynthesis, it is essential that the plants be subjected to the same treatment for some time previous to the experiment. Just what form the curve DB takes is therefore uncertain. The foregoing also illustrates how exceedingly difficult it is to obtain constants of biological reactions if such constants are to have even remotely the same meaning as our physical chemical values.

The temperature coefficient of photosynthesis has been determined by Warburg[140] over a range of temperatures. He used the unicellular alga *Chlorella* and his method had the advantage that it avoided the necessity of determining the internal temperature of a leaf, a procedure that is associated with considerable experimental difficulty and some uncertainty. In view of the fact that the temperature coefficient shows decided changes at different temperatures the method of indicating this for 10° C. is not strictly correct. For the sake of comparison, however, Warburg calculated his results on this basis. He found that when the concentration of carbon dioxide and light intensity are high the temperature coefficient is not constant. Thus he found the following temperature coefficients, calculated for Q_{10} as the indicated temperature differences:

TABLE 23

TEMPERATURE COEFFICIENTS OF PHOTOSYNTHESIS AT DIFFERENT TEMPERATURES. (Warburg.)

Temperature difference, degrees C.	5–10°	16–25°	5.4–10°	10–20°	20–30°	15–25°	25–32°
Q_{10}	4.7	2.0	4.3	2.1	1.6	1.06	1.
Relative light intensity	16	16	45	45	45	1.8	1.

It is apparent from this that the temperature coefficient decreases with increasing temperature. When the light intensity is low the temperature coefficient is about unity. The latter is in agreement with Blackman's results. Osterhout and Haas[141] working with *Ulva rigida* found that the temperature coefficient of photosynthesis between 17° and 27° is 1.81.

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[140] Warburg, *Biochem. Zeit.*, **100**, 258 (1919).

[141] Osterhout and Haas, *Jour. Gen. Physiol.*, 1, 295 (1919). van Amstel, *Rev. trav. bot. Néerlandais,* **13**, 1 (1917).

The fact that the temperature coefficient varies with temperature has been employed to substantiate the view held by a number of investigators that the photosynthetic process involves two reactions (Willstätter and Stoll, Warburg, Osterhout and Haas and others). These various hypotheses differ somewhat in detail, but the facts upon which they are based are about as follows. At high illumination intensity a rise of ten degrees, from 15° to 25°, causes the velocity of photosynthesis to double. Here a chemical reaction is evidently determining the rate of the process; this reaction Warburg has designated as the "Blackman reaction." At low illumination intensity the rate of photosynthesis is independent of the temperature between 15° and 25°. This is what could be expected if under these conditions a photochemical reaction, as, for instance, the photolysis of a chlorophyll-carbonic acid complex were determining the velocity. The assumption here is that all photochemical reactions have low temperature coefficients. The photosynthetic process is made up of two reactions, one an ordinary chemical reaction, which determines the rate of the process at high illumination intensities and has a high temperature coefficient, secondly a photochemical reaction which determines the rate of the process at low illumination intensities and has a low temperature coefficient. These theories are taken up in greater detail under the discussion of the chemistry of the photosynthetic process. There is no satisfactory explanation of the high temperature coefficients at low temperatures. It should be stated, however, that high values of Q_{10} at low temperatures are of very common occurrence in biological processes.[142]

A great deal more experimental work is required in order to elucidate fully the relation between these two steps in the photosynthetic process, the "dark" and the "light" reaction. In fact it cannot be accepted as established that two such reactions actually exist or that their assumption will prove adequate to explain what still appears to be an exceedingly complex system. That the temperature coefficient varies with different temperatures and with other external conditions there is little doubt. Many of these facts have been brought to accord with the general principles first outlined by Blackman's theory of limiting factors. This theory and its modifications, however, tell us little of the kinetics of the photosynthetic process; they are the first attempts at an accurate expression of the relation of this process to the various factors which influence it. In a sense this mode of attack is analogous to the thermodynamic treatment of chemical reactions, though we are not in possession of the exact data which such a method requires. But the thermodynamic treatment is largely independent of any molecular hypothesis we may have formed regarding any process. In photosynthesis we are constantly endeavoring to determine the nature of the molecular changes involved and to this end data are frequently applied which have but very indirect

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[142] Kanitz, A., "Temperatur und Lebensvorgänge," Berlin, 1915, p. 27.

bearing. As a consequence much controversy and confusion has arisen which could have been avoided if the points at issue had been kept more clearly in mind.[143]

Willstätter and Stoll [144] observed that there was considerable difference in the influence of temperature on the rate of photosynthesis between leaves of high and low chlorophyll-content. The leaves of low chlorophyll-content exhibit a lower acceleration with increasing temperature than do the leaves of high chlorophyll-content. Thus leaves of *Ulmus* with low chlorophyll-content showed a temperature coefficient of 1.34 and with high chlorophyll-content of 1.53 for 15° to 25°. Willstätter and Stoll call attention to the fact that in these experiments an increase in illumination intensity was without effect on the photosynthesis of leaves rich in chlorophyll; in fact the light intensity could be reduced by ⅜ without affecting the rate. They explain this on the ground that in these leaves the chlorophyll-content was relatively of much higher concentration than the enzyme which they consider plays an important rôle in the photosynthetic process. This enzyme is a protoplasmic (internal) factor and induces a "dark" reaction. On the other hand, in the leaves low in chlorophyll, which have a low Q_{10}, the enzyme ("dark reaction") is relatively in excess and chlorophyll is relatively in minimum concentration. The rate of the reaction is here determined by the chlorophyll-content and an increase or decrease in light intensity is of decided influence on the rate of photosynthesis. These observations are quite in harmony with the ideas expressed above, of the influence of those factors which are relatively in minimum concentration. Willstätter and Stoll's experiments are particularly valuable, because of their careful control of the chlorophyll factor and their realization of the importance of the internal factor. The nature of this internal factor they regard as being enzymatic. While it must be admitted that virtually nothing is known of this "enzyme," nevertheless the internal factor is doubtless a protoplasmic activity and in so far as we largely depend upon the conception of enzyme activity to account for protoplasmic activity the term seems permissible. Moreover, the effect of temperature on this factor corresponds to that which has been observed for enzymatic reactions. Thus Willstätter and Stoll point out that both types of leaves, those high and low in chlorophyll-content, are equally equipped with the enzymatic factor. Temperature exercises an accelerating influence on the activity of this internal factor. However, this accelerating influence will result in a higher photosynthetic rate only in those leaves which have a high chlorophyll-content, that is, in those leaves which can make use of high intensities of light. In the leaves low in chlorophyll-content the accelerating influence of increased temperatures on the internal factor will not result in a higher rate of

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[143] Brown and Heise, *Philippine Jour. of Sci.*, **12**, **1**, 85 (1917). Brown, *ibid.*, **13**, 345 (1918). Smith, *Ann. Bot.*, **33**, 517 (1919).

[144] Willstätter and Stoll, "Untersuchungen ü. d. Kohlensäureassimilation," Berlin, 1918, pp. 56, 112, 143.

photosynthesis, because the rate here is determined by the chlorophyll-content, namely, the amount of radiant energy which the leaf is capable of utilizing.

On this basis the difference in the temperature coefficients of the two types of leaves becomes intelligible. With the leaves low in chlorophyll-content, a light reaction was determining the rate, resulting in a low Q_{10}. In the other case, the leaves of high chlorophyll-content, this factor was relatively less in minimum concentration, and the influence of temperature on the enzymatic reaction was more clearly expressed. Willstätter and Stoll also found that the temperature coefficient of photosynthesis was lower at higher temperatures.

It is not surprising that the temperature coefficient of photosynthesis should vary from that of simple chemical reactions. The rate of photosynthesis is influenced by a number of factors. As an example of this we may mention the lower solubility of carbon dioxide at higher temperatures which may result in a lessened supply of this gas in the chloroplasts.

An interesting phenomenon discovered by Miss Henrici and described in the section of the Time Factor is related to the effect of starch formation on the photosynthetic rate. The formation of starch in the leaf is greatly affected by temperature. The presence of starch in the chloroplast also is of influence on the rate of photosynthesis. Temperature can, therefore, influence the rate of photosynthesis in this rather indirect manner of affecting the starch formation in the chloroplasts. The threshold of photosynthesis in alpine shade plants and lichens is considerably below 0°. This is probably due to their low freezing point arising from the high osmotic pressure of the cell sap which contains no starch but much soluble sugar. Even when ice crystals appear in the interior of the cells photosynthesis still occurs; this is especially true of the lichens.

e. Chlorophyll.

That chlorophyll is of paramount importance in the photosynthetic process was concluded by the first investigators of the phenomenon. Only those portions of plants containing chlorophyll are capable of reducing carbon dioxide to carbohydrates. Frequently plants are colored red or brown by an admixture of other pigments, but these usually also contain chlorophyll. There are lower organisms capable of reducing carbon dioxide, certain bacteria which do not contain chlorophyll, but the reactions here are very different from those taking place in the chlorophyllous plants. Some of these bacteria do not require light as a source of energy for the carbon dioxide reduction, but are apparently capable of accomplishing the reduction by means of chemical energy as, for example, the oxidation of hydrogen or ammonia. In those plants which use light as a source of energy for the photosynthetic reaction, chlorophyll is an essential component. There exists, however, some difference of opinion as to whether all plant parts containing chloroplasts exhibit the capacity for photosynthesis. In view of the fact that frequently the chloroplasts are rendered inactive by injurious external agencies, it is difficult to establish this point experimentally.[145]

While, then, it is generally accepted that chlorophyll is essential for photosynthesis, the quantitative determination of the rôle of this factor is exceedingly difficult, because it is impossible to vary experimentally the concentration of the chlorophyll in the plants under investigation. In considering chlorophyll alone as a factor in photosynthesis, it is well to bear in mind that this is done only for the sake of analysis; photosynthesis can take place only when the chlorophyll is associated with the living plasma in the chloroplast. We shall discuss, then, the effect on photosynthesis of plants containing varying amounts of chlorophyll, bearing in mind always that chlorophyll is but one part of a complex mechanism to the successful functioning of which other parts are essential.

On account of the impossibility of varying the concentration of chlorophyll experimentally in any one plant, the method which has been followed most generally has been to compare leaves of the same species but of different chlorophyll-content, as for example, normal varieties, rich in chlorophyll, with the yellow varieties, poor in chlorophyll. While this is probably the best method of approach now available to the problem of the influence of variation in chlorophyll-content on photosynthesis, it is not altogether satisfactory. Two varieties of the same species of a plant may differ in chlorophyll-content according to differences in certain environmental conditions, e.g., light intensity and soil, or this difference in chlorophyll-content may arise from more deep-seated hereditary causes. The question arises whether associated with the differences in chlorophyll-content there may not be other differences which also affect photosynthesis. Chlorophyll is itself a product of the metabolism of the plant; for its formation, in most plants, light is essential as is also oxygen. But the respiratory and metabolic activity of the plant is of considerable significance in the photosynthetic activity thereof. It is, therefore, not entirely improbable that the factors which produce differences in chlorophyll-content affect other portions of the photosynthetic mechanism. Thus the differences in photosynthetic activity which in some cases have been ascribed to differences in chlorophyll-content may actually be due to a complex of factors.

Lubimenko[146] found that the light requirements of "shade" plants for photosynthesis is considerably lower than that of "light" plants, i.e. the former type of plant can accomplish the same amount of photosynthetic work with a lower illumination intensity than the latter type. This fact

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[145] Dehnecke, Dissertation, Köln (1880). Friedel, *Compt. rend.*, **142**, 1092 (1906). Ewart, *Journ. Linnéan Soc.*, **31**, 436 (1896).

[146] Lubimenko, *Rev. gen. Bot.*, **17**, 381 (1905); **20**, 162, 217, 253, 285 (1908); *Ann. Sci. Nat.* (9), **7**, 321 (1908); *Compt. rend.*, **145**, 1347 (1907). Griffon, *Ann. Sci. Nat.* (8), **10**, 1 (1899).

Lubimenko endeavored to correlate with differences in chlorophyll-content, for he found that "shade" plants contain more chlorophyll than the "light" plants. The "shade" plants show an optimal light intensity for photosynthesis; if the light intensity is increased above this optimal intensity the rate of photosynthesis decreases. This, it is assumed, is due to the fact that the "shade" plants have a high chlorophyll-content and consequently a high absorption coefficient. On the other hand, "light" plants, with a low chlorophyll-content show maximum photosynthesis at the highest light intensities. This can be interpreted, that in "light" plants the amount of light absorbed when illuminated by highest light intensities, is not sufficient to produce an inhibition in the activity of the chloroplasts. The optimum temperature and optimum light intensity for photosynthesis of a plant decreases with increasing chlorophyll-content according to this view. In the course of its development a plant can regulate the amount of light it absorbs by changes in its chlorophyll-content. It is, however, not only the light intensity which determines the chlorophyll-content, but also temperature. With increasing temperatures the intensity of light required for maximal chlorophyll formation decreases.

Plester[147] studied the relation of carbohydrate formation (by means of the half leaf method) to the chlorophyll-content of the light green or yellow and normal varieties of a number of plants. He found that the light green varieties have a lower chlorophyll-content than the normal varieties per equal area of leaf surface; the former ranged from about 30 to 50 per cent of the latter. From Plester's results it cannot be concluded that there is any direct ratio between the rate of photosynthesis and chlorophyll-content. The light green varieties showed a lower rate of photosynthesis than the normal varieties, ranging from 34.2 to 59.5 per cent. On the other hand in some cases the light green or yellow varieties showed a much higher rate of photosynthesis than would correspond to their chlorophyll-content. This points to the fact that other factors besides chlorophyll-content are playing a rôle. Also, the light green varieties had a lower rate of respiration than the normal plants, though there was no direct parallelism between respiration and chlorophyll-content. A closer relationship seems to exist between the rate of photosynthesis and that of respiration. Hence, to a measure at least, the lower rate of photosynthesis in the light green varieties is compensated for by a lower rate of carbohydrate consumption. It is, however, still a question whether the lower rate of respiration is simply the result of a lower supply of carbohydrates, or whether the other conditions or factors which produce a higher rate of respiration (protoplasmic factors, enzymes) do not also affect the rate of photosynthesis.

Plester also found, contrary to Willstätter and Stoll (see below) that plants which contain besides chlorophyll admixtures of red pigments, had a lower rate of photosynthesis than the same species without these pig-

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[147] Plester, *Beitr. Biol. d. Pflanzen*, 11, 249 (1912).

ments. But in this case also, those plants that had a lower rate of photosynthesis also had a lower rate of respiration. Undoubtedly, however, in these cases questions of light intensity and absorption coefficient enter which make the interpretation of the results very difficult.

The results of Henrici,[148] who made a study of the effect of altitude on photosynthesis and chlorophyll-content of the various plants, are also of interest in this relation. The photosynthetic activity and chlorophyll-content was compared of *Anthyllis vulneraria, Bellis perenis, Primula farinosa,* and *Taraxacum officinale,* grown at different altitudes, the low land material at Basel at 450 m., the alpine material at 1400 to 2700 m. Differences in the structure of leaves, particularly of the chlorophyll bearing portions, with changes in altitude have been observed repeatedly.[149] Henrici found that the plants grown at high altitudes contain less chlorophyll than those of the same species from lower altitudes. This is presumably due to the greater intensity of light at higher altitudes. However, whether the lower chlorophyll-content of plants grown under high illumination intensity can be directly ascribed to the destructive action of such light (especially the red-yellow rays) on chlorophyll, does not seem entirely established.[150] There is no variation in the chlorophyll-content during the course of the day nor do plants taken from one altitude to another change in this respect within two weeks. The photosynthetic relations of the plants grown at different altitudes is more complex than would be expected from simply a difference in the chlorophyll-content and is a function of light and temperature. While photosynthesis in the alpine plants (low chlorophyll-content) commences at higher light intensity than the low altitude plants (high chlorophyll-content), the temperature minimum of the former is much lower than the latter. With high light intensity, photosynthesis of the alpine plants is higher than that of the low land plants at any temperature. With low light intensity the photosynthesis of the alpine plants is higher than that of the low land plants only at low temperatures. At higher temperatures, more light is required in order that the photosynthesis of the alpine plants equal that of the low land plants. These results are in agreement with the findings of Willstätter and Stoll discussed below. The alpine plants can endure a wide range of temperature, and, with their low chlorophyll-content, also high illumination intensities. These plants are apparently well adapted to the conditions of high altitudes, though the conditions of such an adaptation lie not only in a single factor such as the chlorophyll-content, but as well in other factors as the temperature relations of the plants. Henrici's experiments show marked specific differences in the adaptation of the various species of plants used. Her work also serves to demonstrate the great difficulty, if not the impossibility, of obtaining

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[148] Henrici, Dissertation, Basel, 1918.

[149] Bonnier, G., *Ann. Sci. nat. Bot.* (7), **20**, 247 (1895).

[150] Dangerard, P. H., *Compt. rend.,* **151**, 1386 (1910). Ewart, *Jour. Linnéan Soc.*, 31, 436 (1896).

a quantitative analysis of the influence of the various factors affecting photosynthesis under field conditions.

A great advance in the knowledge of the chlorophyll factor in photosynthesis is due to the researches of Willstätter and Stoll.[151] These workers, on the basis of their thorough investigations of the chemistry of chlorophyll and other plant pigments devised reliable quantitative methods for the determination of chlorophyll, a contribution of the first importance toward the solution of these problems. A description of these methods will be found in Chapter 7 of this book.

According to Willstätter and Stoll chlorophyll is composed of two components designated as *a* and *b*:

$$\text{Chlorophyll } a\text{: } [MgN_4C_{32}H_{30}O\ ]CO_2CH_3 \,.\, CO_2C_{20}H_{39}$$
$$\text{Chlorophyll } b\text{: } [MgN_4C_{32}H_{28}O_2]CO_2CH_3 \,.\, CO_2C_{20}H_{39}.$$

Accompanying the chlorophyll pigments in the chloroplasts there are two yellow pigments, carotinoid *c* and *x*:

Carotin: $C_{40}H_{56}$, Xanthophyll: $C_{40}H_{56}O_2$.

The chlorophyll-content of leaves ranges as follows:

0.15-0.35 gram per 100 grams fresh weight.
0.6-1.2 gram per 100 grams dry weight.
0.3-0.7 gram per 1 sq. m. leaf surface.

The yellow pigments range from 0.07 to 0.20 per cent of the dry weight or about 0.0 to 0.07 gram per sq. m. of leaf surface. The ratio of the two yellow components, $Q\,\frac{c}{x}$, is fairly constant, equal on an average to 0.60. The ratio of the chlorophyll components, $Q\,\frac{a}{b}$, also varies but slightly and is equal to 2.9.

In this work of Willstätter and Stoll there are two points of particular importance bearing on the question of the relation of chlorophyll-content to photosynthetic rate. These are: 1, that the chlorophyll-content does not change during the course of photosynthesis, and 2, that the ratio of chlorophyll *a* and *b* also remains nearly constant during photosynthesis.

The second point is about as significant as the first, for in most of the subsequent analyses the two components are determined together. If during photosynthesis the ratio of the two components showed a great variation, it would not be permissible to compare mixtures composed of the two. Willstätter and Stoll studied the relation of photosynthesis to chlorophyll-content of leaves in different stages of development and in leaves which exhibit rather extreme conditions such as yellow varieties,

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[151] Willstätter and Stoll, "Untersuchungen über die Assimilation der Kohlensäure," Berlin, 1918.

etiolated and chlorotic plants. The conditions under which the experiments were carried out were such that neither temperature, carbon dioxide-concentration nor light intensity were determining the rate of photosynthesis. This is, of course, necessary in order to emphasize or demonstrate the influence of other factors, in the cases studied, more especially chlorophyll-content. In most of the experiments excised leaves were used.

Willstätter and Stoll introduced as a measure of the ratio of chlorophyll-content to rate of photosynthesis the term "Assimilationszahl" or photosynthetic number. This is a measure of maximal photosynthesis expressed in grams of CO_2 absorbed per hour by a leaf mass containing 1 gram of chlorophyll under favorable conditions of temperature. Or briefly it is grams of carbon dioxide absorbed per hour per gram of chlorophyll; this is the photosynthetic number which for the sake of brevity we shall designate by P_c.

$$P_c = \frac{\text{hourly photosynthesis, grams } CO_2}{\text{grams chlorophyll.}}$$

This term expresses the highest possible rate of photosynthesis under the particular conditions of temperature and carbon dioxide-concentra-

TABLE 24

PHOTOSYNTHETIC NUMBER, P_c, OF VARIOUS PLANTS, 5 PER CENT CO_2, ABOUT 48,000 LUX, DETERMINED BY WILLSTÄTTER AND STOLL.

		From 10 g. Fresh Leaves:		Photosynthesis: gram CO_2 per Hour, per 1 g. Dry Weight	
Species	Temp.	Dry Weight Grams	Chlorophyll Mg.		P_c
Aesculus Hippocastanum	25°	2.94	24.7	0.054	6.4
Acer Negundo	25°	2.22	24.8	0.086	7.7
Acer Pseudoplatanus	25°	3.58	40.0	0.058	5.2
Ampelopsis quinquefolia	25°	2.00	28.8	0.089	6.2
Hostia plantaginea	30°	1.00	10.0	0.088	8.8
Hydrangia opulodes	30°	1.21	9.2	0.050	6.5
Pelargonium zonale	30°	0.96	12.5	0.097	7.4
Primula	30°	0.90	11.4	0.117	9.1
Prunus Laurocerasus	30°	3.40	12.2	0.029	8.1
Rubus	25°	3.60	32.4	0.052	5.8
Sambuscus nigra	25°	2.75	22.2	0.034	6.6
Tilia cordata	25°	3.19	28.1	0.028	6.6
Ulmus	25°	2.94	16.2	0.022	6.9
Cucurbita Pepo	25°	1.3	17.5	0.164	12.1
Clerodendron trichotomum Thumb	25°	2.07	15.0	0.089	12.3
Fragaria vesca	25°	3.00	17.7	0.078	10.6
Helianthus annuus	25°	1.72	16.5	0.134	14.0
" "	25°	1.94	15.0	0.129	16.7
" "	25°	1.67	20.8	0.137	10.9
Populus pyramidalis hort.	25°	3.19	19.0	0.060	10.0
Pelargonium peltatum (from green-house)	30°	.60	8.2	0.198	14.5

tion. Willstätter and Stoll in discussing the theoretical value of P_c emphasize the temperature factor; but it seems essential to define the carbon dioxide-concentration, for different plants show specific differences in the concentration of carbon dioxide they can utilize. In Willstätter and Stoll's experiments some of the leaves which had a very low chlorophyll-content did not attain maximum photosynthesis because the light intensity was not sufficiently high.

In Table 24 are given the values of P_c for normal leaves, that is, excluding those low in chlorophyll, young and old leaves. Approximately constant values of P_c for a given temperature would indicate that under these conditions the rate of photosynthesis was determined by the chlorophyll-content.

The values of P_c in the first section of Table 24 shows a fair constancy, for 25° ranging from 5.2 to 7.7, for 30° from 6.5 to 9.1. In these cases there is a rough parallelism between the rate of photosynthesis and chlorophyll-content. In the lower section of Table 24 are given the values of P_c for plants which have a notably rapid growth and high photosynthetic rate. In these P_c is considerably higher. It is already evident from these values that there is no simple quantitative ratio between chlorophyll-content and rate of photosynthesis.

In this connection it has been an interesting question as to what are the relations in very young leaves between the rate of photosynthesis, chlorophyll formation and respiration. The latter activity is notably high and decreases with time and the development of the leaf.[152] Under favorable conditions of temperature and light the development of chlorophyll is quite rapid. Thus, for example, Willstätter and Stoll observed increases in chlorophyll-content, at 25° and 48,000 Lux, in 80 minutes as follows: *Tilia cordata*, 32 per cent of the original, *Acer negundo*, 29 per cent, *Quercus Robur*, 22 per cent, *Populus pyramidalis hort.*, 15 per cent, *Acer Pseudoplatanus*, 10 per cent. With this increase in chlorophyll-content there is also an increase in photosynthesis. There is, however, not a direct proportionality between chlorophyll-content and photosynthetic rate. The latter, 8 to 10 days after the first determination, made when the leaves were just unfolding, show about a constant value for equal areas but a decreased value on the basis of dry weight. On the basis of chlorophyll-content after about nine days there is a decrease in photosynthesis, that is, P_c decreases. These relations are given in Table 25 which show an increase in P_c after about nine days and thereafter again a decrease. The table is taken from the results of Willstätter and Stoll.

A perusal of Table 25 will show an increase with time of the dry weight of the leaves; on the basis of the dry weight there is a decrease in photosynthesis. The leaves also show a consistent increase in chlorophyll content, but this is not associated with an increase in photosynthesis after the first nine days. This results in a considerable variation of the P_c.

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[152] Willstätter and Stoll, l. c., p. 87.

These facts point to the existence of some other internal factor and give weight to the theory that the rate of photosynthesis, under constant and favorable external conditions is determined not only by the chlorophyll-content but also by another internal factor. The latter apparently is more rapid in development than chlorophyll, resulting in a high P_c during the first days of the life of the leaf. After full development of the leaf and increase in the chlorophyll-content this other factor is relatively less active than the chlorophyll factor.

TABLE 25

RATE OF PHOTOSYNTHESIS, CHLOROPHYLL-CONTENT AND PHOTOSYNTHETIC NUMBER, AT 25°, 5 PER CENT CO_2, ABOUT 48,000 LUX, DETERMINED BY WILLSTÄTTER AND STOLL.

		From 10 g. Fresh Leaves:		Photosynthesis: g. CO_2 per Hour		
Date	Species	Dry Weight grams	Chlorophyll mg.	Per 1 g. Dry Weight	Per 1 sq. dm.	P_c
Apr. 29	Aesculus Hippocastanum	2.10	10.1	0.054	0.043	11.1
May 7		2.06	15.1	0.088	0.039	12.1
June 3		2.94	24.7	0.054	0.033	6.4
Oct. 8		3.62	31.2	0.041	0.044	4.8
May 1	Sambuscus nigra	1.85	11.7	0.078	0.046	12.2
May 8		2.25	23.1	0.101	0.057	9.8
July 14		2.56	23.5	0.057	0.032	6.2
May 4	Tilia cordata	2.18	8.3	0.040	0.015	10.6
May 12		2.15	11.5	0.085	0.024	16.0
June 5		3.52	28.8	0.058	0.029	7.1
June 25		3.19	28.1	0.058	0.028	6.6
May 5	Acer Pseudoplatanus	2.60	10.7	0.035	0.017	8.6
May 14		2.55	18.7	0.078	0.022	10.7
June 24		3.58	40.0	0.058	0.026	5.2
May 10	Ampelopsis quinquefolia	1.84	7.4	0.042	0.018	10.5
May 19		1.98	15.4	0.105	0.038	13.5
June 8		2.00	28.8	0.089	0.028	6.2
May 11	Quercus Robur	2.76	6.6	0.026	0.013	10.9
May 20		2.64	8.6	0.051	0.024	15.8
June 9		4.14	21.6	0.047	0.038	9.0
June 20		4.50	25.0	0.044	0.041	7.8

Very similar results were obtained by Willstätter and Stoll in a study of the photosynthetic activity of leaves of different ages. They compared the activity of a light-green leaf from the end of a branch with that of a dark-green leaf from the base of the same branch. Some examples of the differences between old and young (but almost full grown) leaves are given in Table 26.

The results of Willstätter and Stoll of the photosynthetic activity of leaves in different stages of development show that although the chlorophyll-content of the leaves increases with age and that the photosynthetic activity also increases, the two are by no means parallel.

In autumn, with the change in color of the leaves, the conditions become very complex. In general there is a decrease in photosynthesis (g. CO_2 per hour) when this is calculated on the basis of leaf area, dry or fresh weight. With the yellowing of the leaves there is also a decrease in the chlorophyll-content. Under conditions of maximal photosynthesis (25°, high CO_2 and light intensity) the activity of the leaves turning yellow, on the basis of chlorophyll-content (i.e. the P_c) is not very different from the normal leaves. This is due to the fact that synchronous with the decrease in chlorophyll there is a decrease in the activity of the protoplasmic factor. As a consequence the P_c also decreases with the yellowing of the leaves. In some cases the two factors, chlorophyll-content and protoplasmic activity, do not decrease at the same rate so that it may occur that the P_c of autumnal leaves is higher than in midsummer. Willstätter and Stoll observed all possible variations in the P_c with the yellowing of the leaves, rising, constant and falling values. Such values, it must be recalled, represent the photosynthetic activity on the basis of chlorophyll-content. In absolute terms, the amount of carbon dioxide reduced in leaves turning yellow is a tenth or less than that of normal leaves.

TABLE 26

PHOTOSYNTHESIS OF LEAVES FROM THE SAME PLANT BUT IN DIFFERENT STAGES OF DEVELOPMENT, AT 25°, 5 PER CENT CO_2, ABOUT 48,000 LUX, FROM THE DETERMINATIONS OF WILLSTÄTTER AND STOLL.

			10 g. Fresh Leaves:		Photosynthesis: g. CO_2 per Hour:		
Species	Date	Description of Leaves	Dry Weight grams	Chlorophyll mg.	Per 1 g. Dry Weight	Per 1 sq. dm. Leaf Area	P_c
Acer pseudoplatanus	June 23	4th-6th leaf from end of branch	3.3	8.3	0.030	0.016	11.8
	" 24	From base of branch ..	3.58	40.0	0.058	0.026	5.2
Tilia cordata	June 25	Young, light-green	2.56	6.5	0.036	0.018	14.2
	" 26	Lower dark-green from same branch	3.19	28.1	0.058	0.028	6.6
Laurus nobilis	June 30	Light-green leaves	3.10	12.7	0.024	0.019	5.9
	July 1	Dark-green leaves of previous year	4.95	21.2	0.016	0.023	3.7

We have already mentioned experiments in which the photosynthesis of normal and yellow varieties of various plants was compared (Plester). These demonstrated that there was no direct proportionality between chlorophyll-content and rate of photosynthesis. Willstätter and Stoll made similar experiments using 5 per cent carbon dioxide and high light intensity. The yellow varieties of the plants used by them contained 3 to 15 per cent, and even less, of the chlorophyll of the normal varieties. It was found that on the basis of chlorophyll-content the yellow varieties show a much greater photosynthetic activity than the normal ones. The P_c of the former was in some cases almost 20 times that of the latter ranging from 6 to 12 for the normal varieties against 50 to 120 for the yellow varieties.

TABLE 27

Photosynthesis of the Green and Yellow Varieties of Elm, 5 Per Cent CO_2, About 24,000 Lux. (Willstätter and Stoll.)

						Photosynthesis: g. CO_2 per Hour:	
Variety	Temp.	Wt. of Leaves grams	Dry Weight	Leaf Surface sq. cm.	Chlorophyll mg.	8 g. Fresh Leaves	1 sq. m. Surface
Chlorophyll, poor ..	25°	8.0	2.00	321	0.95	0.075	2.3
" " ..	15°	Same leaves	2.00	321	0.95	0.056	1.7
Chlorophyll, rich ..	25°	8.0	2.35	421	13.0	0.089	2.1
" " ..	15°	Same leaves	2.35	421	13.0	0.058	1.4

A most striking case of the disproportionality between chlorophyll-content and photosynthetic rate was found by Willstätter and Stoll in the elm. These results are reproduced in Table 27.

In the case of the elm (Table 27) though there is a great difference in the chlorophyll-content of the two varieties, the rate of photosynthesis shows but slight differences, and at 15° the one poor in chlorophyll has a higher rate of photosynthesis on the basis of equal surface.

Experiments of this nature may be of considerable value in analyzing the photosynthetic mechanism. Temperature variations do not affect the rate of photosynthesis of the yellow varieties as much as of the normal ones. Differences in light intensity have apparently a profound effect on the yellow varieties. Undoubtedly it will be essential to gain more knowledge of the nature of the internal protoplasmic factor before a clear understanding can be had of the whole photosynthetic process. The time factor seems to become apparent in the yellow varieties more slowly than in the normal ones.

Willstätter and Stoll point out that the relatively high photosynthetic rates of yellow varieties cannot be attributed to the carotinoids. The content of the latter pigment in the yellow varieties is in many cases considerably less than in the normal varieties. What is the function of the yellow pigments of the leaf is still unknown.[153] It has been given a rôle in photosynthesis as well as in the respiratory process, though most of these ideas are largely hypothetical.

The photosynthetic activity of etiolated plants, or more accurately, of plants in which the chlorophyll is just developing, demonstrates the disproportionality between photosynthesis and chlorophyll-content. Willstätter and Stoll investigated the photosynthesis of etiolated leaves under conditions of maximal activity, i.e. 25°, 5 per cent CO_2 and high light intensity, about 48,000 Lux, using cultures of *Phaseolus vulgaris* and *Zea mays*. The etiolated plants, as soon as the first traces of chlorophyll are formed in the light, are remarkably active. For example, *Phaseolus,* with a chlorophyll-content of 0.7 mg. per 10 grams fresh leaves, had a P_c of 133, while the control plants grown in light with 18.6 mg. chlorophyll on the same basis showed a P_c of 9.4. In general the P_c of etiolated leaves is much higher than that of young leaves which developed in the light. This high photosynthetic rate of etiolated leaves, or those which have only just become green, holds not only when calculated on the basis of chlorophyll-content but as well as an absolute measure. Etiolated leaves which had been in the light used in the experiment 2 to 4 days, showed a higher rate of photosynthesis, on the basis of either, dry weight, fresh weight or area than the control plants raised in light. This greater activity of the etiolated plants also holds for increase in dry weight which was about twice that of the control plants, after the former had developed about 75 per cent of the normal chlorophyll-content. Willstätter and Stoll interpret these facts by assuming that, while chlorophyll formation is inhibited in the dark, the development of the protoplasmic or enzymatic factor is not suppressed; in fact the absence of light appears to favor the development of this factor. As a result of the accumulation or higher development of this enzymatic agent in etiolated leaves, these, after they develop a small quantity of chlorophyll are able to surpass leaves grown in the light.

Miss Irving[154] has also studied the photosynthetic activity of etiolated plants. She endeavored to determine whether etiolated plants were capable of utilizing for photosynthesis the carbon dioxide produced by their respiration. Her results do not show a decrease in carbon dioxide evolution when the plants were illuminated. This is in disagreement with the results of Willstätter and Stoll. It is possible that Miss Irving's results may be explained on the basis that the light intensity employed in her experiments was very low (light from a north window) though no intensities are given.

Another condition of interest in relation to chlorophyll-content and photosynthesis is that of chlorotic plants. When plants are grown in such a manner that no iron salts become available to the leaves, they remain

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[153] Palmer, L. S., "Carotinoids and Related Pigments," Chemical Catalog Co., 1922, p. 262.

[154] Irving, *Ann. Bot.*, **24**, 805 (1910). Ewart, *Jour. Linnéan Soc.*, **31**, 554 (1897).

very pale green or colorless with restricted development of the chloroplasts. This condition continues even under conditions of high illumination intensity. The condition of chlorosis can be caused by a number of circumstances, but probably the most common is the absence of iron.

Willstätter and Stoll cultivated plants with nutrient solutions containing no iron. While other types of leaves also poor in chlorophyll such as the light green or yellow varieties, autumnal and etiolated leaves, showed high photosynthetic activity on the basis of their chlorophyll-content, the chlorotic leaves had a very low rate of photosynthesis. Thus chlorotic *Helianthus* leaves with a chlorophyll-content for 10 grams fresh leaves of 1.9, 2.9, 3.8 mg. had a P_c respectively of 13, 21.7 and 19.5 compared to the normal leaves with 11.6 mg. and a P_c of 11.5. From this it is apparent that in chlorotic leaves the chlorophyll is only partially utilized and that in such leaves not only is the chlorophyll-content low but other essential parts of the photosynthetic mechanism are imperfectly developed. Chlorosis, then, means besides insufficient chlorophyll, inadequacy in other parts of the photosynthesis apparatus as well. Chlorophyll itself contains no iron and we have no adequate explanation of the nature of the condition termed chlorosis nor of the rôle of iron in the activity of the chloroplasts. Benjamin Moore [155] has reported the presence of iron in the colorless portion of the chloroplasts and considers iron essential for their formation. His conclusions of the rôle of iron, are, however, not entirely convincing and further investigation in this field seems necessary. In this connection the observations of Curtel [156] are of interest who found that chlorotic plants have a lower rate of respiration and transpiration than normal plants.

Willstätter and Stoll also showed that while the rate of photosynthesis in the extreme light-green and yellow varieties was low, the photosynthetic number was very high. On the other hand, in albino or variegated leaves, which do not contain the yellow pigments, the rate of photosynthesis is low as is also the P_c.

The anthocyanin pigments which in some leaves accompany the chlorophyll are apparently without direct influence on the rate of photosynthesis. The photosynthetic activity of fruit skins is very similar to that of leaves.

An important fact brought out by the investigations of Willstätter and Stoll is that leaves of the light green or yellow variety, in their photosynthetic activity, are affected more by differences in light intensity, while the leaves rich in chlorophyll are more affected by changes in temperature. This is one of the facts which has led to the assumption of the existence of an internal factor which Willstätter and Stoll consider to be of enzymatic nature. They interpret varying effects of light and temperature on leaves of different chlorophyll-content as follows. The excess or larger quantity of chlorophyll in normal leaves does not result in a photo-

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[155] Moore, *Proc. Roy. Soc.*, B **87**, 556 (1914). "Biochemistry," Longmans, Green & Co., London, 1921, p. 53.

[156] Curtel, *Compt. rend.*, **130**, 1074 (1900).

synthetic activity much greater than that of the yellow varieties. Hence the latter are constituted as well in regard to the activity of the protoplasmic factor as the former. Increase in temperature augments the activity of this internal protoplasmic factor. But the activity of this internal factor is only one step in a series of reactions. An increase in its activity will result in an augmentation of the rate of the total reaction only if the other steps are proceeding at a higher rate, i.e. if the rate of the reaction of the internal factor is determining the total rate. Therefore, an increase in temperature will become effectual and result in an augmented photosynthetic rate only in those leaves which have enough chlorophyll to absorb the radiant energy necessary to maintain this increased rate. In leaves poor in chlorophyll an increase in temperature results in but a slight rise of photosynthetic rate, because here the reactions depending upon the absorption of radiant energy by the chlorophyll are determining the total reaction.

In normal leaves which usually have more light available than they can utilize in photosynthesis, the internal factor is relatively in minimum concentration and is determining the rate of the process, at least under experimental conditions with ample carbon dioxide supplied. In the leaves poor in chlorophyll the amount of absorbed radiant energy is determining the rate of the process and the internal factor is relatively in excess. Further discussion of the rôle of the internal factor will be found in another section.

The work of Willstätter and Stoll clearly establishes the disproportionality between chlorophyll-content and photosynthetic rate. These studies also demonstrate the complexity of the reactions constituting the photosynthetic process. They emphasize the importance of agents about which we know as yet practically nothing beyond the fact that they exist, namely the protoplasmic or enzymatic factors. These investigations, moreover, make evident the fruitfulness of the quantitative chemical treatment of physiological problems and the value of chemical and physical conceptions in interpreting such complex phenomena.

f. Water Supply.

That water enters chemically into the photosynthetic reaction was clearly established by de Saussure. The effect of the water-content of the plant and of the water vapor in the atmosphere on the stomatal openings through which the carbon dioxide passes to the centers of photosynthetic activity has also been recognized for a long time. Thus water is an essential compound in the chemical reactions comprising the photosynthetic process and through its action on the stomata indirectly influences the rate of this important function of the plant. The latter fact has been brought out by Kreusler's [157] investigations. Moreover, the fact that the water-content of a leaf influences the carbohydrate ratio thereof, that is, the

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[157] Kreusler, *Landw. Jahrb.*, 14, 913 (1885).

ratio of polysaccharides to soluble monosaccharides, serves to emphasize the important rôle which water plays in photosynthesis, for it is well established that the accumulation of starch has an inhibitory influence on photosynthesis.

Déhérain and Maquenne[158] as well as Iljin have shown how greatly the rate of photosynthesis of leaves is affected by their water-content. In the investigations of Iljin the rate of photosynthesis was related to the degree of opening of the stomata. The latter was measured by means of a Darwin porometer. The methods employed cannot be considered as yielding strictly quantitative results, but indicate that alterations in the size of the stomatal openings can have a profound influence on the rate of photosynthesis.

Iljin[159] has also investigated the effect of water loss in leaves on the disappearance of starch from the guard-cells of the stomata. It has been found that leaves which had become flaccid or wilted through loss of water and had again become turgid and of normal appearance when an adequate supply of water was given, did not under the latter circumstances attain their original photosynthetic activity. The cause for this lies in a disturbance of the stomatal function. The rate of photosynthesis depends to a large measure upon the rate at which carbon dioxide is supplied to the chloroplasts. The carbon dioxide reaches the chloroplasts through the stomatal openings. If these are not open or are partially closed, obviously the ingress of carbon dioxide into the leaf will be hampered and the rate of photosynthesis will be decreased. Now Iljin found that leaves which had recovered after severe loss of water and appeared normal, still on careful examination revealed the fact that a large proportion of the stomata had been killed and were closed. Also, the opening and closing of the stomata under normal conditions is apparently accomplished by alterations in the osmotic pressure of the cell sap of the stomatal guard-cells. These changes in osmotic pressure are, in part at least, due to the transformation of starch into soluble carbohydrates and vice versa. Thus, in a humid atmosphere, the osmotic pressure of the guard-cells increases and the stomata open; while in a dry atmosphere the soluble carbohydrates are converted into starch, which can be detected in the guard-cells, the osmotic pressure decreases and the stomata close. This mechanism is probably dependent upon the activity of an enzyme and Iljin has been able to demonstrate that ample water supply favors soluble carbohydrate formation while desiccation favors the formation of starch. This is quite in harmony with other observations of the effect of water content on the carbohydrate ratio in plants.[160] However, if the desiccation is carried too far the activity of the enzyme is apparently impaired, consequently the regulatory action of the guard-cells is inhibited and the stomata do not

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[158] Déhérain and Maquenne, *Compt. rend.*, **103**, 167 (1886). Iljin, *Flora*, **116**, 306 (1923). Kreusler, *Landw. Jahrb.*, **14**, 951 (1885).

[159] Iljin, *Jahrb. wiss. Bot.*, **61**, 670 (1922).

[160] Spoehr, H. A., Pub. No. 287, Carnegie Inst. of Wash., 1919, p. 57.

react to changes in the water-content of the plant. No doubt there are some features of the mechanism of the movement of the stomata still to be explained, but Iljin's investigations have indicated one important influence of water on the rate of photosynthesis.

In experimental work it is therefore highly important that due consideration be given the question of the humidity of the atmosphere to which the plant is subjected. In mosses and leaves possessing no stomata this factor is according to some authors [161] not of such importance, while others have found considerable variation in photosynthetic activity with changes of water-content. Lichens and some mosses can be dried to a powdery consistency, in which condition the power of photosynthesis is, of course, temporarily lost; on the addition of water these plants regain almost immediately their photosynthetic activity. In the leaves of higher plants loss of water beyond a certain point results in death, and while such dried and powdered leaves still exhibit a post mortal respiration with the formation of carbon dioxide the power of photosynthesis is apparently permanently lost. It should be stated, however, that this question has not yet been definitely answered and that by the use of proper methods it may be possible to obtain photosynthesis with leaf material which has previously been dried. This has been shown to be possible by the investigations of Molisch [162] which are discussed in the section under Internal Factors.

Dastur,[163] from investigations in which the starch-iodine test was employed concludes that with advancing age there occurs a decay of photosynthetic activity. This is at first noticeable in the mesophyll cells of the margin and intravascular regions of the leaves, supposedly the regions in the leaf farther removed from the vascular system supplying water. There is some question, however, whether the iodine test is reliable for determining photosynthetic activity especially where small differences are concerned.

For plants growing under natural conditions the humidity of the air and water supply of the soil are of great importance for the photosynthetic activity of the plant. These factors to a large measure determine the opening of the stomata and consequently the absorption of carbon dioxide by the leaf. In general it is the latter factor more than any other, namely, the amount of carbon dioxide which the plant takes up, that determines the rate of photosynthesis and hence the vegetative development of the plant. In xerophytic plants, such as the cacti, which exercise special economy of their water-content, the gaseous exchange is as a consequence also greatly affected. This applies naturally not only to the absorption of carbon dioxide for photosynthesis but as well to the absorption of oxygen for respiration. The result is that these plants exhibit certain modifica-

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[161] Jumelle, *Rev. gén. Bot.*, **4**, 168, 318 (1892). Bastit, *ibid.*, **3**, 521 (1891). Jönsson, *Compt. rend.*, **119**, 440 (1894). Henrici, *Verh. Naturf. Ges. Basel.*, **32**, 157 (1921).

[162] Molisch, H., *Zeit. f. Bot.*, **17**, 577 (1925); *Bot. Zeitg.*, **62**, 1 (1904).

[163] Dastur, *Ann. Bot.*, **38**, 779 (1924).

tions in their metabolic activities in which organic acids, such as malic acid, play an important rôle. While there is no reason for believing that in these plants, the chemical reactions constituting the photosynthetic process are different from other plants, owing to the modified gaseous exchange the study of photosynthesis in xerophytes offers specially complex conditions.[164]

But water supply is of importance to the photosynthetic process not only on account of its indirect influence through affecting the stomatal openings but also in a very direct manner. This question is more exhaustively discussed in the chapter on the Chemistry of Photosynthesis. Suffice it to recall that de Saussure demonstrated that water enters into the chemical reactions constituting photosynthesis. He showed that of the dry material synthesized by a plant less than half could be ascribed to the weight of the carbon in the carbon dioxide taken up by that plant. The rest he regarded as coming from the water.

The reaction for photosynthesis is usually written in the following manner with the molecular weight relations as indicated:

$$\underset{264}{6\ CO_2} + \underset{108}{6\ H_2O} \rightarrow \underset{180}{C_6H_{12}O_6} + \underset{192}{6\ O_2}.$$

The basis for this is that the ratio of the volume of carbon dioxide absorbed to that of oxygen emitted is very close to unity which would indicate the formation of a compound of the composition $C_nH_{2n}O_n$. The value of n is, of course, not established thereby, but from the widespread occurrence of hexoses in leaves the assumption is frequently made that the equation represents empirically the course of the reaction. Similarly the relation of the weight of carbon dioxide absorbed to that of material formed in photosynthesis in many instances supports this view. From the equation it is apparent that the molecular ratio of water to carbon dioxide in photosynthesis is: $H_2O : CO_2 = 108 : 264$. It is rather surprising that in much of the writing on photosynthesis the authors disregard the fact that we are dealing with carbonic acid and not simply with CO_2, that the water actually enters into the chemical reaction and is not only a solvent.

From theoretical as well as experimental considerations, therefore, we can conclude that water is essential for the photosynthetic process. Each 100 grams of material synthesized, calculated as $C_6H_{12}O_6$ requires 60 grams of water.

Interesting in this connection are also the osmotic relations of the photosynthetically active cells. Treboux[165] has studied the effect of different concentrations of various substances which are considered as nontoxic to the photosynthetic activity of *Elodea*. A noticeable effect was first observed with a 0.1 per cent solution of KNO_3. Solutions of KCl,

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[164] Richards, H. M., Pub. No. 209, Carnegie Inst. of Wash., 1915. Spoehr, H. A., *Biochem. Zeit.*, **57**, 95 (1913).

[165] Treboux, *Flora.*, **92**, 53 (1903). Jacobi, *Flora*, **86**, 326 (1899).

$NaNO_3$, sucrose and glycerine isotonic with 0.5 per cent KNO_3 showed an equal inhibiting effect. This inhibiting effect increases with concentration. Concentrations which did not cause plasmolysis produced no permanent effects; that is, the plant regained its original rate when the salt solution was replaced by water. If plasmolysis had occurred (2.5 per cent KNO_3) the plant did not recover its original rate when placed in water. Somewhat contrary to these results are those of Kny[166] who found that *Spyrogyra* cells which had been in a 40 per cent solution of sucrose for less than an hour, showed evolution of oxygen when placed in a 20 per cent solution and were illuminated. When the concentration of the sucrose solution was gradually increased from 10 to 40 per cent, some cells still showed photosynthesis after 24 hours. There appears to be no doubt, however, that plasmolysis greatly decreases the rate of photosynthesis, though the activity may not be entirely prevented.

Fromageot[167] concludes from his studies with *Ulva lactuca* in different concentrations of sea-water that there is a distinct optimal concentration for photosynthesis and that this corresponds to that of sea-water ($\Delta = 1.94$).

g. The Time Factor.

Investigations of the effect of temperature on photosynthetic rates have shown that the maximum rate cannot be maintained for any length of time, but that with time this maximum rate shifts to a lower temperature. This is apparently due to the fact that there are two opposed reactions involved. This has been shown graphically in Figure 14. If the point M is taken as the optimum, this point is not a fixed one. Its position will be determined by all factors which affect the rates of the opposing reactions O A and D B, and these factors are manifold. In the case of photosynthesis the curve of inactivation, D B, is a function not only of temperature but of other factors as well, such as light intensity and the accumulation of the products of photosynthesis. Similarly the curve of acceleration, O A, is a function not only of temperature but also of chlorophyll-content and certain internal factors. As a consequence the position of the optimum point, M, is dependent upon the previous treatment of the plant material in regard to temperature and illumination intensity and the rate at which the temperature is raised to this optimum point.

Blackman and Matthaei have shown that for cherry laurel the rate of photosynthesis remains fairly constant at temperatures below 25°. Above this temperature the initial rate cannot be maintained, but decreases with time. It is highly probable that different species of plants differ as to the point where this time-factor first becomes apparent.

As has been stated, the inactivation of photosynthetic activity can be brought about not only by higher temperatures but also by long exposure

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[166] Kny, *Ber. bot. Ges.*, **15**, 396 (1897). Klebs, *Biol. Centralb.*, **7**, 166 (1887).
[167] Fromageot, *Compt. rend.*, **177**, 779 (1923).

to high illumination. This has been demonstrated by Ursprung[168] with the formation of starch in the chloroplasts. Starting with a starch-free leaf, the formation of starch on illumination and the gradual accumulation thereof with continued illumination of the leaf can be clearly followed by the depth of color produced when the leaf is treated with iodine. If exposure to bright sunlight is continued the amount of starch in the leaf gradually decreases again. Thus a leaf of *Phaseolus* after 5 hours of illumination showed very deep coloration of the starch-iodine, while after 8.5 hours of illumination the reaction was faint. This phenomenon can be produced by almost any source of light of sufficient intensity, namely, sunlight, electric arcs and filament lamps, and the time required is proportional to the intensity of the light. By using a spectrum the effect is first brought about in the red-orange portion, the region showing, under the circumstances, the highest photosynthetic rate. With higher intensity the shorter wave-lengths bring it about in correspondingly shorter time. It is therefore apparently proportional to the photosynthetic activity. Ursprung has given the name of *solarization* to this phenomenon on account of its analogy to the effect produced in photographic plates under similar circumstances.[169]

We have here another case of the inactivation of the chloroplasts. These organs after long exposure to intense light cease to function although they are not killed, and, after a period of darkness, again produce starch normally. It is possible that the phenomenon is in some manner associated with the oxygen produced in photosynthesis.[170]

It must be borne in mind that solarization as observed by Ursprung, has been confined to the presence of starch; it would be highly desirable if similar experiments would be carried out in which more complete analyses of the carbohydates in the leaf were made and consideration given to the influence of temperature.

The inhibiting effect on photosynthesis of long exposure to light of high intensity has also been investigated by Ewart.[171] From these results it would appear that the inhibiting effect is due to destruction of the chlorophyll. The question arises whether the inhibition of photosynthesis under conditions of continued exposure to high illumination intensity is due merely to the destruction of the chlorophyll or whether the chloroplast plasma is also injured. Pantanelli[172] inclines to the opinion that both pigment and stroma are affected; that the fatigue effects observed by him in bright light are possibly due to a combination of chlorophyll destruction and injury to the chloroplast plasma. It is evident that our limited knowledge of the relation of pigment to stroma in the chloroplasts and of the mechanism of these latter bodies prevents a more thorough understanding

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[168] Ursprung, *Ber. bot. Ges.*, **35**, 57 (1917).
[169] Plotnikow, "Handbuch d. Photochemie," Berlin-Leipzig, 1920, p. 645.
[170] Pringsheim, *Jahrb. wiss. Bot.*, **12**, 288 (1879).
[171] Ewart, *Ann. of Bot.*, **11**, 439 (1897); **12**, 379 (1898).
[172] Pantanelli, *Jahrb. wiss. Bot.*, **39**, 167 (1903).

of the phenomenon of the inhibition of photosynthesis by high light intensity.

It has been known for a long time that the photosynthetic rate decreases with the accumulation of the products of photosynthesis. This is especially noticeable when the leaves which are being observed have been removed from the rest of the plant. Boussingault[173] first noticed the gradual decrease in the amount of carbon dioxide reduced by leaves which had been removed from a plant. This is undoubtedly associated with the fact that in excised leaves the capacity for translocation of synthesized carbohydrates does not exist. Under normal conditions the material formed in the leaves travels to other parts of the plant where it is consumed or stored. When the leaves are cut from the plant this is not possible, and accumulation readily occurs.

Saposchnikoff[174] has demonstrated the inhibitory power of an accumulation of carbohydrates and that these cannot increase beyond a certain point. When the leaves of *Vitis vinifera* contain 23 to 29 per cent carbohydrates of the dry weight there was a cessation of photosynthesis. Leaves which had accumulated a certain amount of carbohydrates showed a decreased photosynthetic rate, while a decrease in this accumulation of carbohydrates resulted in an increased photosynthetic rate. It is evident that the rate of the different steps comprising the photosynthetic process exercises a profound effect upon the total reaction and the movement of the products away from centers of activity is of importance for continuous action. This applies not only to the accumulation of carbohydrates but apparently also to oxygen, for pressures of oxygen above $\frac{1}{50}$ atmosphere tend to decrease the photosynthetic rate when high light intensity is used.

These facts are of particular importance in experimental work, when excised leaves are used, though they must be taken into consideration under all circumstances. When the rate of photosynthesis is high as under conditions of high carbon dioxide concentration, strong illumination and elevated temperatures, this effect is especially to be watched for.

Ewart[175] made similar observations with a variety of plants, but found that the plants which have thus been inactivated do not always regain their photosynthetic capacity by being kept in darkness. The case of *Allium cepa* is of interest because this plant does not form starch. When leaves of this plant are exposed to bright light for a long time, 14 days, or for a shorter period while being fed sugar, the evolution of oxygen finally ceases. This inactivation apparently does not injure the cells or chloroplasts. After a few days in darkness the capacity for photosynthesis is regained. It is possible that this phenomenon is associated with the osmotic relations of the cell, for when the cell sap reaches a certain concentration photosynthesis ceases.

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[173] Boussingault, "Agronomie Chimie et Agriculture," Vol. **4**, pp. 286, 312 (1868).

[174] Saposchnikoff, *Ber. bot. Ges.*, **11**, 391 (1893); **9**, 293 (1891); **8**, 233 (1890).

[175] Ewart, *Jour. Linnéan Soc.*, **31**, 429 (1896).

There is as yet not sufficient information to enable a clear conception to be formed as to the manner in which the accumulation of starch inhibits the photosynthetic activity of the chloroplasts. In fact, in view of the behavior of plants which form no starch and show an inhibition through high concentration of soluble carbohydrates, it is possible that the accumulation of starch is not directly associated with the cause of the inhibition, but is merely an accompanying phenomenon. At the same time, if we accept the theories of Wurmser, of Briggs, and of Warburg, that the primary photochemical action takes place on the surface of the chloroplast, it is conceivable that the accumulation of starch in the chloroplast would materially affect this action. In starch-laden leaves, the starch grains are frequently larger than the chloroplasts, the latter being partially covered by the starch. Under these conditions the surface of the chlorophyll solution is materially reduced. As a consequence as much carbon dioxide could not reach the chlorophyll as when the chloroplasts are starch-free, and the amount of carbon dioxide entering the photochemical reaction would be reduced with a resulting decrease in photosynthesis. Thus, any substance which displaces carbon dioxide from the surface would tend to inhibit the rate of photosynthesis.

Miss Henrici[176] has described some interesting curves showing the indirect effect which temperature and light may exert on the photosynthetic rate. These curves for alpine phanerogamic shade plants and lichens do not show the single maximum, either as a function of temperature or illumination intensity when only one factor is changed. There are, in fact, two maxima. These plants form starch under conditions of higher light intensity or higher temperature than normally occur in the winter. For example, while at 0° in these alpine plants there is never any starch, when these are exposed to light of high intensity starch appears. The threshold of temperature and illumination intensity for photosynthesis is much lower than that of starch formation. As a result when either factor is increased the rate of photosynthesis rises to a maximum; during this time no starch is formed. With continued increase of either factor the rate decreases; this decrease is synchronous with the appearance of starch in the chloroplasts. With further increase of either light or temperature the rate of photosynthesis passes a minimum value, then rises to a second maximum and finally drops rapidly again. Plants which habitually form no starch do not behave in this manner and, as has been stated, the feeding of soluble sugar and consequent starch formation, rapidly decreases the photosynthetic rate. These effects are apparently closely associated with those observed by Ursprung.

Prolonged exposure to sunlight may cause temporary inhibition of the photosynthetic capacity of plants. The causes of this action are still obscure, though it appears that the plasma of the chloroplasts is more sensitive under certain conditions than the protoplasm of the cell.[177]

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[176] Henrici, *Verh. Naturf. Ges. Basel.*, **32**, 107 (1921).
[177] Pringsheim, *Jahrb. wiss. Bot.*, **12**, 326 (1882).

h. Internal Factors.

The external conditions which influence photosynthetic activity, namely, temperature, light intensity, and partial pressure of carbon dioxide can be easily determined and the intensity of each of these influences can also be controlled experimentally. As a consequence the effect of these factors on the photosynthetic process can be studied experimentally, and although the analysis of the reactions involved has not proved to be a simple task nor is as yet complete, it has been possible to gain considerable information. The study of the influence of these external factors has served to emphasize the complexity of photosynthesis and to bring to light the fact that there are conditions or influences within the cell which are of equal or greater significance in determining the rate of photosynthesis. The most evident of these is probably the chlorophyll-content of the plant and the relation of this to photosynthetic activity has already been discussed. The existence of these internal factors becomes evident from the fact that under circumstances the photosynthetic rate varies independently of the external factors such as temperature, light intensity and carbon dioxide-concentration. Moreover, with constant external conditions, the rate of photosynthesis does not run parallel with the chlorophyll-content. So that, besides the latter factor, there must be some other, internal factor which is determining the rate of the reactions.

Conclusions regarding the existence of an internal factor have been arrived at through inference rather than by direct experimental demonstration. Our knowledge regarding internal factors has not progressed very far; in fact, a good deal of the subject is still purely hypothetical. It is an exceedingly difficult experimental task; external conditions can be altered at will, but to deal with material or conditions inside of the cell, material about which we know virtually nothing, presents unusual obstacles. Moreover, it is uncertain whether there are several internal factors, or whether different properties of the same thing have been described.

Pantanelli [178] came to the conclusion that a very important rôle in the photosynthetic process is played by the protoplasmic activity of the colorless components of the chloroplasts. This was based upon the observation that the photosynthetic activity becomes inhibited in time and after a period of darkness again attains its original rate. The inhibition in Pantanelli's experiments was probably not due to an accumulation of the products of photosynthesis, for the duration of illumination was short, nor was apparently the chlorophyll-content decreased though the illumination intensity was high. The photosynthetic activity shows the phenomenon of fatigue and recovery very similar to that of the animal muscle. With this inhibition of photosynthetic activity there occurs a retardation of the protoplasmic streaming. A disturbance in protoplasmic streaming con-

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[178] Pantanelli, *Jahrb. wiss. Bot.*, **39**, 184 (1903).

current with photosynthetic inactivity has been observed frequently.[179] This is associated with the aggregation of the chloroplasts into a central clump. In this condition the chlorophyll in the chloroplast is destroyed if illumination is continued. It is interesting that protoplasmic streaming, after retardation by light of high intensity, recommences sooner than does the recovery of the original rate of photosynthesis. While the aggregation of the chloroplasts probably plays an important rôle in the inhibition of the photosynthetic activity, it cannot be said that this fact in itself explains the phenomenon. In the fatigue effects observed by Pantanelli the destruction of chlorophyll probably plays no part. But as to the real cause of the temporary inhibition of photosynthesis no definite statement can be made. It is very doubtful that in photosynthesis chlorophyll is successively decomposed and reformed, for Willstätter and Stoll could observe no alteration in the chlorophyll-content during photosynthesis. And when chlorophyll is decomposed, as occurs during very intense illumination, the reforming thereof is a slow process. Therefore it seems at least plausible that in the fatigue effects the colorless portion of the chloroplasts plays a part. It is doubtful whether we can make much advance in these problems until we know more about the relation of chlorophyll to the colorless portion of the chloroplast. There is, on the one hand, the theory that the chlorophyll and the plasmatic stroma are morphologically separate, and, on the other, that the two are chemically united. In view of the ease with which chlorophyll can be extracted from the plant it is doubtful whether the union of the pigment and the proteinaceous stroma can be of a more stable nature than exists in emulsions.

An exceedingly important fact in this connection is that photosynthetic activity is apparently intimately associated with the vital activity of the plant. Any disturbance in the respiratory activity of the plant also affects the photosynthetic activity. This is particularly true of narcotics and poisonous substances. Whether the photosynthetic activity is affected by the action of these substances on the protoplasm or on certain enzymes elaborated by the protoplasm is not certain. From the results of Molisch who was able to detect the evolution of oxygen in leaves that had been dried, and the protoplasm consequently killed, it would appear that enzymes play an important rôle. It may be said that ascribing these functions to an enzyme about which we know virtually nothing, rather than to the protoplasm is just shifting the burden and constitutes no real advance in our knowledge; it is nevertheless a step in the same direction in which Buchner's discovery of zymase aided in elucidating the phenomenon of fermentation.

The absence of oxygen also has a profound effect on the rate of photosynthesis. Boussingault[180] showed that in an atmosphere of hydrogen, nitrogen or methane plants lose the power of photosynthesis and attributed

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[179] Ewart, *Ann. Bot.*, **12**, 385 (1898); *Journ. Linnéan Soc.*, **31**, 439 (1896).

[180] Boussingault, "Agronomie, chimie agricole et physiologie," Paris, 1868, **4**, p. 329.

it to a disturbance of respiration. Later Pringsheim[181] studied the effects of lack of oxygen by subjecting the plants to a stream of hydrogen and carbon dioxide. Under these conditions protoplasmic streaming soon ceases, and if the cells are then illuminated no photosynthesis occurs. Cells in this condition of "inanition" can be revived by the supplying of oxygen. Even in continuous illumination in an atmosphere of hydrogen and carbon dioxide, i.e., starting with active photosynthesis, cells of *Chara* gradually showed stoppage of photosynthesis and protoplasmic streaming. If this condition of "inanition" is not continued too long photosynthesis and streaming can be revived by addition of traces of oxygen. Pringsheim interpreted these facts on the basis that photosynthesis and respiration were intimately associated. He argued that with photosynthesis independent of respiration, the former process in an atmosphere containing carbon dioxide and with sufficient light intensity should continue to evolve oxygen, and respiration and protoplasmic streaming could continue. This, his experiments showed, is not the case. Ordinarily photosynthesis produces more oxygen than is used in respiration.

Pringsheim's[182] next deduction does not seem as well founded. He concluded that in photosynthesis no oxygen is formed within the cell. But rather that in the decomposition of carbonic acid in the cell a substance, possibly hydrogen peroxide, is formed which passes out by diosmosis and on the surface of the cell decomposes with the liberation of oxygen. He maintained that we have no experimental evidence of the formation of oxygen within the cell. That all our conclusions are based upon methods of gas analysis which can yield results only of the final end products. Of the chemical nature of the hypothetical oxygen compound Pringsheim did not venture a conjecture but emphasized that the carbon dioxide decomposition and oxygen evolution are two separate processes. This he sought to support by his observations of oxygen evolution from dying cells.

There is as yet not sufficient experimental evidence to permit the formulation of a theory of either the kinetics of oxidation or of reduction in living cells. In connection with the fact, which seems quite well established, that a small amount of oxygen is essential for the reduction of carbon dioxide in the photosynthetic process, similar conditions which have been observed in the catalytic hydrogenation by means of platinum are of interest. Adams and others[182a] have called attention to the fact that oxygen is essential to the activity of platinum black as a hydrogenation catalyst, though the rôle of oxygen in these reactions is not yet definitely established.

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[181] Pringsheim, *Sitzb. preuss. Akad. Wiss.*, 1887, 763. Friedel, *Compt. rend.*, **131**, 477 (1900).

[182] Pringsheim, *Jahrb. wiss. Bot.*, **17**, 178 (1886).

[182a] Carothers and Adams, *Jour. Am. Chem. Soc.*, **47**, 1047 (1925). Waldschmidt-Leitz and Seitz, *Ber. Chem. Ges.*, **58** B, 563 (1925).

It does not seem pertinent to follow Pringsheim's further deductions. But his experimental results indicate this fact. That a normal cell containing chlorophyll in an apparently unchanged condition and exposed to bright light and an atmosphere containing carbon dioxide is incapable of photosynthesis in an atmosphere free of oxygen. It appears, therefore, that besides the conditions of light, chlorophyll, temperature, and carbon dioxide supply, other conditions are essential and these are intimately associated with the respiration of the protoplasm. The latter is an important internal factor affecting photosynthesis, in addition to the chlorophyll-content and the number of chloroplasts. The exact manner in which these two processes of respiration and photosynthesis may be linked is still an open problem.

There have been observed some quantitative relations between photosynthetic activity and respiration. The leaves of the light-green or yellow varieties of the plants investigated by Plester[183] showed a lower rate of photosynthesis than the varieties rich in chlorophyll. Similarly the former had a lower rate of respiration. Thus, while it is evident that there is no parallelism between chlorophyll-content and rate of photosynthesis, the quotient respiration—photosynthesis is more constant. This quotient for the light-green varieties was found to be as follows: *Ptelea* = 1.77, *Catalpa* = 1.72, *Mirabilis* = 2.0, *Ulmus* = 2.0, *Populus* = 2.1, *Atiplex* = 1.3. The latter plant exhibits rather erratic photosynthetic rates.

A correlation between respiration and photosynthetic rates was also noticed by Henrici.[184] She found in a study of alpine and low land plants, that those plants which had a high photosynthetic rate also had a high rate of respiration and vice versa. Boysen-Jensen[185] found that plants which have a high rate of respiration also have a high rate of carbohydrate formation. Similar results have also been obtained by Spoehr and McGee.[186]

Kny[187] has shown that photosynthesis soon ceases when the chloroplasts are separated from the cytoplasm of the cell, although there appears to be no direct parallelism between injury to the cytoplasm and photosynthetic inhibition. Disorganization of the cell nucleus is not detrimental to photosynthetic activity.

Ewart[188] has also made observations on photosynthesis as affected by an atmosphere of hydrogen and carbon dioxide. Even in mosses, which show this effect more slowly than other plants, stoppage of photosynthesis finally follows exposure to such an atmosphere. When oxygen is made available the plants recover their photosynthetic ability if they have not been kept in the mixture of hydrogen and carbon dioxide too long.

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[183] Plester, *Beit. Biol. der Pflanzen.*, **11**, 249 (1912).

[184] Henrici, "Chlorophyllgehalt und Kohlensäure-Assimilation bei Alpen-Ebenpflanzen," Inaug. Diss., Basel, 1918, p. 90.

[185] Boysen-Jensen, *Botanisk Tidsskrift*, **36**, 219 (1918).

[186] Spoehr and McGee, Carnegie Inst. of Wash. Pub. No. **325**, 76-98 (1923).

[187] Kny, *Ber. bot. Ges.*, **15**, 388 (1897).

[188] Ewart, *Linnéan Soc.*, **31**, 403 (1897).

Willstätter and Stoll[189] found that various plants exhibit a wide variation in their resistance to lack of oxygen. It is perhaps not without significance that the mosses which are resistant to the absence of oxygen have no stomata and hence the gaseous interchange with the atmosphere is more difficult. According to Willstätter and Stoll the partial pressure of oxygen can be reduced to one hundredth of that in air without disturbing photosynthesis if the rest of the atmosphere is nitrogen. After complete displacement of oxygen for two hours the leaves were no longer capable of photosynthesis on illumination. This is quite in agreement with the older observations. Under these conditions the leaves show no visible signs of injury. After exposures to an oxygen-free atmosphere of shorter duration (one hour) some plants as *Cyclamen europaeum, polytrichum juniperinum,* the photosynthetic activity is decreased but not entirely inhibited. Of special interest are the observations that the plants just mentioned when kept in an oxygen-free atmosphere for 15-24 hours, on illumination, show no photosynthesis, but after about 30 minutes begin to evolve oxygen and increase in this activity to a high rate. The long continued exposure to an oxygen-free atmosphere is not without permanent injurious effect on the photosynthetic mechanism; the plant does not again attain its original photosynthetic rate. The longer the exposure to an oxygen-free atmosphere, the lower is the subsequent rate of photosynthesis and the more incomplete is the recovery. Lack of oxygen therefore produces a permanent injury of the protoplasm; the degree of this injury depending upon the length of time the plant is deprived of oxygen and the individual constitution of the plant.

Willstätter and Stoll conclude that oxygen is absolutely essential for photosynthesis, but that a very small quantity of oxygen suffices for supplying the photosynthetic apparatus. Moreover, they consider that free oxygen is not necessary but that oxygen held in an easily dissociable form can do the work. The conception of oxygen loosely bound in the plants is largely based upon those cases which were kept in an oxygen-free atmosphere and on illumination again recovered their photosynthetic activity. It is suggested that oxygen is removed from the plant in two stages: 1, the replacement of the free oxygen by an oxygen-free atmosphere, and 2, the removal of loosely bound oxygen, through the dissociation of an oxygen compound when the oxygen tension of the atmosphere becomes less than that of this hypothetical compound. The second stage is slower than the first. Hence in plants which have lost oxygen chiefly by the first stage, a recovery of photosynthesis is possible on illumination. Only the more resistant plants can withstand the second stage.

On the basis of their work on the relation of chlorophyll-content to photosynthetic rates, Willstätter and Stoll came to the conclusion that there is no direct ratio between these two. Besides chlorophyll as an internal factor influencing the rate of photosynthesis, there is, according

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[189] Willstätter and Stoll, "Untersuchungen über die Assimilation," Berlin, 1918, p. 349.

to this conception, also an enzymatic agent. This latter protoplasmic factor is contained in both the chlorophyll-rich and light-green or yellow varieties in about the same amount. Temperature affects primarily this factor. Now the temperature coefficient of photosynthesis is considerably higher in leaves rich in chlorophyll than in leaves poor in chlorophyll. This Willstätter and Stoll explain on the basis that an increase in temperature and consequent augmented activity of the protoplasmic factor, can result in a higher rate of photosynthesis only under conditions in which the other steps in the photosynthetic process are also raised, or at least are not lower than the ones determined by the enzymatic factor. That is, it must be possible for the plant to absorb enough light to supply the greater energy necessary to carry on these other steps at a rate which is equal to that of the enzymatic factor, now augmented by a rise in temperature. Therefore, higher temperature can result in a higher rate of photosynthesis only if there is sufficient chlorophyll present which can absorb this increased amount of energy required. These latter conditions are met only in leaves rich in chlorophyll. In leaves poor in chlorophyll the activity of the enzymatic factor may also be raised by higher temperatures but it will not result in a total increase in photosynthesis, because the leaf cannot absorb sufficient energy to increase the rate of the purely photochemical steps in the series of reactions comprising photosynthesis.

Willstätter and Stoll found that etiolated leaves after they had developed a fraction of the chlorophyll-content of normal leaves surpassed the latter in photosynthetic activity. They assume that this higher activity of etiolated leaves is due to a greater development of the internal enzymatic factor. It is possible, on this assumption, that light is not so favorable for the development of this enzymatic factor as darkness or at least in high intensity exerts an inhibiting effect. Although there is hardly sufficient evidence to permit of speculation, is it not possible that if light does exert an inhibiting effect on this internal factor, that the "time factor" of photosynthetic activity may be explained on this basis? Also it may be possible that the fatigue effects, described by Pantanelli and discussed above, are to be explained on the ground that light, while essential for the photochemical reactions, is injurious to the protoplasm in high illumination intensities. The deleterious effects of light, particularly the blue-violet rays, on enzymes and proteins have been repeatedly demonstrated.[190]

In fact Ewart[191] has shown that "it is possible to produce a condition of permanent light rigor, i.e., death, in the chlorophyll grains over an exposed region of a cell of *Chara* without affecting the vitality of the cell, i.e. the plasma of the chlorophyll grain appears to be more sensitive than the general protoplasm of the cell."

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[190] Reynolds-Green, *Trans. Roy. Soc. London,* **188**, 167 (1897). Emmerling, *Ber. chem. Ges.,* 34, 3811 (1901). Chauchard, *Compt. rend.,* **158**, 1575 (1914). Pincussen, *Biochem. Zeit.,* **134**, 457 (1922). Schanz, *Arch. Ges. Physiol.,* **164**, 445 (1916).

[191] Ewart, *l. c.,* 443.

The high photosynthetic rates observed by Willstätter and Stoll in leaves poor in chlorophyll make Pringsheim's protective theory very improbable. Pringsheim,[192] starting from the erroneous hypothesis that direct light accelerates respiration, developed the theory that chlorophyll acts as a protection against light. He supposed that light might induce photosynthesis in colorless cells as well as those containing chlorophyll; the pigment acting as a protection against light and permitting the reaction to become more prominent in the green cells than in the colorless ones. The theory is quite untenable, for the cells containing chlorophyll are, if anything, more sensitive to light than the colorless ones.

From the fatigue effects of Pantanelli, the inhibition experiments of Ewart and others and the disproportionality between chlorophyll-content and photosynthesis as demonstrated by Willstätter and Stoll, it is apparent that the protoplasm of the cell plays an important rôle in the process. Now, of course, it must be realized that the nature of this protoplasmic factor or enzyme has not been established. Willstätter and Stoll consider it probable that it behaves as a dissociable oxygen compound (dissozierbare Sauerstoffverbindung).

In relation to the question of the dependence of photosynthesis on protoplasmic activity mention should be made of the attempts which have been made to detect photosynthetic activity in dead leaves. The first reports regarding this subject were not clear and decidedly contradictory. Friedel [193] was the first to claim that photosynthesis was possible in dried leaf material to which water had again been added, but was himself unable to substantiate his first findings. Recently Molisch has again made a very careful study of this problem. For the detection of photosynthesis he used a very sensitive luminescent bacteria method to the technique of which he had previously made many valuable contributions. In the earlier experiments on the photosynthetic activity of dried leaves, the latter were ground in water and the mixture was then filtered. Molisch altered the procedure so that the entire paste was used; this proved to be an important point, for material prepared in this manner after being kept in the dark for a time and subsequently illuminated, even for a very brief period, showed the evolution of oxygen. The leaves were dried at 30 to 35° for 3 to 4 days and in some cases were kept in a desiccator over calcium chloride or sulphuric acid for weeks. Some leaves which had been heated to 84° for 5 hours still showed the capacity to form oxygen, but the leaves could not endure a temperature of 96° for one hour. When heated to higher temperatures in the moist condition the leaves lose the capacity to form oxygen in the light. Similarly, if the leaves are treated with ether and dried they no longer are able to form oxygen. Leaves which have been killed by freezing are also able to emit oxygen when illuminated.

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[192] Pringsheim, *Jahrb. wiss. Bot.*, **12**, 288 (1882).

[193] Friedel, *Compt. rend.*, **132**, 1138 (1901). Harroy, *ibid.*, **133**, 890 (1901). Herzog, *Zeit. physiol. Chem.*, **35**, 459 (1902). Molisch, *Bot. Zeitg.*, **62**, 1 (1904); *Zeit. f. Bot.*, **17**, 577 (1925).

Dried leaves can be kept for a long time, up to three months, without completely losing the capacity for oxygen formation in the light. That chlorophyll is essential for the formation of oxygen in the light by killed leaves is shown by the fact that etiolated leaves do not possess this property, although the amount of chlorophyll that is necessary is exceedingly small.

Molisch demonstrated this phenomenon with a wide variety of plants. Some plants with a relatively high acid content on being dried and tested for oxygen evolution gave very slight or negative results. When the acids are neutralized during the preparation of the leaf material positive results are obtained, and it is not improbable that the negative results in the first case are to be attributed to the effect of the acid on the chlorophyll during the process of drying.

Molisch regards that these experiments on the oxygen production of killed leaves in the light, demonstrate the existence of an enzyme which is essential for the photosynthetic process; that the life of the plant is not necessarily essential for the photosynthetic activity any more than the living yeast plant is essential for the fermentation process. While the results of Molisch perhaps do not entirely remove the photosynthetic process from the living activity of the plant to the same degree that the isolation of zymase did for the process of alcoholic fermentation, they nevertheless are a distinct contribution toward establishing the enzymatic nature of photosynthesis, a trend which has already been fostered by the results of Willstätter and Stoll, Pringsheim and others.

Returning to the influence on photosynthesis of lack of oxygen, it is apparent that this is largely one of degree; some plants are easily permanently inhibited while others withstand absence of oxygen for long periods. All show some injury. In viewing these facts critically it is well to bear in mind that it is extremely difficult to free any plant from the last traces of a gas. Plants differ greatly in their rate of oxygen consumption as well as in the rate of gaseous interchange with the surrounding atmosphere. Also it is difficult to free a gas from the last traces of oxygen; this is apparently impossible by the use of glowing copper. Moreover, the injurious effects of lack of oxygen may well be due to injury of the protoplasm caused by the accumulation of toxic substances. It is well known that plants deprived of oxygen continue to consume carbohydrates, but that the course of metabolism is greatly altered, usually resulting in the formation of ethyl alcohol. Different plants vary in the rate of alcohol formation. So also does the protoplasm of different plants vary in resistance to toxic substances, such as alcohol, produced by the plant itself, or administered from without.

Apparently photosynthesis is more sensitive to protoplasmic disturbances than is respiration. Thus Wurmser and Jacquot [194] found that when certain marine algae were subjected to higher temperatures (36° to 45°)

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[194] Wurmser and Jacquot, *Bull. Soc. Chim. biol.*, **5**, 305 (1923); **6**, 169 (1924).

for from 1 to 15 minutes, the rate of photosynthesis was always depressed when the plants were returned to the temperature of their previous environment, 16°. The depression is greater the higher the temperature and the longer the exposure to the higher temperature. Similar effects were produced with glycerine, which are taken to show that the photosynthetic apparatus is more delicate than that of respiration. Warburg[195] has also shown that the photosynthetic rate is reduced by hydrocyanic acid and urethanes of extreme dilutions in which the respiratory activity is not affected or in certain cases is even stimulated. The effect of narcotics on photosynthesis is discussed in Section i. of this chapter.

Warburg[196] found that, while respiration is not influenced by different partial pressures of oxygen (probably above a certain minimum) photosynthesis is less at higher pressures. This decrease in the photosynthetic rate is noticeable only at high illumination intensities. A change in the partial pressure of oxygen from $\frac{1}{50}$ to 1 atmosphere reduces the photosynthetic rate by about one-third. From this it would appear that the reaction

$$nCO_2 + nH_2O \rightarrow (CH_2O)n + nO_2,$$

can be checked by a higher concentration of oxygen. This may be of considerable significance in determining the kinetics of the reaction and should be investigated more fully.

In the discussion of the temperature coefficient of photosynthesis attention has already been called to the fact that the photosynthetic process is composed of two reactions. One of these is a photochemical reaction with a low Q_{10}, the other an ordinary chemical reaction with a Q_{10} of about 2, in approximate agreement with the van't Hoff rule. There is a variety of evidence which is in harmony with this conception. It is the ordinary chemical reaction which is associated with the protoplasmic activity of the plant. It is this reaction which is affected by changes in temperature and is sensitive to narcotics. The nature of this reaction is not known, but has been the subject of considerable speculation. Warburg[197] has called this reaction the "Blackman Reaction" and advanced a theory that the reaction resulted in the formation of a photochemical acceptor, a compound of carbon dioxide sensitive to light. Willstätter and Stoll proposed the formation of a peroxide in the reduction of carbonic acid, and that the splitting off of oxygen from the peroxide constitutes the "Blackman Reaction." Later Warburg considered that his original theory was not tenable and on the basis of measurements of the inhibiting action of certain narcotics on the decomposition of hydrogen peroxide by *Chlorella* supported Willstätter and Stoll's theory. This phase of the

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[195] Warburg, *Biochem. Zeit.*, **100**, 264 (1919); **103**, 196 (1920).

[196] Warburg, *l. c.*, **103**, 193 (1920).

[197] Warburg, *Biochem. Zeit.*, **100**, 230 (1919); **103**, 188 (1920); **146**, 486 (1924). Willstätter and Stoll, "Untersuchungen über die Kohlensäure Assimilation," 1918, p. 395.

photosynthesis problem requires still a great deal more investigation before any definite conclusion can be arrived at.

Finally, as internal factors which affect the rate of photosynthesis there come into play the position of the chloroplasts, the accumulation and translocation of the material synthesized. These are discussed in the sections devoted to chloroplasts and to the time factor.

i. Effect of Various Substances, of Age, Electricity, etc.

It has long been a question whether other substances could be substituted for carbon dioxide in the photosynthetic process. There are a number of substances which when fed to plants produce starch, but most of these are of practically an equal energy content as hexose sugars, so that radiant energy is not necessary and the starch formation is not a photosynthesis. We are concerned first with those substances which require reduction, in which the reaction step to sugar is endothermic and hence would require the absorption of energy. The first to suggest itself in this relation is the lower oxide of carbon.

Carbon monoxide: de Saussure found that the behavior of plants in this gas is the same as in an atmosphere of nitrogen.[198] There were no evidences of photosynthesis. Virtually the same conclusions were arrived at by Boussingault [199] and by Stutzer.[200] Richards and MacDougal [201] found that larger quantities of carbon monoxide are toxic for phanerogams. Krascheninnikoff also found that it could not be used, while the results of Bottomley and Jackson [202] indicate that this gas can, to a measure at least replace carbon dioxide in photosynthesis. Recently Wehmer [203] has reported that carbon monoxide up to a concentration of 50 per cent exerts no injurious effect on the development of seedlings.

Formic Acid: Usher and Priestley [204] report the formation of oxygen from *Elodea* in 0.02 per cent solution of this acid, on illumination. Spoehr [205] has also found that in an atmosphere containing small quantities of formic acid but no carbon dioxide, leaves of the sunflower show an appreciable gain in dry weight and form starch in bright sunlight. Plants cannot endure high concentrations of this acid, and in the dark the toxicity soon becomes evident.

Ketene, $CH_2 : CO$, *and Carbon Suboxide* C_3O_2, have as far as we know not been investigated and on account of their poisonous and reactive properties probably offer little of interest. Collie [206] suggested that deriva-

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[198] Ostwald's "Klassiker d. Exakten Wissenschaften," No. 16, p. 27.

[199] Boussingault, "*Agronomie chimie agricole*," *etc.*, **4**, 300 (1868).

[200] Stutzer, *Ber. chem. Ges.*, **9**, 1570 (1876). Just, *Wallnys Forsch. Agrik. physik.*, **5**, 79 (1882). Krascheninnikoff, *Rev. gén. bot.*, **21**, 173 (1909).

[201] Richards and MacDougal, *Bull. Torrey Bot. Club*, **31**, 57 (1904).

[202] Bottomley and Jackson, *Proc. Roy. Soc.*, **72**, 130 (1903).

[203] Wehmer, *Ber. bot. Ges.*, **43**, 184 (1925).

[204] Usher and Priestley, *Proc. Roy. Soc.*, **78** B, 322 (1906).

[205] Spoehr, *Plant World*, **19**, 15 (1916).

[206] Collie, *Jour. Chem. Soc.*, **91**, 1806 (1907).

tives of these substances may play an important rôle in the formation of organic compounds found in plants.

Formaldehyde: This substance has played a very important rôle in the theoretical discussions of the photosynthetic process. The behavior of plants toward formaldehyde is considered in Chapter 5.

Anesthetics: The inhibiting effect of chloroform on the photosynthesis of aquatic plants was first described by Claude Bernard and has been repeatedly verified. If the anesthetization is not continued too long permanent injury to the chloroplasts can be avoided and they regain their activity. Bonnier and Mangin,[207] like Bernard,[208] showed that photosynthesis is more sensitive to the action of ether and chloroform than is respiration and that by carefully regulating the quantities of these substances administered, a separation of the two processes can be attained. Thus it is possible to stop photosynthesis and allow the respiration to continue. The separation is, however, not a very sharp one, for with the amounts of ether or chloroform required to stop photosynthesis, the rate of respiration is also affected; either it is somewhat inhibited or with small quantities of the anesthetic it is accelerated. Different species of plants show a wide variation in their reaction to ether and chloroform. A thorough investigation of the effect of anesthetics has been made by Irving. From this it is apparent that small doses of chloroform affect the gaseous exchange so that light produces little effect thereon. Very small concentrations of ether and chloroform exert a stimulating influence on respiration, but it is very doubtful whether any such acceleration occurs in photosynthesis. From the investigations of Willstätter and Stoll it is apparent that quantities of chloroform which inhibit the rate of photosynthesis effect a decomposition of the chlorophyll in leaves of *Prunus laurocerasus.* With ether, while recovery of the photosynthetic activity is not complete, there is apparently no appreciable alteration in the chlorophyll and carotinoid components.

Narcotics: morphine, cocaine, and quinine, inhibit photosynthesis. Treboux reports a 0.005 per cent solution of quinine hydrochloride of very slight effect, while a 0.15 per cent solution of the same salt inhibits completely.[209] Antipyrene is equally poisonous. Warburg has investigated the effect of urethanes. The inhibiting action of a homologous series depends apparently upon the absorption of these substances. Photosynthesis is far more sensitive to these substances than respiration. The latter is stimulated by weak solutions, which inhibit photosynthesis. The concentrations affecting respiration are about twenty times as strong as those affecting photosynthesis. The following table from Warburg illus-

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[207] Bonnier and Mangin, *Ann. Sci. Nat.* (7), 3, 14 (1886). Schwartz, *Unters. a. d. Bot. Inst. Tübingen*, **1**, 102 (1881). Ewart, *Jour. Linnéan Soc.*, **31**, 408 (1896). Irving, *Ann. Bot.*, **25**, 1077 (1911). Bose, "The Physiology of Photosynthesis," London, 1924, p. 60.

[208] Bernard, "Leçons sur les phénomènes de la vie," 1878, p. 278.

[209] Jacobi, *Flora*, **86**, 323 (1899). Treboux, *ibid.*, **92**, 56 (1903). Warburg, *Biochem. Zeit.*, **100**, 264 (1919); **103**, 196 (1920).

trates this difference; the experiments were carried out with high carbon dioxide-concentration, high light intensity, and at 25:

Narcotic	Photosynthetic Inhibition of 50 Per Cent by millimoles per liter	Respiration Inhibition of 50 Per Cent by millimoles per liter
Methyl-urethane	400	1200
Ethyl-urethane	220	780
Propyl-urethane	50	100
Butyl-urethane (iso)	17	43
Amyl-urethane (iso)	12	32
Phenyl-urethane	0.5	6

Inorganic Compounds: Some inorganic salts, even in great dilution, have a very decided effect on photosynthesis. The toxicity of solutions is not only a matter of the concentration but also of the total amount of the toxic salt available. For example, Treboux [210] found that twigs of *Elodea* in 100 cc. of 0.000015 per cent $CuSO_4$, 0.00016 per cent $ZnSO_4$ and 0.00015 per cent $CoSO_4$ were but very slightly affected; in 500 cc. of solutions one-tenth these concentrations, the plants were soon killed. The salts just mentioned, and those of mercury, are very toxic. The inhibiting effect on photosynthesis decreases gradually with concentration and appears to be about the same as the toxicity for the plant cells. Ferrous sulfate and ferric chloride, in not too dilute solutions, accelerate photosynthesis. The action may be due to the fact that these solutions have an acid reaction. Mercury vapor is very toxic.

Briggs [211] studied the effect of a deficiency of various mineral nutrients on photosynthesis. In cultures lacking one of the essential elements, namely, potassium, magnesium, iron and phosphorous, photosynthetic activity of *Phaseolus vulgaris* was decidedly less than in leaves grown in solutions with all the essential nutrients present. The same depression was observed whether external conditions such as light or temperature were limiting factors. Briggs endeavored to explain this on the basis that a lack of any of the essential elements resulted in a reduction of the reactive surface of the chloroplasts; the reactive surface affecting both the temperature and light reactions. Briggs also found that the photosynthetic activity of a plant grown in what is usually considered a complete mineral nutrient solution to be less than that of a plant grown in soil. Very little is known regarding the rôle of mineral nutrients in photosynthesis.[212]

Galwialo [213] reports that in leaves which have been cut into pieces and placed into water containing carbon dioxide, the rate of photosynthesis soon decreases and that photosynthesis can be revived by the addition to

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[210] Treboux, *l. c.*, 56. Rumm, *Ber. bot. Ges.*, **11**, 79 (1893). Frank and Krüger, *ibid.*, **12**, 8 (1894).

[211] Briggs, *Proc. Roy. Soc.*, **94** B, 20 (1922).

[212] Stoklasa and Matousek, "Beiträge zur Kenntniss der Zuckerrübe," Jena, 1916. André, *Compt. rend.*, **162**, 563 (1916).

[213] Galwialo, *Biochem. Zeit.*, **158**, 65 (1925).

the water of salts extracted from roots of the plants. He concludes that there are two elements necessary for photosynthesis, electrolytes and a ferment, that in a mixture of these components from the plants (maize, pea, bean, potato) and the electrolytes in water solution a reduction of carbon dioxide in sunlight is attainable. In the absence of more exact experimental data it is, however, difficult to judge of the value of these results.

Acids and Alkalies: Very dilute solutions of acids (0.0001 N.) exert a stimulating effect on photosynthesis. Treboux[214] found that this was the case with HCl, HNO_3, H_2SO_4, CrO_3, H_3PO_4, acetic, succinic, oxalic, tartaric and citric acids, as well as with $KHSO_4$ and KH_2PO_4. Higher concentrations are inhibitory. Bose reports that one part of HNO_3 in two billion parts of water caused an increase of nearly 200 per cent in the rate of photosynthesis. This author has also observed a stimulating effect of traces of iodine and of the extract of thyroid gland.

The effect of alkalies on the photosynthetic process is complicated because this becomes involved in the carbon dioxide-concentration available to the plant.

Potassium Cyanide in very dilute solutions produces inhibition of photosynthesis in aquatic plants. Lund and Holt[215] found complete but reversible inhibition in 0.00008 molal solution with fronds of the Pacific Coast kelp *Nereocystis.* Higher concentrations (0.0006 molal) produce permanent injury in the light, but when the plants are kept in these solutions in the dark no permanent injury was observed. Warburg also observed that the photosynthetic activity is affected by much lower concentrations of cyanide than is respiration. He found reversible inhibition of photosynthesis by *Chlorella* in a 0.0001 normal solution of prussic acid while respiration was not affected by a 0.01 normal solution. Warburg further reports that while his algæ were very sensitive to low concentrations of cyanide in the absorption of carbon dioxide, the cyanide did not prevent the utilization of the carbon dioxide produced in respiration. This fact is brought out when different light intensities are used: with high light intensity the rate of photosynthesis normally greatly exceeds that of respiration, and in a 0.05 normal HCN solution photosynthesis is greatly inhibited. As the light intensity is decreased, and the rate of photosynthesis consequently also decreases, an illumination intensity is reached at which photosynthesis equals respiration (compensation point). At this point all the carbon dioxide produced in respiration, but no more, is utilized in photosynthesis. A 0.05 normal solution of HCN does not affect this photosynthesis. In fact, according to Warburg, the degree of inhibition of cyanide solutions depends greatly upon the intensity of light. In a 0.0001 normal cyanide solution with high light intensity

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[214] Treboux, *l. c.*, 65. Wieler and Hartlieb, *Ber. bot. Ges.*, **18**, 348 (1900). Bose, *l. c.*, 64.

[215] Lund and Holt, *Proc. Soc. Expt. Biol. and Med.*, **20**, 232 (1923). Warburg, *Biochem. Zeit.*, **100**, 267 (1919); **103**, 199 (1920).

(about 19,000 Lux) photosynthesis was inhibited 65 per cent. The same concentration of cyanide with weaker light (1800 Lux), which normally utilized slightly more than the carbon dioxide that was produced by respiration, did not inhibit photosynthesis. Warburg has made use of these observations in his theoretical deductions. Further investigation of the subject with other methods and plants seems desirable.

Age: For a long time it was thought that leaves showed active photosynthesis only after they had attained a certain size and condition of development. While different species of plants vary greatly in their rate of development it is highly probable that the foregoing conclusion is erroneous. Undoubtedly the development of the capacity for photosynthesis depends very much upon the conditions of temperature and light under which the plants are growing, yet it appears from the results of Willstätter and Stoll that photosynthesis is quite high in leaves which are just unfolding. Young leaves have an exceedingly high rate of respiration which decreases to one-quarter of this rate when the leaf matures. The results of photosynthesis measurements depend in many instances upon a proper determination of respiration and such results vary according as to whether they are calculated on the basis of area, fresh weight or dry weight. Willstätter and Stoll's experiments show that the rate of photosynthesis after about nine days of growth is almost constant for equal areas of leaf surface. Calculated on the basis of dry weight and in some cases on the basis of fresh weight, the rate of photosynthesis decreases with age. On the basis of chlorophyll-content there is a decided decrease in photosynthetic activity. It is important, therefore, to consider carefully on what basis photosynthesis measurements are determined and also the methods which are employed in making such measurements.

Willstätter and Stoll also compared young leaves with those of the previous year of *Laurus nobilis* and of *Taxus baccata* and found that on the basis of fresh weight there was little difference, while on the basis of dry weight the older leaves had a lower photosynthetic rate. In the autumn, when the leaves are just beginning to change in color, on the basis of dry or fresh weight as well as on the basis of area, they show a decided decrease in photosynthesis. It is, however, hazardous to draw any very general conclusions regarding the effect of age and development on the photosynthetic activity of plants. The marked individual and specific peculiarities of different species together with their adaptive modifications resulting in differences of structure as well as composition of the leaves become evident in photosynthesis. In general, functional activity decreases with age, but owing to the complication of factors which is involved in producing this diminished activity, a quantitative expression of the causes underlying it is as yet impossible.[216]

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[216] Ewart, *l. c.,* 452. Kreusler, *Landw. Jahrb.,* **14,** 913 (1885). Willstätter and Stoll, *l. c.,* 86.

Electricity: For more than a century the question of electro culture and the influence of electric currents on the production of crops has, in the hands of different investigators, been the subject of almost continuous investigations. Few of these researches have been devoted to the more immediate causes of the reported acceleratory action of electric currents on the growth of plants and the production of crops. There are a number of general theories, such as that of Lemström, that the electric current "produces an augmentation of the energy with which the circulation of the juices is going on." Also the acceleratory action has been variously ascribed to the formation of nitric and nitrous acids, ammonia and ozone.

Relatively little work has been done on the effect of electric currents on photosynthesis. Thouvenin has reported a decided acceleration of the oxygen evolution by some aquatic plants in the light. Several Daniel's cells in series were connected so that the two poles were at opposite ends of a branch of *Elodea*. A current of 0.0005 to 0.001 ampere increased the rate of oxygen emission taking place in the light. Plants in which the photosynthesis was inhibited by chloroform were not affected by the electric current. Pollacci reported an acceleration of starch formation in land plants under the influence of direct current. His conclusion that the electric current can replace radiant energy is of great interest, but probably needs further confirmation. The direction of flow of the current through the plant is apparently also of significance. The entire subject is in need of thorough working over with a view of coördinating and extending the isolated observations and of establishing more accurately all the chemical and electrical conditions involved.[217]

Henrici[218] has made the interesting observation that the rate of photosynthesis is influenced by the degree of ionization of the atmosphere. In her experiments the air containing carbon dioxide was either discharged or its conductivity increased by means of thorium oxide. The rate of photosynthesis is greatly accelerated in an atmosphere of high conductivity. Under favorable conditions the rate of photosynthesis in ionized air is 1.5-4 times that in discharged air. Thus, in an atmosphere of high conductivity it is possible to obtain a rate of photosynthesis with a light intensity which in unionized air produces no photosynthesis. However, no matter how great the conductivity, it is not possible to obtain photosynthesis without light. The influence of the atmospheric ionization is apparently closely associated with the carbon dioxide in the air. Individual plants and different species exhibit a wide variation in their reaction to ionized and unionized air and a great deal more experimental

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[217] Lemström, "Electricity in Agriculture and Horticulture," London. Thouvenin, *Rev. gen. bot.*, **8**, 432 (1896). Pollacci, *Atti. Ist. Bot. Pavia* (2), **13** (1907): *Bot. Cent.*, **99**, 544 (1905). Koltonski, *Beih. Bot. Cent.*, **23**, 1, 204 (1908). Bose, "Physiology of Photosynthesis," p. 72. Kny, *Ber. bot. Ges.*, **15**, 398 (1897). Waller, *Ann. Bot.*, **39**, 516 (1925).

[218] Henrici, *Arch. Sci. Phy. Nat.* (5), **3**, 276 (1921). Spoehr, *Bot. Gaz.*, **59**, 366 (1915).

work is necessary in order to employ these observations for the formulation of a theory of the rate of atmospheric ionization in photosynthesis.

3. The Compensation Point

The light intensity at which the respiratory and photosynthetic activities compensate each other, i.e., where there is an equilibrium, or steady state, $C_nH_{2n}O_n + nO_2 \rightleftarrows nCO_2 + nH_2O$, and the gaseous exchange is consequently zero, has been designated by Plaetzer[219] as the compensation point. As this point is dependent upon the rate of respiration it varies greatly in different plants. Plaetzer studied only aquatic plants and employed the bubble counting method with the precautions prescribed by Kniep for plants with an intercellular system. (See Chapter 4.) In using plants without an intercellular system the titration method of Winkler was employed for determining the oxygen in the water.

The effect of temperature on the compensation point as determined by Plaetzer is of considerable interest. It appears that with decreasing temperature the compensation point is lowered. Thus, the compensation point, expressed in Hefner candles, changes with temperature as follows:

Spirogyra,	174	at 20° ;	26.7	at 5°.	*Fontinalis,*	150	at 20° ;	40	at 5°.
Cladophora,	253.3	" " ;	62.9	" "	*Cinclidotus,*	400	" " ;	75	" "

It would appear from the above that with a given light intensity photosynthesis increases with decreasing temperature. In fact, Plaetzer was able to demonstrate that a light intensity which at 20° represented the compensation point of *Cinclidotus*, at 5° produced a decided evolution of oxygen due to photosynthesis. That is, with the same light intensity which at 20° just balances the energy transfers, at 5° there is a gain for the plant. It should be noted, however, that the actual total rate of photosynthesis at 5° is lower than at 20°, but in relation to the respiration it is higher. Paradoxical as this appears at first glance, it must be clearly borne in mind that we are dealing here with light of low intensity, i.e. at these temperatures the light is the limiting factor. It must be recalled again that in photosynthesis the three chief external factors, any one of which may, according to circumstances, be "limiting" are carbon dioxide-concentration, light intensity and temperature. Now then, given an ample supply of carbon dioxide, if a plant at a definite temperature, is exposed to a light intensity just sufficient to surpass the compensation point, oxygen will be emitted. If the light intensity is increased, oxygen emission (photosynthesis) will also increase; if the temperature is increased photosynthesis will not increase. That indicates that the intensity of illumination—not the temperature—determines the rate of photosynthesis, i.e. light is the limiting factor. If the light intensity is further increased, a point will be finally reached at which photosynthesis, even with the most intense light, will not increase. Here light is no

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[219] Plaetzer, *Verhandlungen Physik-Med. Ges. Würzburg N. F.*, **45**, 31 (1917).

longer the determining or limiting factor. At this point (still provided there is ample carbon dioxide) temperature becomes the determining factor and an increase in temperature increases the rate of photosynthesis.

The results of Plaetzer on the compensation point must be regarded in the light of these facts. The observation that photosynthesis decreases with increasing temperature can hold only for a limited range of light intensity. When this is increased so that it no longer is a limiting factor at the lower temperature, but rather the temperature constitutes the limiting factor, then photosynthesis will increase with increasing temperature.

Perhaps a clearer insight of the phenomenon of apparent decreasing photosynthesis with increasing temperature when light is the limiting factor can be obtained from Plaetzer's quantitative data. Let *i* represent the light intensity which at 5° produces compensation between respiration and photosynthesis. It will be recalled that at 20° *i* no longer is the compensation point and that with light intensity *i* at 20°, carbon dioxide is given off by the plant, i.e., oxygen is taken up and respiration overbalances photosynthesis. The question then arises, is the absolute amount of oxygen produced in photosynthesis with light intensity *i* at 5° the same as at 20°. The following simplified data answer the question:

a = respiration (CO_2 emitted) at 20° in the dark.
b = respiration (CO_2 emitted) at 5° in the dark, i.e., the respiration which at the compensation point *i* is equal to photosynthesis, hence also b = photosynthesis at 5°.
c = oxygen consumed at 20° under the influence of light intensity *i*.

Then a = b + c if the light intensity is the limiting factor. If this equation does not hold the action of *i* is different at 5° than at 20°, i.e., greater or less. Plaetzer's results are:

$$\begin{aligned} a &= 4.3 \\ b &= 1.38 \\ c &= 2.97 \\ b + c &= 4.35. \end{aligned}$$

In the equation a = b + c the assumption is, of course, made that the rate of respiration is the same in the dark as in the light. This is still a debated question and no conclusive evidence is as yet available. If *i* has the same influence, x, on respiration at 5° and at 20°, then c would remain unchanged, for (a + x) — (b + x) = c. The nature of x is still unknown, though it is possible that the difference between a = 4.3 and b + c = 4.35 indicates a slight stimulation of respiration. The main result shows quite definitely, however, that with light constant and the limiting factor, a change in temperature does not alter the absolute rate of photosynthesis, while respiration is directly influenced.

Plaetzer furthermore showed with *Cladophora* that with increasing temperature, the compensation point (i.e. light intensity required to maintain respiration—photosynthesis at an equilibrium) rises more rapidly than the rate of respiration, determined in the dark. Thus, with an increase in temperature from 5° to 25° the rate of respiration showed a proportionate rise of 1 : 4.8. The light intensity required to maintain the compensation point over this same range of temperatures showed a corresponding proportion of 1 : 6.69.

The explanation of this phenomenon is in all probability not a simple one. Aside from the possibility (not yet established positively) that light has a stimulating action on respiration, it would appear that photosynthesis, under these conditions, did not increase in proportion to the increase in light intensity. Such a direct proportionality could exist only under conditions where light was the limiting factor. Now it is highly probable that with the increasing light intensity a point was reached where light was no longer the limiting factor and temperature played the rôle. Since it has been found that with high illumination and high carbon dioxide-concentration the temperature coefficient of photosynthesis decreases with increasing temperature (4.3 at 5° to 1.6 at 32°) it would be expected that the rate of photosynthesis would not increase at the same rate as respiration with increasing temperature. From the recent work of Harder,[220] it is apparent, moreover, that where one factor ceases to be a limiting factor and another factor commences to play this rôle is not a definite point, but rather that there is a gradual transition from one condition to another. This fact must also be taken into consideration in interpreting the phenomenon just described. Finally the relative decrease in the rate of photosynthesis as compared to that of respiration with increasing temperature must also be viewed in the light of the influence of the "time factor." The latter, as well as a discussion of limiting factors have been taken up in a previous section.

The position of the compensation point of a plant in regard to temperature is naturally of great importance to the life of the plant and its relation to the environment. The fact that with decreasing temperature respiration decreases more rapidly than photosynthesis has also been recorded by Harder.[221] His results indicate that *Cladophora*, at low light intensity, has a higher photosynthetic activity at low temperatures than at higher temperatures. Harder points out that conditions must exist in nature where at higher temperatures the plant gains no material through photosynthesis on account of the excessive respiratory activity, while at lower temperatures with the same light intensity nutritive material is formed in the plant. These conditions would, of course, apply particularly to the seas of the polar zones where the light intensity is low. Harder gives the following ratio of:

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[220] Harder, *Jahrb. wiss. Bot.*, **60**, 531 (1921).
[221] Harder, *Jahrb. wiss. Bot.*, **56**, 281 (1915).

$\frac{\text{Photosynthesis}}{\text{Respiration}}$ for different temperatures:

20 — 22	0.5882	0.4227	0.4280
2 — 3.5°	1.603	0.9207	2.059

Plaetzer found, that while the different species vary greatly in the light intensity at which compensation is attained, the same species seems to possess a definite compensation point. Harder,[222] on the other hand, was able to demonstrate that the compensation point is a variable quantity, varying more than 100 per cent, for any one species depending upon the previous conditions of illumination of the plant. Thus, ivy leaves which had grown in the direct sunlight showed a compensation point of 2477 Lux, while similar leaves, grown in the shade, one of 1133 Lux. Harder was able, moreover, to demonstrate that with continued darkness the compensation point is reduced although the difference between the two sets of plants, sun and shade plants, is maintained. Thus, two sets of *Fontinalis antipyretica,* the one taken from a sunny, the other from a shady habitat, possessed, after corresponding periods in darkness the following compensation points:

Date	Sun Plants	Shade Plants
Sept. 20	152 Lux	95 Lux
26	118	79
30	111	74
Oct. 2	84	64
5	59	< 41
10	27	about 10

Harder also showed that by starting with the same culture of *Cladophora* and keeping one portion in diffuse light and another in direct sunlight great differences in the compensation point result within seven days.

Similar results have been obtained by Boysen-Jensen.[223] In *Sinapis alba,* a light plant, the compensation point lies at 1.0 (Bunsen units × 100); while in *Oxalis acetosella,* a shade plant, the compensation point lies at 0.2.

It is evident, therefore, that the compensation point is by no means of constant value for any single species of plant and that it is essential that the previous condition of the plant as to illumination must be taken into consideration before attempting to draw conclusions from the value of the compensation point. Harder's results show that under circumstances in *Fontinalis* photosynthesis more than overbalances respiration at an illumination of 10 Lux, while under other circumstances 150 Lux is not sufficient to accomplish this. To show that the compensation point can

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[222] Harder, *Ber. bot. Ges.,* **41**, 194 (1923).

[223] Boysen-Jensen, *Bot. Tidsskr.,* **36**, 219 (1918). Staelfelt, "Meddel. fran Statens Skogsförsöksanst.," Stockholm, **18**, No. 5 (1921).

rise very high, Harder quotes Klebs who found that the leaves of the beech tree which had been kept under constant and high illumination still emitted carbon dioxide at 6250 Lux. The great importance of a consideration of such circumstances to plants in nature needs no special elaboration.

The investigations of O. Warburg[224] on the efficiency of the photosynthetic process are pertinent to the subject of the nature of the compensation point. He found wide fluctuations in the quotient: absorbed radiant energy to chemical work performed, depending upon the previous treatment of the plants studied. It was established that when the plants are cultivated under conditions of high light intensity they are capable of utilizing only a small amount of the absorbed radiant energy. On the other hand, when the plants are cultivated under conditions of low light intensity they are capable of utilizing a relatively large proportion of radiant energy absorbed. By cultivation under conditions of either high or low light intensity, one type of plant can apparently be converted into the other within a few days, and its photosynthetic efficiency altered accordingly. It is doubtful on the basis of the work of Willstätter and Stoll, that this alteration can be entirely at least ascribed to alterations in chlorophyll-content, as they found no direct proportionality between this and photosynthetic rates.

It is apparent, therefore, that merely increasing the light intensity or the period of illumination of a plant does not result in a higher photosynthetic efficiency.

Some very interesting observations of the effect of hydrocyanic acid on the compensation point have been made by Warburg[225] on the green alga *Chlorella*. A 0.0001 normal solution of hydrocyanic acid exerts a decidedly inhibiting effect on the photosynthetic activity; on removing the plant from the HNC solution normal photosynthesis is again attained. Respiration, on the other hand, is not affected in the same manner. A 0.01 N. solution of HNC slightly stimulates the oxygen consumption and carbon dioxide emission and only after several hours exerts a poisonous or inhibiting effect. By studying the influence of various concentrations of HNC on photosynthesis Warburg found that oxygen evolution is inhibited by very small amounts of HNC, but that there is a point beyond which even relatively high concentrations of the poison have no effect. Warburg's results seem to indicate that in 0.05 normal HNC solution the plants are incapable of taking up and reducing carbon dioxide even in high light intensity. That is, plants with a compensation point of 500 Lux in 0.05 normal HNC solution produced no more oxygen at a light intensity of 19,000 Lux than with 500 Lux. Also the compensation point is not affected by the HNC, for the carbon dioxide produced by respiration is reduced by means of light of low intensity at the same rate in plants exposed to cyanide as in plants under normal conditions. Thus it appears that relatively high concentrations of HNC (0.05 normal) do

[224] Warburg, *Z. physik. Chem.*, **102**, 246 (1922).
[225] Warburg, *Biochem. Zeit.*, **103**, 199 (1920).

not affect the mechanism of photochemical reaction, as is indicated by the maintaining of the compensation point in plants treated with HNC, but that this substance does affect the photochemical reactivity of the carbon dioxide which the plant normally takes up from the surrounding medium.

An example of the effect of low concentration of cyanide on photosynthesis, as found by Warburg, is given below:

Approximate Light Intensity in Lux.	Cyanide Conc. in moles per liter	Length of Experiment in minutes	Photosynthesis
1,800	0	30	61
1,800	0.0001	30	61
19,000	0	5	540
19,000	0.0001	5	192

In these plants the compensation point was about 500 Lux. It appears, therefore, that the light intensity of the compensation point does not determine the point where the inhibiting action of HNC commences; the critical light intensity is higher. In 0.0001 normal solution of HNC, carbon dioxide is absorbed and reduced, but the rate is considerably decreased as compared with plants not treated with cyanide. It seems highly probable that an interpretation of Warburg's results will finally be found in the effect of HNC on the absorptive capacity of the material in the plant which first takes up the carbon dioxide from the surrounding medium.

Chapter 3

The Products of Photosynthesis

The substances which are known to be produced in photosynthesis are oxygen and carbohydrates. It was, in fact, through the formation of oxygen that photosynthesis was discovered. Oxygen and carbohydrates must be regarded as the final products of what is apparently a series of reactions comprising the photosynthetic process. We have as yet no definite and experimentally satisfactory evidence regarding the intermediate products in these reactions. The intermediate or first formed product never accumulates to any extent so that it has been extremely difficult to ascertain the precise manner in which carbonic acid is converted into carbohydrates and oxygen. For years it has been the object of chemists and physiologists to describe the chemical reactions involved, but probably the greatest obstacle has been a lack of reliable knowledge concerning the first formed product. In the absence of direct observational evidence many theories have been proposed to "boost" us over this obstruction.

In this chapter we shall confine the discussion to the products which are known to be produced as a result of photosynthesis. In the chapter on the chemistry of photosynthesis the different theories which have been formulated to describe the various steps in the process are discussed. As has been stated, oxygen and carbohydrates are the two chief products of photosynthesis, and while the formation of each of these is chemically closely related, their production in photosynthesis has to a considerable extent been investigated separately. The quantitative relation between the liberation of oxygen and the formation of carbohydrates has received little attention.

1. The Liberation of Oxygen

While it can be demonstrated very easily that the atmosphere surrounding a plant which is photosynthetically active becomes richer in oxygen and depleted of carbon dioxide, the composition of the gas which is emitted by the plant can be more accurately determined with aquatic than with land plants. The gas which is emitted from an aquatic plant during photosynthesis is not pure oxygen, but contains 25 to 85 per cent of this gas and varying amounts of nitrogen and carbon dioxide. The percentage of oxygen in the escaping gas increases with the rate of photosynthesis and vice versa; this phenomenon has already been discussed in Chapter 2.

The quantitative relations of the amount of carbon dioxide absorbed to that of oxygen emitted have been subjected to careful examination. These values, termed the photosynthetic quotient, have been found to be very close to unity and are also discussed in greater detail in Chapter 2. Oxygen emission commences immediately on illumination of the green parts and stops when the light exposure ceases. According to Kostytschew[1] the amount of oxygen emitted is considerably less than that of carbon dioxide absorbed during the first few minutes of illumination; after a short period of illumination (15 to 30 minutes) the $\frac{CO_2}{O_2}$ ratio attains unity.

2. The Carbohydrates of the Leaf

The early work on photosynthesis was to a great extent confined to investigations on the gaseous exchange of plants, and, owing largely to the undeveloped state of organic chemistry, little advance was made regarding the substances which were formed from the decomposition of the carbon dioxide. Ingen-Housz, Senebier and de Saussure realized that the carbon dioxide absorbed during photosynthesis was converted into material which was appropriated by the plant for the formation of new tissue and was used to maintain respiration. But the chemistry of these substances was treated only very meagerly.

The first decisive step in identifying carbohydrates with the photosynthetic process was made by Sachs. Since the time of Ingen-Housz and Senebier it was realized that photosynthesis was dependent upon chlorophyll. Von Mohl, the discoverer of protoplasm in plants, in 1837, had made an intensive study of chlorophyll, had described the chloroplasts and recognized starch in them. A great deal of confusion existed regarding the nature and function of the chloroplasts, but von Mohl developed the conception that the starch which was associated with the chloroplasts was of the nature of reserve food material. These investigations together with those of other anatomists, notably Nägeli, determined the structure, and to some degree also the function, of the chloroplasts. Gries[2] then demonstrated that the starch in the chloroplasts disappears when the leaves were kept in the dark. It was, however, not certain whether starch or chlorophyll was the first to appear in the illuminated plant so that the genetic relationship of these two substances was still uncertain.

With these facts as a background Sachs[3] undertook his classical researches on the function of chlorophyll. These established the fact that

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[1] Kostytschew, *Ber. bot. Ges.*, **39**, 319 (1922).

[2] Gries, *Ann. Sci. Nat. Bot.*, **8**, 179 (1857).

[3] Sachs, *Jahrb. wiss. Bot.*, **3**, 184 (1863); *Bot. Zeitg.*, **20**, 365 (1862); **22**, 289 (1864); *Arbeiten aus dem bot. Inst. Würzburg*, **3**, 1 (1884).

starch is a product of photosynthesis and is formed in the chloroplasts. These organs have the power of forming starch in the light and again dissolving it in the dark. Sachs developed the macrochemical method of determining the amount of starch in a leaf on the basis of the coloration produced with iodine. By means of this method he also demonstrated the necessity of light, chlorophyll and carbon dioxide for starch formation. He showed that starch represents a reserve food material and his investigations indicate that starch formation is the result of an accumulation of the products of photosynthesis.

That starch is deposited in the chloroplasts, even in the dark, when leaves are floated on solutions of soluble carbohydrates was demonstrated by Böhm.[4] This was an exceedingly important observation. Already at that time, as still, there existed an active controversy regarding the first product of photosynthesis. Sachs took the stand that starch was the first visible product. This method of producing starch in plants kept in the dark, from soluble carbohydrates has been extensively investigated.[5] Starch is formed under these circumstances in the plastids, whether they contain chlorophyll or not. Starch formation in photosynthesis is therefore probably a secondary reaction and cannot be regarded as a direct product of photosynthesis, but is formed when the concentration of the simple, soluble carbohydrates attains a sufficiently high concentration.

Plants form starch in the dark, not only from the carbohydrates, glucose, fructose, galactose, mannose, sucrose and maltose, but also from other organic compounds. Thus alcohols such as mannitol, dulcitol, erythritol and glycerine also are capable of being used for starch formation in the plant. The pentose, arabinose, is also capable of conversion into starch according to reports of Polonovski[6] and Morillez. The fact that such a variety of substances can be utilized by the plant for starch formation would indicate that it is fallacious to draw conclusions regarding the first product of photosynthesis from the observation that any particular substance produces starch. Nor can it be concluded that all substances which form starch are normally the products of photosynthesis.

The independence of starch formation and photosynthesis is further illustrated by the fact that some plants, although they carry on active photosynthesis, never produce starch. Böhm showed this to be the case and Meyer made a classification of the plants which form starch and those which do not. The plants which form no starch contain disaccharides and monosaccharides. There are also some plants which store their carbohydrate in the form of inulin instead of starch. Böhm also showed that some plants in which normally no starch is found, e.g. *Galanthus,*

[4] Böhm, *Bot. Zeitg.*, **41**, 33, 49 (1883).

[5] Meyer, *Bot. Zeitg.*, **44**, 81, 105, 129, 145 (1886). Laurent, *Bull. Soc. bot. Belgium*, **26**, 243 (1887). Klebs, *Unters. bot. Inst. Tübingen.*, **2**, 489 (1888). Bokorny, *Biol. Centrabl.*, **17**, 1 (1897). Saposchnikoff, *Ber. bot. Ges.*, **7**, 259 (1889). Winkler, *Jahrb. wiss. Bot.*, **32**, 525 (1898).

[6] Polonovski and Morillez, *C. A.*, **19**, 2064.

Hyacinthus, *Ornithogalum* and *Iris* can be induced to starch formation when the leaves are kept on a 20 per cent solution of sucrose for 8 to 10 days.

The question as to which of the carbohydrates is the first one to be formed in the photosynthetic process has been a matter of controversy for a great many years and is not yet definitely settled.[7] That the starch in leaves is to be regarded as transitory reserve material and is a condensation product of previously formed sugar was repeatedly suggested, while others clung to the opinion that depending on the internal conditions in the leaf either sugar or starch is formed. Schimper was probably the first to express the idea that glucose was the precursor of starch and that starch was formed only when the glucose had attained a certain concentration. The concentration of glucose necessary for starch formation varies widely in different plants; in some plants it is normally never attained while in others it is reached after brief periods of photosynthesis, so that in the latter plants starch is a regular component of the chlorophyll bearing cells.

From the investigations of Winkler[8] it follows that with few exceptions all chloroplasts and leucoplasts which are sufficiently well developed are capable of starch formation when the concentration of glucose or sucrose is high enough. According to these observations the minimal concentration for starch formation in most cases is 0.2 per cent sucrose. The optimal concentration is about a 10 per cent sucrose solution, higher concentrations are only very slightly more effective and a 30 per cent sucrose solution produces no starch. The concentration of sugar which induces starch formation varies with different plants and no general rule covering all plants can be made. Accurate determinations of this nature, with due consideration of the influence of temperature, would be very desirable.

Most leaves contain monosaccharides, disaccharides as well as polysaccharides. The proportion in which these are present is affected by various factors and constitutes an equilibrium: monosaccharide $\rightleftarrows$ disaccharide $\rightleftarrows$ polysaccharide. The transformation of one into another is the result of reversible enzymatic reactions. The equilibrium is affected not only by concentration of one of the members of the group but also by temperature.

Before discussing the conversions which the different groups of carbohydrates undergo in the plant it will be necessary to describe briefly the carbohydrates which have been found in plants. It may be stated that the entire subject of the carbohydrates of foliage leaves would profit by an extensive and critical experimental investigation. Owing to the large number of members of this group, the difficulty of separating these, the variety of other compounds which interfere in their purification and separation and the possibility of changes being produced by enzymes and other substances during the analysis, the work on this problem is ex-

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[7] Pringsheim, *Jahrb. wiss. Bot.*, **13**, 441 (1882), for older literature. Meyer, *Bot. Zeitg.*, **43**, 505 (1885); **44**, 147 (1886). Schimper, *ibid.*, **43**, 779 (1885).

[8] Winkler, *Jahrb. wiss. Bot.*, **32**, 525 (1898).

ceedingly arduous. The problem is, in fact, very similar to that which Osborne and his collaborators have pursued with such success on the proteins in plants. As yet we have reliable evidence regarding only those carbohydrates which are present in relatively large quantities. Those which may be of special importance in connection with the mechanism of photosynthesis and to the physiology of the leaf in general may be present in only very low concentration. It does not follow that just because a substance is present in large quantities it plays a proportionately important rôle in the chemical changes taking place in the cell. In fact it is probably the more stable and less reactive substance which accumulates and is thus detectable; the highly reactive molecules probably determine the course of the reactions, though they are naturally much more difficult to obtain. Few attempts have been made to obtain evidence of the existence of such transitory substances, with the possible exception of formaldehyde.

A larger number of carbohydrates have been obtained in pure form from plants. In most cases, however, these have been obtained not from organs in which photosynthesis was taking place, such as leaves, but in more specialized organs in which the carbohydrates were deposited in large quantities. We are here more particularly interested in the immediate products of photosynthesis, while the carbohydrates in storage organs such as fruits and tubers may well represent conversion products of the substances formed in photosynthesis. As a matter of fact only few of the carbohydrates have been isolated in pure form from leaves and the evidence of their existence is based upon indirect proof, such as reducing power and optical rotation. The experimental difficulties of determining the first product of photosynthesis and, in fact, of any of the components in leaves have already been alluded to. These difficulties are, first of all, of an analytical nature, that is, of devising methods by means of which the great mixture of substances can be quantitatively determined; and secondly, of ascertaining the nature of the transformations which the carbohydrates in the leaf undergo quite independently of photosynthesis. The green leaf is not only an organ for the manufacture of carbohydrates which are used and transformed in other portions of the plant. It is a living organism, in it the carbohydrates undergo a great variety of changes yielding scores of products. To separate these various chemical reactions and endeavor to follow any one of them is, needless to say, a most difficult task.

For the purpose of orientation we shall discuss briefly the carbohydrates which are of special importance in relation to the photosynthetic process and the carbohydrate transformations in the leaf. For a more exhaustive discussion of the chemistry of the carbohydrates reference must be made to special works on this subject.[9] When regarded

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[9] Lippmann, E. O. von, "Die Chemie der Zuckerarten," 1904. Armstrong, E. F., "The Simple Carbohydrates and the Glucosides," 1924. Maquenne, L., "Les Sucres et Principaux Dérivés," 1900. Czapek, F., "Biochemie der Pflanzen," Vol. I, 1913.

from the point of view that carbohydrates are the first discernible products of photosynthesis and that they give rise to the tremendous number of compounds found in nature, the importance of carbohydrates in the chemistry of living things becomes apparent. Thus many of our most prized economic substances as fats, oils, rubber, alkaloids, glucosides, etc., are the result of carbohydrate transformations through the metabolic activity of the plant. The mode of formation of these substances is essentially a chemical problem. As yet only a feeble beginning has been made in unravelling the tangle of chemical reactions involved in the formation of the multiplicity of compounds produced by plants.

a. Dioses and Trioses.

Glycollic aldehyde, $CH_2OH . CH : O$, the simplest true carbohydrate, has not been found in plants.[10] It is formed as an intermediate product in the condensation of formaldehyde to carbohydrates by means of alkalies and as Pribram and Franke [11] showed it is also produced by the action of ultra-violet light on formaldehyde solutions. Glycollic aldehyde is a possible intermediate product in the synthesis of hexoses in the plant.[12] This is based upon the fact that glycollic aldehyde is easily condensed to tetroses and hexoses; the reaction proceeds much more readily than the condensation of formaldehyde.[13]

Glycollic aldehyde is also condensed to glycogen in the liver of the animal organism.[14]

Glyceric aldehyde, $CH_2OH . CHOH . CH : O$. This sugar has also not been found in plants, though we can find no record that it has been searched for. It is very easily condensed to a mixture of optically inactive hexose sugars. Fischer [15] long ago suggested that, in view of the difficulty of gaining reliable evidence of the existence of formaldehyde in plants, it may prove more fruitful to look for glycerose, a mixture of glyceric aldehyde and dioxyacetone. It has been assumed that the trioses are intermediate products in the formation of hexoses from glycerine by the plant.

A triose of the constitution $CH_2(OH) . CH(OH) . O . CH_2(OH)$ has been isolated by Buston and Schryver [16] from cabbage leaves. The

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[10] Euler, *Ber. chem. Ges.*, **39**, 50 (1906).

[11] Pribram and Franke, *Monatsh. Chem.*, **33**, 415 (1912).

[12] Fincke, *Biochem. Zeit.*, **61**, 157 (1914). Lippmann, *Ber. chem. Ges.*, **24**, 3306 (1891). Reinke, *ibid.*, **14**, 2148 (1881).

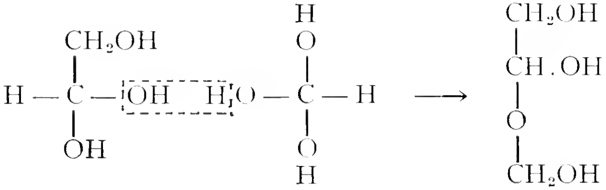
[13] Fischer and Landsteiner, *Ber. chem. Ges.*, **25**, 2549 (1892). Nef, *Ann. Chem.*, **357**, 291 (1907); **376**, 40 (1910). Fenton, *Jour. Chem. Soc.*, **65**, 899 (1894); **67**, 48, 774 (1895); **69**, 546 (1896); **71**, 375 (1897); **73**, 71 (1898); **81**, 426 (1902); **87**, 817 (1905).

[14] Barrenscheen, *Biochem. Zeit.*, **58**, 300 (1914).

[15] Fischer, *Ber. chem. Ges.*, **23**, 2138 (1890). Witzemann, *Jour. Amer. Chem. Soc.*, **36**, 1766, 1908 (1914).

[16] Buston and Schryver, *Biochem. Jour.*, **17**, 470 (1923).

possibility is suggested that it is formed by the condensation of glycollic aldehyde and formaldehyde; both in the hydrated form:



$$\begin{array}{c} CH_2OH \\ | \\ H-C-OH \\ | \\ OH \end{array} + \begin{array}{c} H \\ O \\ | \\ HO-C-H \\ | \\ O \\ H \end{array} \longrightarrow \begin{array}{c} CH_2OH \\ | \\ CH.OH \\ | \\ O \\ | \\ CH_2OH \end{array}$$

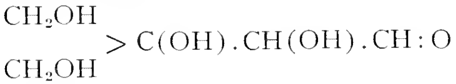
The substance crystallizes in needles and melts at 148°; it is readily soluble in cold water and hot glacial acetic acid, but is insoluble in most organic solvents. It does not reduce Fehling's solution and forms no phenylosazone, and is not easily hydrolyzed. It forms a tribenzoyl derivative melting at 52-53°.

b. Tetroses.

Tetroses do not appear in plant leaves. The alcohol erythretol has been found as an ester of orsellic acid in erythrin, $C_{20}H_{22}O_{10} . 1\frac{1}{2}H_2O$, in certain algæ and lichens.

Digitoxose, $C_6H_{12}O_4$, has been obtained as a product of hydrolysis of the glucoside digitoxin which is found in the leaves of digitalis. From the investigations of Kiliani it has the constitution $CH_3 - (CHOH)_3 - CH_2 - CH:O$, and is a partially reduced methyl pentose. Digitoxose crystallizes from alcohol on the addition of several volumes of ether in prism and plates, melting at 101° and $(\alpha)_0 = +46°$. It is easily soluble in water, alcohol and acetone. It forms one oxime, melting at 102°, soluble in water and alcohol but not in ether; it does not form a crystalline phenylhydrazone or phenylosazone. The cyanhydrine, on hydrolysis forms an acid, the lactone of which crystallizes in needles melting at 158° and possessing the formula $C_7H_{12}O_5$.[17]

Apiose, $C_5H_{10}O_5$, has been obtained by the hydrolysis of the glucoside apiin present in the leaves of parsley, celery, etc. According to Vongerichten[18] it is a β-oxymethyltetrose:



$$\begin{array}{l} CH_2OH \\ CH_2OH \end{array} > C(OH).CH(OH).CH:O$$

It has not been obtained in crystalline form, has a slight positive rotation and is not fermentable. On reduction with phosphorous and hydriodic acid it yields isovaleric acid which indicates that there is a forked chain in the molecule. Apiose forms a phenylosazone, melting at 155-157° crystallized from alcohol, and is soluble in acetone and somewhat soluble

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[17] Kiliani, *Arch. Pharm.*, **233**, 299 (1895); **234**, 273 (1896); **235**, 425 (1897); **237**, 446 (1899); **251**, 562 (1913); **254**, 255 (1916).

[18] Vongerichten, E., *Ann. Chem.*, **318**, 121 (1901); **321**, 71 (1902); *Ber. chem. Ges.*, **39**, 235 (1906).

in ether. Apiose benzylphenylhydrazone, crystallized from benzol, melts at 135°, and the p-bromphenylosazone, from alcohol, melts at 212°.

c. Pentoses.

Pentoses are very common components of leaves and other portions of plants. In the combined form, as pentosans, they have been found widely distributed, while free pentoses have been found only in small quantities. In fact, the existence of the free sugars as components of leaves has not been universally accepted. The pentoses usually occur as components of complex substances such as glucosides, nucleinic acids and most commonly as anhydride-like condensation products of unknown molecular weight. In the latter form they are found as pentosans, hemicellulose, gums and mucilage. The chemistry and structure of these latter compounds has as yet been but imperfectly worked out. The pentose and hexose sugars in the leaves differ in that the concentration of the latter in the free or uncondensed form is usually much higher than the former. On the other hand, of the total amount of sugars present not infrequently one-half are pentoses. While, then, of the reducing sugars present in plants, the pentoses may constitute but a very small portion or be entirely absent, of the total non-reducing sugars or polysaccharides of the plant, there is usually a considerable proportion of pentoses. They are usually found in the anhydride form as components of the cell walls and vessels in the structural elements of plants and may constitute a portion of the protoplasm. Thus, they are found in straw, wood, pith, seed hulls, the expressed juice, and in many gums.

The amounts of pentosans in different plants have been determined by Tollens [19] and his students. Many of these determinations are, however, not to be taken as precise values, as the analytical methods employed have since been found not to yield very exact results. Fresh oak leaves have been found to contain about 10 per cent of pentosans, pine needles about 7 per cent, sphagnum 15 per cent, lichens 3 to 8 per cent, mosses 3 to 17 per cent, and the woody portion of American conifers 6 to 10 per cent, while American deciduous trees contain 10 to 24 per cent.

In mangold leaves, Davis, Daish and Sawyer [20] found the free pentoses to vary from about 0.4 to 0.9 per cent according to the season and Spoehr [21] found slightly lower amounts in *Opuntia phaeacantha*.

d. Methods of Analysis of Pentoses.

Owing to the fact that in the analysis of plant material, as for example, the carbohydrates of leaves, we have to deal with a very complex

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[19] Tollens, *Ber. chem. Ges.*, **2**, 1751 (1890); **24**, 694, 3575 (1891); **25**, 2912 (1892). De Chalmot, *Am. Chem. Jour.*, **15**, 21, 276 (1893); **16**, 218 (1894). Stone, *J. Am. Chem. Soc.*, **19**, 183 (1897). Lippmann, "Chemie der Zuckerarten," I, 52 (1904).

[20] Davis, Daish and Sawyer, *Jour. Agri. Sci.*, **6**, 406 (1914).

[21] Spoehr, Carnegie Inst. of Washington, Pub. 287, 51 (1919).

mixture of substances, the identification and quantitative determination of any particular substance becomes very difficult. The admixture of various substances makes the ordinary reactions or tests uncertain or completely inhibits them. Plants always contain a mixture of carbohydrates, their separation and quantitative determination cannot be attained with great precision. This is also due to the fact that the proportion of different carbohydrates is often such that one is present in very much lower concentration than another. Thus, the amount of glucose and fructose is usually very much greater than that of either arabinose or xylose; this adds to the difficulty of the determination of the latter. In many cases a qualitative identification of the different carbohydrates present is of considerable value.

For the identification of the pentoses and methyl pentoses and the differentiation of these a number of color reactions have been devised. Most of these reactions depend upon the fact that when treated with strong acids pentoses yield furfural, methylpentoses yield methylfurfural and hexoses oxymethylfurfural. These reactions are especially valuable for the determination of the presence or absence of a group of carbohydrates. At the same time, because the reactions are at times affected by the presence of other carbohydrates, a negative result does not always establish the absence of a particular carbohydrate. We cannot take up the detailed manipulation and interpretation of the various tests but must refer to the original literature and special publications on the subject.[22]

Phloroglucin reaction. To the solution to be tested is added sufficient hydrochloric acid to make about an 18 per cent solution, a small quantity (about 0.1 gram) phloroglucin is added and the mixture is heated in the boiling water bath for 10-15 minutes. In the presence of pentoses a red-violet coloration is produced. For identification the absorption spectrum of the colored solutions produced is invaluable. The solution is shaken out with amylalcohol (free from furfural!) and the extract examined spectroscopically. One absorption band, between the D (sodium) and E (thallium) lines, is characteristic for pentoses. The reaction is also produced by glucuronic and galacturonic acids. In the presence of impurities and hexoses a precipitate of varying density may be formed. The cooled solution may, under these circumstances, be filtered and the precipitate washed. The filter paper is washed with alcohol and the clear solution examined spectroscopically. The reaction is very sensitive, the absorption band is not produced by hexoses or methyl pentoses, but these in large excess, diminish its sensitivity. The absence of the characteristic band does not definitely exclude the presence of pentoses.[23]

Orcin reaction. There are various forms of this reaction; the modifica-

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[22] Van der Haar, A. W., "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," Berlin, 1920. Abderhalden, E., "Handbuch der Biochemischen Arbeitsmethoden," II, p. 95, Berlin, 1910. Tadokoro, *Jour. Col. Agri. Hokkaido Univ.*, **10**, pt. 6, 141 (1923).

[23] Wheeler and Tollens, *Ann. Chem.*, **254**, 329 (1889). Tollens, *Ber. chem. Ges.*, **29**, 1202 (1896). Pinoff, *ibid.*, **38**, 766 (1905).

tion of Bial has found much favor. The reagent consists of a solution of 1 gram orcin in 500 cc. 25 per cent hydrochloric acid and 1.5 cc. of a 10 per cent aqueous ferric chloride solution. The test is carried out by adding sufficient reagent to the solution to be tested so that it contains about 18 per cent hydrochloric acid and is boiled for a few minutes. The solution turns blue-green or blue. It is shaken out with amylalcohol (free from furfural) and examined spectroscopically. There are two absorption bands between the Fraunhofer lines C and D. The weaker of these, the one nearer the D line, is the only one which is produced only by pentoses. The reaction is very sensitive but must be considered as a test also for methylpentoses, glucuronic and galacturonic acids. It was at first claimed the latter two acids do not give the test. This is only the case when the acids are present in exceedingly small quantities. There is a difference in the rate of reaction which also accounts for the difference in color formation. Xylose reacts faster than arabinose and this faster than glucuronic acid.[24]

Naphthoresorcin test for Glucuronic Acid. A means of differentiating between pentoses and glucuronic acid has been devised by Tollens and somewhat modified by Neuberg and Saneyoshi. Equal volumes of the solution to be tested and concentrated hydrochloric acid are boiled one minute with 1 cc. of a 1 per cent alcoholic solution of naphthoresorcin. In the presence of pentoses and hexoses the solution rapidly colors. After cooling the solution is shaken out with an equal volume of benzol and the latter solution examined spectroscopically. Glucuronic acid yields a red-violet solution with an absorption band at the D (sodium) line. Tollens [24a] obtained positive results with this test with some plant material. Presumably the naphthoresorcin test applies also to galacturonic acid.

A number of other color reactions have been devised for the detection of pentoses and methylpentoses depending upon the formation of furfural and methylfurfural.[25] Important among these is the reaction of furfural with aniline acetate which gives a red color; methylfurfural gives a yellow color.

The identification of the pentoses can be accomplished under favorable conditions by the isolation of the sugar itself. But as crystallization is often prevented by the presence of other substances, recourse must be taken to special reactions.

l-Arabinose. This sugar forms several characteristic substituted phenylhydrazine compounds. The p-bromphenylhydrazone is quite insoluble in water, recrystallized from alcohol or acetone it softens when heated rapidly at 150° and melts at 165°.[26] The diphenylhydrazone formed by

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[24] Allen and Tollens, *Ann. Chem.*, **260**, 305 (1890). Bial, *Deut. med. Wochschr.*, **28**, 253 (1902). Lefevre and Tollens, *Ber. Chem. Ges.*, **40**, 4520 (1907).

[24a] Tollens, *Ber. chem. Ges.*, **41**, 1788 (1908). Neuberg and Saneyoshi, *Biochem. Zeit.*, **36**, 56 (1911). Mandel and Neuberg, *ibid.*, **13**, 148 (1908). Van der Haar, *ibid.*, **88**, 203 (1918).

[25] Schiff, *Ber. chem. Ges.*, **20**, 540 (1887).

[26] Fischer, *Ber. chem. Ges.*, **27**, 2490 (1894).

heating an alcohol solution of the components is sparingly soluble and melts at 204-205°.[27]

l-Xylose. The hydrazones of l-xylose are in general more soluble than those of l-arabinose. A characteristic compound of l-xylose is the double salt cadmium bromide xylonate, $(C_5H_9O_6)_2Cd \cdot CdBr_2 \cdot 2H_2O$, which is sparingly soluble. Arabinose does not form an analogous compound. The xylose in about a 20 per cent solution is treated with an equal weight of bromine and twice the weight of cadmium carbonate, heated gently and allowed to stand for twelve hours. Then the mixture is evaporated almost to dryness, a small amount of water is added, filtered and washed. The filtrate is concentrated to a small volume and an equal volume of alcohol is added. After some time the crystals are formed which can be analyzed for cadmium and bromine and have an $(\alpha)\frac{20}{D} = +7.4°$. The reaction succeeds even when in the sugar mixture only about one-fifth is xylose.[28]

d-Ribose is still an exceedingly rare pentose and has been found as a constituent of the pentoside vernin and also of the nucleinic acid investigated by Levene and Jacobs.[29]

e. Quantitative Methods of Pentose Determination.

The formation of furfural from pentoses on treatment with hydrochloric acid has been used very extensively for the quantitative determination of this group of carbohydrates. The method consists essentially in heating the sample in a distillation apparatus with 12 per cent hydrochloric acid, adding more acid as this distills over and determining the furfural in the distillate. For a number of reasons the method is not entirely satisfactory and many modifications of the original Stone and Tollens [30] method have been devised. These modifications consist largely in the method of determining furfural in the distillate, in the method of heating the sugar mixture with the acid and in the method of distillation.

One of the first difficulties in the quantitative determination of pentoses by means of the furfural method is occasioned by the fact that not only pentoses but also hexoses yield furfural-like substances. The amount of the latter formed from sucrose has been variously reported as yielding 0.2 to 1.5 per cent. Hexoses form oxymethylfurfural and methylpentoses methylfurfural. These substances behave so much like furfural, that in the analytical methods employed they can ordinarily

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[27] Neuberg, *Ber. chem. Ges.*, **33**, 2243 (1900); *Zeit. Physiol. Chem.*, **35**, 38 (1902). Müther and Tollens, *Ber. chem. Ges.*, **37**, 312 (1904). Tollens and Maurenbrecher, *ibid.*, **38**, 500 (1905).

[28] Bertrand, *Bull. Soc. chim.* (3), **5**, 546, 554 (1891). Widtsoe and Tollens, *Ber. chem. Ges.*, **33**, 136 (1900). Nef, *Ann. Chem.*, **403**, 253 (1914).

[29] Levene and Jacobs, *Ber. chem. Ges.*, **42**, 2102, 2469, 2474, 3247 (1909). Levene and Clark, *Jour. Bio. Chem.*, **46**, 22 (1921). Braun, *Ber. chem. Ges.*, **46**, 3949 (1913).

[30] Stone and Tollens, *Ber. chem. Ges.*, **21**, 2150 (1888).

not be distinguished.[30a] Davis and Sawyer have shown that a mixture of 0.01 gram of arabinose plus 25 grams of sucrose yield 20 per cent more furfural than without the sucrose, while 0.02 gram arabinose plus 0.25 gram sucrose (the proportion usually present in the plant extract which they were examining) was 15 per cent high for pentose alone. The presence of hexose sugars in great excess is therefore apt to cause serious error in the determination of pentoses by means of the furfural method.

Another fact which must be taken into consideration in this method is that strong hydrochloric acid has a destructive effect on furfural so that low results are obtained. The passage of a slow stream of steam through the distillation mixture is sufficient to carry over the furfural and thus avoid the destruction thereof by the strong acid according to Pervier and Gortner.[31]

A variety of methods have been employed for the determination of furfural. The reagents which have been used include phenylhydrazine, barbituric acid, sodium bisulfite, Fehling's solution and phloroglucin. The latter in which the insoluble compound formed with furfural is weighed has found most general use. On the basis of the difference in solubility of the furfuralphloroglucide and the methylfurfuralphloroglucide in 96 per cent alcohol at 60° the amounts of pentoses and methylpentoses can be differentiated. A critical discussion of the details of these methods is given in the book of van der Haar and also in the papers by Pervier and Gortner. The latter have also devised a method in which potassium bromate is used which has some advantages over the phloroglucine method.

In general the method of pentose determination by means of furfural formation is not altogether satisfactory, as it is apparently very difficult for different workers to obtain reproducible results.

An alternative method of determining the pentoses in plant material is based upon the fermentation of the hexose sugars and the determination of the remaining unfermentable sugars.[32] Here, however, great care must be exercised to obtain strains of yeast which do not affect the pentoses and by special tests to determine that the residue is completely fermented, as galactose ferments very slowly under certain conditions, and that the unfermentable residue is actually pentose and not some other unfermentable sugar. If this has been established the pentoses then can be determined by means of copper methods.

[30a] Warnier, *Rec. trav. chim.*, **17**, 377 (1898). Brauns, *Pharm. Weekblad*, **46**, 326 (1909). Kröber, Rimbach and Tollens, *Zeit. angew. Chem.*, **15**, 508 (1902). Kluyver, Dissertation Delft., 1914. Davis and Sawyer, *Jour. Agri. Sci.*, **6**, 406 (1915).

[31] Pervier and Gortner, *Ind. and Eng. Chem.*, **15**, 1167, 1255 (1923); **16**, 97 (1924). Extensive bibliography.

[32] Davis and Sawyer, *Jour. Agri. Sci.*, **6**, 406 (1915). Spoehr, Pub. No. 287, Carnegie Inst. Washington, p. 36 (1919).

f. Methylpentoses. $CH_3.(CH.OH)_4.CH:O$.

The occurrence of methylpentoses in plants corresponds in general to that of the pentoses, though they are apparently not as widely distributed nor as plentiful. Free methylpentoses in plants are very rare though no very careful nor extensive search has been made.[33] They frequently accompany pentoses as methylpentosans in the structural elements of plants and are also components of a number of glucosides or methylpentosides. Very little is known regarding the mode of formation or rôle in the carbohydrate economy of the plant.

l-Rhamnose, is a component of a number of glucosides, the best known of which is probably quercitrin present in oak bark and also widely distributed in other plants. It is the methyl derivative of l-lyxose. It forms an insoluble b-naphthylhydrazone, melting at 192-193°.

d-Isorhamnose (isorhodeose) is a component of the glucoside convolvulin; it is a methyl derivative of d-xylose.

Fucose as a methylpentosan is a component of many sea-weeds. It was the first of these substances to be the subject of extensive investigation. It is the methyl derivative of d-arabinose and has the constitution corresponding to l-galactose. It forms a p-bromphenylhydrazone, melting at 178°.

Rhodeose, the optical antipode of fucose, is also a component of the glucoside convolvulin.[34]

Methylpentoses can be identified by means of a color reaction with hydrochloric acid and acetone. The substance is heated with 10 cc. of 38 per cent hydrochloric acid and 2 cc. acetone. A violet color is produced which in the spectroscope covers the D (sodium) line. Pentoses produce the same color, but this color disappears if the tube is allowed to stand at ordinary temperature for one-half to one hour, while the color of the methylpentose remains.[35]

For the quantitative determination of methylpentoses, which usually occur accompanied by pentoses, the methylfurfuralphloroglucide can be employed. In order to differentiate between the pentoses and the methylpentoses advantage is taken of the fact that methylfurfuralphloroglucide is soluble in 96 per cent alcohol at 60°, and can thus be separated from the furfuralphloroglucide formed from the pentoses.[36]

g. Hexoses. Asymmetric Synthesis.

The hexose sugars are by far the most important carbohydrates which occur in plants. Not only are these sugars the most common components of plants, but also for the life activities of both plants and animals the

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[33] Requier, *Jour. Pharm. Chim.* (6), **22**, 540 (1905).

[34] Czapek, "Biochemie der Pflanzen," I, 270, 642 (1913). Lippmann, "Chemie der Zuckerarten," I, 159 (1904).

[35] Rosenthaler, *Zeit. anal. Chem.*, **48**, 167 (1909).

[36] Ellett and Tollens, *Zeit. Rübenzuckerind.*, **42**, 19 (1905); *Ber. chem. Ges.*, **38**, 492 (1905). Mayer and Tollens, *Ber. chem. Ges.*, **40**, 2434 (1907).

hexose sugars play an exceedingly important rôle. In general glucose may well be considered as the chief source of energy which enables the cells of most plants and animals to carry on their various forms of vital activity. It should be stated at once, however, that this is concluded from the almost universal presence of glucose or its condensation products in greater or less concentration in the cells. Whether it is glucose itself which is oxidized or a product of transformation of glucose, cannot be considered as definitely established. In fact, glucose represents the most stable form of the hexose carbohydrates and it is not improbable that before oxidation it is transformed into a more easily broken down form as is required by the cell. Certain it is that glucose is the most common of all the hexose sugars, but it must also be remembered that glucose is probably also the most stable of the hexoses found in nature.

It is a very striking fact, and one which has puzzled chemists for a long time, that of the 32 theoretically possible isomeric aldo-, 2-keto-, and 3-ketohexoses there are found in nature only a very limited number. These isomeric compounds have the same composition, all apparently contain a straight chain of six carbon atoms and differ principally in the spatial distribution of the hydrogen, oxygen and hydroxyl groups attached to each carbon atom.

The hexoses which are found in plants are: the aldoses, d-glucose, d-mannose and d-galactose, the 2-ketohexoses, d-fructose and d-sorbose and there is some evidence that there are also small quantities of the 3-ketohexoses, d-glutose. Of these d-glucose is found in greatest abundance, followed by d-fructose in varying proportions. These two hexoses are also found in large quantities in the form of their various respective di- and polysaccharides, notably sucrose, maltose, trehalose, raffinose and especially starch and inulin. The other hexoses in the free form are relatively rare components of leaves or other plant parts. It is very difficult to account for the fact, at least on the basis of our present knowledge of carbohydrate synthesis, that only this small number of carbohydrates appear in the plant as products of photosynthesis. Each one of the hexoses found represents but one of two space isomers, they are all (with the exception of d-glutose) optically active substances. This is one of the most important points which must be met in any theory of the mechanism of the synthesis of carbohydrates in plants. It has led to extensive speculation, for not only in these genetically first organic compounds is optical activity a striking characteristic, but it is apparently maintained throughout the entire organic world.

How to account for this optical activity of the products of photosynthesis is a problem for the solution of which we have not as yet sufficient experimental data. However, the following possibilities suggest themselves:

1. That all the theoretically possible hexoses are formed and that these are rapidly converted into the five or six actually found. While there is some evidence of a conversion of hexoses within the cell, as for in-

stance the change of glucose into fructose and vice versa, this is not of the nature of the conversions assumed. The hexose sugars found in plants are all of the dextro series. We have no evidence that the optical antipodes of these, the levo series, are ever formed in the plant. At the same time, it is perhaps well to bear in mind that there have been very few thorough researches directed definitely to establish the absence of the levo sugars. With the great mixture of substances existing in leaves and the difficulties entailed in separating these, such criteria as the optical rotation may not serve adequately to detect small amounts of the levo compounds. There is little reason for believing that the levo series would have a greater rate of activity than the dextro. On the other hand, as is mentioned below, catalysts which are themselves asymmetric favor reaction with one of two optical antipodes. Also, it is a rather common phenomenon that living organisms are capable of using only one of two optical antipodes thus resulting in the resolving of d-l compounds, a fact which dates to the classical researches of Pasteur. Similar properties have been found characteristic of a number of enzymes. Fischer first assumed the formation of an inactive racemic sugar; this was split by the action of the organism, resulting in the one active component remaining. This view, which Fischer [37] abandoned later, has been elaborated to some extent by others. But this is purely speculative, for as far as the evidence goes, we cannot accept the existence of even small quantities of the levo sugars nor of the other hexoses such as gulose, idose, talose, etc.

It is interesting that while of the hexoses only members of the dextro series have been identified, viz. d-glucose, d-mannose, d-fructose and d-galactose, both dextro and levo pentoses have been reported as components of plants. The l-arabinose has been found in the leaves of *Adonis vernalis* by van Ekenstein and Blanksma [38] and is also a component of gums such as gum arabic and cherry gum. *d*-Arabinose has been reported as a component of the glucoside barbalin.[39] This is one of the few instances of the occurrence of both the d an l modification of a carbohydrate in plants.[40]

2. A second possibility which may account for the formation of optically active products in the photosynthetic process are asymmetric

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[37] Fischer, *Ber. chem. Ges.*, **23**, 394, 2138 (1890). Winther, *ibid.*, **28**, 3022 (1895). Werner, "Lehrbuch der Stereochemie," Jena. 1904, p. 63. Pfeffer, *Jahrb. wiss. Bot.*, **28**, 220 (1895).

[38] van Ekenstein and Blanksma, *Chem. Zentr.* (1908), I, 119. Bauer, *Jour. prakt. Chem.* (2), **34**, 47 (1886). Kiliani, *Ber. chem. Ges.*, **19**, 3030 (1886). Tollens, *Landw. Vers.*, **39**, 425 (1891).

[39] Leger, *Compt. rend.*, **134**, 1111, 1584 (1902); **150**, 983, 1695 (1910); **155**, 172 (1912).

[40] Mention should also be made of the occurrence of the inactive form of galactose. Winterstein, *Ber. chem. Ges.*, **31**, 1571 (1898) reports i-galactose together with d-galactose as a product of the hydrolysis of chagual gum, and Tollens and Oshima, *Ber. chem. Ges.*, **34**, 1422 (1901), report obtaining i-galactose from the Japanese nori. The reported l-glucose in Grindelia by Power and Tutin, *Proc. Amer. Pharm. Ass.*, 1905, p. 5, was in all probability d-fructose.

forces outside of the plant. To these belong the polarization of light, the rotation of the earth and magnetic effects of the earth. The manner in which these forces affect chemical reactions, if at all, is not known. Van't Hoff[41] suggested that dextro- or levo-circularly polarized light might affect the synthesis of compounds in nature.

Cotton[42] reported that the two components of circularly polarized light are differently absorbed by the two forms of colored tartrate salts. Byk[43] then called attention to the fact that light is circularly polarized by the surface of the sea, so that d-circularly polarized light predominates and an asymmetric agent is thus present on the earth. In spite of repeated and careful attempts to effect a direct asymmetric synthesis by means of physical-asymmetric influences, these have not been successful.[44]

The resolution of an externally compensated substance into the optically active components by crystallization has been a familiar phenomenon since the classical researches of Pasteur and of Jungfleisch. Kipping and Pope[45] substantiated the discovery of Jungfleisch that there may be a preponderance of the d-salt in the crystallization of sodium ammonium tartrate, though Erlenmeyer[46] has shown that the preponderance of the d or the l form is not a constant occurrence. It has naturally suggested itself whether a partial crystallization of such a solution may not lead to permanent asymmetry. This, under very restricted circumstances, seems to offer one possibility of the natural existence of an asymmetric influence which, if present when the first living organism started, might induce a perpetual asymmetry.[47]

3. A third possibility which suggests itself as a cause for the formation of asymmetric compounds in the plant is that there are asymmetric influences within the leaf. There is considerable chemical evidence which makes such a view theoretically possible. An asymmetric compound once formed, the theoretical difficulties of producing other asymmetric compounds disappear. Asymmetric compounds may give rise to other asymmetric compounds in the course of chemical reactions and this original asymmetry can in a sense be perpetuated. This, of course, does not answer the question of the original asymmetric compound, and the reactions involving asymmetric compounds as reactants cannot in the strictest sense be considered as asymmetric reactions. If, however, we take the leaf as it now is, we know that it contains a variety of asymmetric compounds and that this asymmetry can in a number of ways be imparted to other compounds synthesized.

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[41] Van't Hoff, "Die Lagerung der Atome im Raume," 1894, p. 29. Kimball, "Pyreheliometer and Polarimeter Observations, Bull. Mt. Weather Observatory, **2**, Pt. 2. Nichols, *Phys. Rev.*, **24**, 497 (1908).

[42] Cotton, *Ann. Chim. Phy.* (7), **8**, 347 (1896).

[43] Byk, *Zeit. Physik. Chem.*, **49**, 641 (1904).

[44] Bredig, Mangold and Williams, *Zeit. angew. Chem.*, **36**, 456 (1923).

[45] Kipping and Pope, *Jour. Chem. Soc.*, **73**, 606 (1898).

[46] Erlenmeyer, *Biochem. Zeit.*, **52**, 439 (1913).

[47] Fitzgerald, *Nature*, **58**, 545 (1898).

This asymmetric influence and orienting effect has been observed in a variety of reactions of compounds containing asymmetric carbon atoms. A selective action of one of the components of a racemic mixture has been observed to occur because of differences of the rate of reaction of the two components, so that if the reaction is interrupted before it has been completed, there will be a preponderance of one of the products, which will be optically active.[48]

Another means whereby an asymmetric compound may give rise to other active compounds is through the action of asymmetric catalysts. It has been observed that one member of a racemate has a higher rate of reaction in the presence of an asymmetric catalyst than the other member.[49] This is of particular interest in relation to reactions taking place in living organisms. Here the catalysts are chiefly enzymes which are made up in part of optically active substances. Some of these enzymes are capable of synthesizing optically active compounds from inactive substances.[50] Thus, emulsin forms d-mandelic nitrile from benzaldehyde and hydrocyanic acid. Some of the reactions brought about by enzymes can also be produced by pure chemicals and the rotatory power of the product of the latter reaction is strongly influenced by the activity of the catalyst. In the synthesis of mandelic nitrile, just mentioned, the emulsin can be replaced by optically active alkaloids and in this case l-quinine produces d-mandelic nitrile and d-quinine forms the l-nitrile.[51]

Some enzymes will act only upon one of two space isomers and it is a very familiar fact that living organisms differentiate sharply between compounds which differ but slightly in their space relationship. Thus, while many of these compounds differ from each other in their chemical structure but very slightly, their behavior in the living cell exhibits wide variation. In fact, the interchange in spatial position of a single hydrogen and hydroxyl group determines whether the compound can or cannot be broken down by the living cell. So that while one of these compounds may be completely oxidized to carbon dioxide and water, the other may act as a chemically inert substance in the cell fluid. It was a consideration of these facts which led Fischer to develop his conception of lock and key to portray the relation of an enzyme to the substance on which it acts. In order that an enzyme may act upon a certain sugar the structure of the two must fit and as a simile of this Fischer suggested the relation of the structure of a key to the lock to which it has been fitted.

An indication of the asymmetric influences in plants is offered by the series of investigations on phytochemical reductions by Neuberg and his collaborators. They have shown that the yeast plant when supplied with glucose is capable of reducing a variety of substances. Acetol, $CH_3.CO.CH_2OH$, is reduced to propylene glycol, $CH_3.CHOH.CH_2OH$, the

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[48] Markwald, *Ber. chem. Ges.*, **32**, 2130 (1899). Fischer, *ibid.*, **39**, 530 (1906). Erlenmeyer, *Biochem. Zeit.*, **97**, 261 (1919).

[49] Bredig and Fajans, *Ber. chem. Ges.*, **31**, 783 (1898).

[50] Rosenthaler, *Biochem. Zeit.*, **14**, 238 (1909); **17**, 238 (1909); **19**, 186 (1909).

[51] Bredig and Fisk, *Biochem. Zeit.*, **46**, 7 (1912).

l-modification predominating. Wherever there is a possibility of two isomers being formed in this type of reaction, the reaction goes asymmetrically.[52] As yet we are not in possession of any definite knowledge on the basis of which we can explain this difference in behavior of compounds the free energy of which, as far as we know, is very nearly the same. Doubtless the reactions constituting the breakdown of hexose sugars are of a catalytic nature and it is also highly probable that the catalysts in the form of enzymes are, like the carbohydrates, of unsymmetrical structure. We have considerable chemical evidence that the asymmetrical nature of a catalyst may exert a decided influence on the course of the chemical reaction it is catalyzing. Thus, as has been mentioned, an asymmetrical catalyst may bring about the reaction of only one of two space isomers or, what amounts to about the same thing, react with one at a very much greater rate than with the other. However, the kinetics of these reactions can as yet not be explained satisfactorily, and this must probably await a better understanding of the mode of reaction of carbon compounds and this in turn the nature of the carbon atom.

On the behavior of asymmetric catalysts in inducing reactions which show preference or selection for asymmetric compounds we must probably rely for an explanation of the fact that the carbohydrates formed in photosynthesis are also asymmetric, or more properly, that of the relatively large number of hexose sugars only a few are formed in the photosynthetic process. Such a conception is strengthened by the fact, established by Willstätter and Stoll and others, that photosynthesis is dependent upon the action of an enzyme. In what step of the series of chemical reactions comprising photosynthesis, the enzyme exerts its asymmetric influence it is still impossible to say. There is some evidence for believing that the first step in photosynthesis consists in the union of carbon dioxide with a substance in the leaf. If this substance is already asymmetric it is highly probable that its asymmetry will be imposed upon the product resulting from the action of light on the carbon dioxide compound. But until we are in possession of more precise knowledge regarding this first step in photosynthesis and the subsequent photochemical reactions the discussion of the origin of the asymmetric compounds in leaves is largely speculation.

The same may be said for most attempts at explaining the origin of life on the basis of our present knowledge of physics and chemistry. One of the characteristics of all living things is that they are composed of asymmetric compounds and the behavior of these organisms or the enzymes which make possible many of their life activities, is governed by the space characteristics of the food material. By far the great majority of forms of life known to us derive their energy from carbon compounds. The only means which we know of by which carbon compounds capable of sustaining life are formed is through photosynthesis. This process re-

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[52] Neuberg, *et al.*, *Biochem. Zeit.*, **67**, 32 (1914); **90**, 388 (1918); **91**, 257 (1918); **92**, 96, 111 (1918); **112**, 313 (1920).

quires such an elaborate mechanism, even in the lowest forms of plants, that it is inconceivable that a photosynthetic process elaborating carbohydrates existed without the intermediary of a living organism or in very much simpler organisms than we now know. But there is no evidence whatsoever that this was ever the case. So that speculations on the origin of life must make assumptions which are so far removed from any knowledge of which we are now in possession that the deductions must of necessity have but a very limited significance.

Virtually the only direct chemical evidence we have pertaining to the limited number of hexoses which are found in nature are the experiments of Nef on the effect of alkalies on the hexose sugars. In these investigations Nef found that equilibrium is reached with a mixture of these sugars in an alkaline solution, that certain of the hexoses are never present in such mixtures and that the amounts of those which are present show some similarity to those found in nature. These experiments will be discussed in the chapter on the Chemistry of Photosynthesis.

h. Analysis of Hexoses.

We can here but indicate some of the methods which have been used for identifying and determining hexoses in plant material; for the details of the methods reference must be made to the literature cited. The fermentation with baker's yeast is one of the most common tests for hexose sugars. The sugars fermented are d-glucose, d-fructose and d-mannose; d-galactose is fermented much more slowly by ordinary yeast, while with pure yeast pentoses and methylpentoses are not fermented. A fermentation at 35° for 2-3 days thus accomplishes a separation of these sugars. It is essential to make several blank tests to determine: 1, whether the yeast with water alone produces carbon dioxide; 2, to determine whether the yeast is active; 3, whether the yeast affects pentoses. With these points in view, a positive test points to d-glucose, d-fructose and d-mannose; with the fermentation tubes 10 mg. of glucose can still be detected.

When aldopentoses are treated with acids there is formed furfural which has been used as a test to identify these sugars. Hexoses treated in a similar manner, yield 4-oxymethylfurfural. The color reactions of hexoses when treated with concentrated acids and phenols (e.g. Molisch reaction) depend upon the formation of condensation products of the phenols with the 4-oxymethylfurfural.[53] The latter compound is formed more rapidly with ketoses than with aldoses, and on this fact is based a distinctive reaction between aldoses and ketoses (Pinoff reaction).

The 4-oxymethylfurfural in the presence of concentrated acid undergoes a further change to formic acid and levulinic acid. The formation of levulinic acid when hexoses are treated with 20 per cent hydrochloric

[53] Nef, *Ann. Chem.*, **376**, 117 (1910).

acid has been used as a test for this group of sugars. Levulinic acid forms a crystallizable silver salt which has been used for its identification.[54]

A specific reaction for ketoses has been described by Pinoff;[55] this is a modification of the familiar Molisch reaction. About 50 mg. of the sugar to be tested with 10 cc. alcoholic sulfuric acid (75 cc. + 20 cc.) and 0.2 cc. of a 5 per cent alcoholic solution of alpha-naphthol are heated in a water-bath to 95-98°. In the presence of a ketose the solution becomes violet within one minute and should not be heated more than three minutes. With longer heating the aldoses also develop a faint violet color which increases with time.

The Seliwanoff[56] reaction for ketoses takes advantage of the fact that ketoses form 4-oxymethylfurfural more readily than the aldoses. About 50 cc. of the sugar solution with 10 cc. normal hydrochloric acid and 10 mg. resorcinol are heated in the boiling water bath for 15 minutes. In the presence of ketoses a red coloration is produced. As pentoses, and more slowly also aldoses produce a color the reaction cannot always be relied upon for keto-hexoses.

d-Glucose is probably the most widely distributed organic compound in plants. At some time in their development it is found in almost all plant parts and while the amounts present vary considerably with the season and external conditions, it is highly probable that glucose is present, if only in traces. Which one of the sugars is the first to appear in the photosynthetic process we shall discuss in a later portion of this chapter. Suffice it for the present that most evidence points to the conclusion that carbohydrates are the first products which accumulate in the photosynthetic activity of chlorophyllous cells. The products of photosynthesis are stored in the plant as condensation products of glucose in one form or another, namely, sucrose, dextrine, starch and cellulose. But the movement of the material from one part of the plant to another, as from the leaves to the roots, usually takes place in the form of the simpler carbohydrates, the products of hydrolysis of the storage material, and of these, glucose is a common form.

All living things require a continuous supply of energy for maintaining their activity. A majority of living organisms obtain this energy from the chemical transformations which the carbohydrates are capable of undergoing, and in this function glucose plays a very important rôle. In the higher plants the carbohydrates are usually in this process converted to carbon dioxide and water by oxidation, with the occasional formation of hydroxyacids. Lower organisms, fungi and bacteria, on the other hand, produce a variety of products such as alcohols, lactic, succinic and citric acids. Throughout almost the entire plant and animal kingdoms glucose is a most valuable food. The chemical changes which glucose undergoes

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[54] Wehmer and Tollens, *Ber. chem. Ges.*, 33, 1286 (1900). Levene, *Zeit. physiol. Chem.*, 43, 199 (1904-1905).

[55] Pinoff, *Ber. chem. Ges.*, 38, 3308 (1905).

[56] Seliwanoff, *Ber. chem. Ges.*, 20, 181 (1887). Königsfeld, *Biochem. Zeit.*, 38, 310 (1912).

in its breaking down and oxidation and the many syntheses in which glucose finally becomes a component of a more complex molecule such as glucosides, amines and proteins, cannot be followed here.

While glucose has been obtained from a large variety of plants and plant parts by means of extraction or through hydrolysis of condensation products, the direct evidence of the existence of free glucose in leaves is surprisingly meager. The presence of glucose is usually concluded from the formation of glucose phenylosazone, the optical rotation or even only from the fact that the leaf extract reduces Fehling's solution. In view of the mixture of substances present in leaves any one of these criteria is not sufficient to establish the presence of glucose. However, Deleano [57] has identified glucose and fructose in grape leaves by means of the methylphenylhydrazone and the methylphenylosazone.

An estimation of the amount of glucose in leaves has been obtained usually by methods which employ a combination of the optical rotatory power and reducing power of a mixture. As can be seen from the analytical data which are given in a later portion of this chapter, the amount of glucose in leaves varies greatly with the time of day and in different plants. In general the amount of glucose runs from none to about 3 per cent of the dry leaf material.

Glucose is identified by means of the formation therefrom of saccharic acid on oxidation with nitric acid. Saccharic acid forms characteristic acid-potassium and silver salts.[58] Glucuronic acid also forms saccharic acid. For the identification of glucose are also valuable the following compounds; phenylosazone, p-nitrophenylhydrazone, β-naphthylhydrazone, p-bromphenylosazone and the p-nitrophenylosazone. Which of these compounds is best suited for the identification of glucose depends upon what other sugars are present in the mixture. Van der Haar [59] has worked out the best means of identifying various monosaccharides present in mixtures by means of the different phenylhydrazine compounds.

d-Mannose in the free state has been found in small amounts in plants. There exists little information regarding the presence of mannose in foliage leaves. The anhydride condensation products of mannose are widely distributed as mannans.[60] The alcohol mannitol is widely distributed in plants. It is a curious fact, reported by Knudson,[61] that d-galactose and d-mannose are toxic to the roots of some plants. d-Mannose forms a characteristic phenylhydrazone which is used for identification as is also the p-bromphenylhydrazone and the p-tolylhydrazone.

d-Galactose. The presence of this sugar in plants in the free form has not been definitely established. It is, however, widely distributed as

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[57] Deleano, *Zeit. physiol. Chem.*, **80**, 79 (1912).

[58] Gans and Tollens, *Ann. chem.*, **249**, 217 (1888).

[59] Van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren," 1920, p. 240.

[60] Tsukamoto, *Bull. Coll. Agri. Tokyo*, **2**, 406 (1897). Flatau and Labbé, *Bull. Soc. Chim.* (3), **19**, 408 (1898). Easterfield and Aston, *Proc. Chem. Soc.*, **19**, 191 (1903).

[61] Knudson, *Am. Jour. Bot.*, **4**, 430 (1917).

a component of galactosides and as an anhydride condensation product with other sugars, as galactan, in gums and pectin. The alcohol dulcit has also been found in plants. Galactose is identified by the formation of the sparingly soluble mucic acid formed on oxidation with nitric acid.[62] The o-tolylhydrazone and α-methylhydrazone are also used.

d-Fructose has been found almost as widely distributed in plants as glucose. It is found in the free condition in leaves and often in higher concentration than glucose. As a component of sucrose it is especially common as well as of tri- and polysaccharides such as raffinose, melicitose, inulin and levulan. The isolation and identification of fructose in mixtures of other sugars, such as are obtained from plant material, are associated with some difficulty. The Pinoff reaction is of value in detecting fructose (also sorbose) in a mixture of aldoses and Neuberg[63] recommends the methylphenylosazone. The use of iodine and bromine to differentiate between aldoses and ketoses has been used extensively of late and will be described later in this chapter.

d-Sorbose is the only other ketose which has been identified in plants and this has been found in fruits and has not been reported as present in leaves.[64]

i. Cycloses.

A group of compounds which is very widely distributed in plants, though not found in large quantities, is derived from hexahydrobenzol. These cyclic compounds have the same molecular formula as the hexoses, $C_6H_{12}O_6$, have a sweet taste, are soluble in water, exist in various stereoisomeric forms some of which are optically active, but do not reduce cuproalkaline solutions. The inositols, $C_6H_6(OH)_6$, are the most widely distributed of these compounds. A phosphoric acid compound of i-inisitol is the commonly occurring phytin.

Very little is known regarding the mode of formation or function of the cycloses in plants and little work has been done in this field. From the viewpoint of photosynthesis the cycloses are of interest on account of the theory of Crato who in 1892 suggested that inositol may be the first product of the reduction of carbon dioxide in the photosynthetic process. The ring is opened, yielding a straight chain aldohexose (see page 204).

However, there has been no work done to test the theory; there are no quantitative data available which would throw light on the inositols as direct products of photosynthesis. Crato's[65] theory has in its essentials been restated by Kögel.

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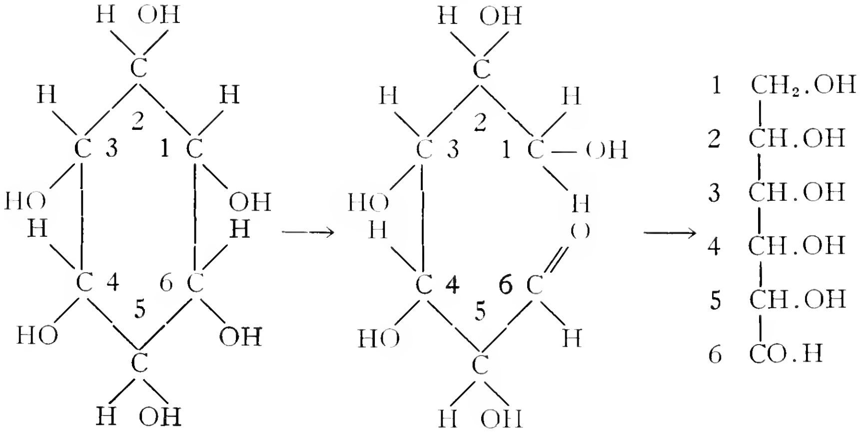
[62] Van der Haar, *Biochem. Zeit.*, **81**, 263 (1917). Braun, *Ber. chem. Ges.*, **49**, 1266 (1916).

[63] Neuberg, *Ber. chem. Ges.*, **35**, 959 (1902).

[64] Döbner, *Ber. chem. Ges.*, **27**, 345 (1894).

[65] Crato, *Ber. bot. Ges.*, **10**, 250 (1892). Kögel, *Biochem. Zeit.*, **95**, 313 (1919); **97**, 21 (1919). For inositols see Armstrong, "Carbohydrates," etc., 1924, p. 112. Maquenne, "Les Sucres," 1900, p. 189. Czapek, "Biochem. der Pflanzen," III, 1921, p. 480. Lippmann, "Chem. der Zuckerarten," I, 1904, p. 1007.



j. Heptoses.

That the monosaccharides found in plants are not confined to five and six carbon atom sugars, as was supposed for a long time, has become evident from the discovery of La Forge and Hudson of naturally occurring heptoses. These authors report the presence of a seven carbon atom sugar, sedoheptose, in the leaves and stalks of *Sedum spectabile*. Sedoheptose is an alphaketoheptose, the structural constitution of which has not yet been definitely established. It is not fermentable, reduces Fehling's solution, and, when heated with dilute acids, forms an anhydride; this anhydride does not reduce. This sugar is related to the naturally occurring heptitol, volmetiol.[66]

Another heptose, d-mannoketoheptose, has been isolated by La Forge[67] from the fruit, avocado, *Persea gratissima*. This is also a non-fermentable sugar. From the same source has been obtained the heptitol, perseitol, which Fischer showed to be the alcohol obtained by the reduction of α-mannoheptose.

k. The Conjugated Carbohydrates.

The monosaccharides are the building blocks of which the more complex carbohydrates are composed. The body of a plant is made up largely of these complex carbohydrates, more especially cellulose. But not only the structural elements of a plant and the conducting systems, but also the leaves and centers of photosynthetic activity contain relatively large quantities of the conjugated carbohydrates. In general when the

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[66] La Forge and Hudson, *Jour. Bio. Chem.*, **30**, 61 (1917); **42**, 367, 375 (1920).

[67] La Forge, *Jour. Bio. Chem.*, **28**, 511 (1917). Maquenne, *Compt. rend.*, **107**, 583 (1888). Fischer, *Ber. chem. Ges.*, **23**, 936 (1890). Fischer and Passmore, *ibid.*, **23**, 2226 (1890).

concentration of monosaccharides increases to a certain point conditions are favorable for the formation of di- and polysaccharides. But the conversion of monosaccharides into conjugated carbohydrates is also influenced by other conditions such as temperature and water content. These reactions are reversible, so that under certain conditions the conjugated carbohydrates in turn are again converted into monosaccharides. Since the development of a plant represents an accumulation of potential energy, there is usually a total net gain of carbohydrates which is in the form of conjugated carbohydrates, composing the structural elements of the plant and deposited in storage organs such as seeds, fruits, tubers, etc. We shall here concern ourselves less with the contents of these latter organs, but rather with those organs in which photosynthesis is taking place, more particularly the leaves.

Besides the monosaccharides, already mentioned, there are found in leaves sucrose, maltose, dextrine, starch, pentosans and cellulose. As we are primarily interested in the more immediate products of photosynthesis, the cellulose content is a matter of secondary consideration. Similarly other conjugated saccharides have been found in plants, for instance, trehalose, raffinose and melicitose, but their presence in leaves is questionable and we have no evidence, consequently, that they need be considered in a discussion of the primary products of photosynthesis.

Sucrose. Conclusions as to the presence of sucrose in leaves are largely based upon indirect determinations, usually the increase in reducing power and the change in optical rotation after inversion. Using these methods, amounts ranging from 0.5 to 10 per cent of the dry leaf material have been reported. The direct determination and isolation of sucrose is associated with greater difficulties. Attempts to isolate sucrose by means of the strontium compound and the calcium saccharate have been only partially successful and have yielded conflicting results.[68]

Sucrose is usually identified through the following properties: it does not reduce cuproalkaline solutions, and has a strong dextro rotation. On inversion with acids or invertin sucrose is split into d-glucose and d-fructose and the resulting mixture turns the plane of polarized light to the left and also reduces cuproalkaline solutions. From the inverted mixture the fructosemethylphenylosazone can be obtained and the presence of glucose can be established by means of the saccharic acid reaction.

Maltose, a disaccharide, composed of two molecules of d-glucose is a familiar product of the hydrolysis of starch. It reduces cuproalkaline solutions and is hydrolyzed into two molecules of d-glucose by acids and by the enzyme maltase.

The presence of this sugar normally in leaves has been a matter of controversy. Brown and Morris [69] maintain that a considerable portion

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[68] Kayser, *Land. Versuchs.*, **29**, 465 (1883). Peligot, *Ann. Chim.* (3), **54**, 377 (1858). Schulze, *Land. Versuchs.*, **34**, 403 (1887); *Ber. chem. Ges.*, **21**, 299 (1888); *Zeit. physiol. Chem.*, **52**, 404 (1907). Deleano, *ibid.*, **80**, 82 (1912).

[69] Brown and Morris, *Jour. Chem. Soc.*, **63**, 667 (1893). Ruhland, *Jahrb. wiss. Bot.*, **50**, 200 (1911). Campbell, *Jour. Agri. Sci.*, **4**, 248 (1912).

of the carbohydrates of the leaves of *Tropaeolum* is composed of maltose; from their analyses as high as 50 per cent in some cases. They identified the maltose by means of the maltosephenylosazone and made quantitative determinations of this sugar by means of a differential in the copper reducing power of the sugar mixture. Other workers have also reported varying amounts of maltose in leaves. Lewis and Tuttle [70] report as high as 1.21 per cent of the expressed sap of leaves of *Pyrola rotundifolia* and slightly less for *Populus tremuloides* and *Linnaea borealis,* though the amount varies greatly with the season. From the work of Brown and Morris it is evident that a great deal of importance is to be attached to sucrose as a possible first product of photosynthesis. This has raised two leading questions: first, whether the sucrose arises from the maltose, and second, whether the maltose is actually present in the living leaf or whether it is formed from the starch during the preparation of the leaf material for analysis.

Parkin [71] has made analyses of leaves of the snowdrop (*Galanthus nivalis*), a plant which does not normally form starch in its leaves. He was unable to detect any maltose, but considerable sucrose.

There is no doubt that the preparation of leaf material for analysis, particularly the drying, is apt to be a source of error, as is discussed in a later portion of this chapter. In the process of drying there is the possibility that enzymes continue to act on certain carbohydrates for some time, and that some enzymes are more easily destroyed than others. Thus, if maltase is destroyed sooner than diastase, the latter would continue to break down starch to maltose, the latter sugar would not be hydrolyzed to glucose and there would be an accumulation of maltose.[72]

There are few reliable methods for the direct identification of maltose, as maltose behaves very much like glucose towards most reagents. Maltose has a higher rotation (+ 137-138°) than glucose (+ 52.7°). Maltose also reduces cuproalkaline solutions, and on hydrolysis yields two molecules of glucose. After hydrolysis the rotation of a solution containing maltose decreases and the reducing power increases. This has been the basis of most quantitative determinations of maltose. In the presence of sucrose the inversion is carried on first with invertin, which effects sucrose and then with acid, which hydrolyzes maltose, and a differentiation can thus be arrived at. Application can also be made of the Barfoed copper acetate solution and of iodine solutions.[73]

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[70] Lewis and Tuttle, *Ann. of Bot.,* **34**, 405 (1920).

[71] Parkin, *Biochem. Jour.,* **6**, 1 (1911).

[72] Davis and Sawyer, *Jour. Agri. Sci.,* **7**, 352 (1916).

[73] Brown and Morris, l. c., 663. Hinkel and Sherman, *Jour. Am. Chem. Soc.,* **29**, 1744 (1907). Cajori, *Jour. Bio. Chem.,* **54**, 617 (1922). Fellenberg, *Mitt. Lebensm. Hyg.,* **11**, 129 (1920). Geelmuyden, *Zeit. anal. Chem.,* **48**, 137 (1909). Castallani and Taylor, *Jour. Trop. Med.,* **25**, 41 (1922). It should be mentioned that whenever iodine is used as a reagent, either in the determination of glucose or starch, it must be borne in mind that much plant material contains substances which absorb iodine. These substances are probably phosphatides, the unsaturated fatty acids of which take up iodine very readily. This is a frequent and very trouble-

Maltose forms a phenylosazone (M.p.206°), which under certain conditions, can be separated from the glucose-phenylosazone. The former is more soluble in water and in acetone so that cold 50 per cent acetone can be used for the separation.[74]

Starch. We have already alluded to the classical researches of Sachs on the formation of starch in the leaf and the great influence these extensive studies had on the development of the problem of photosynthesis. By use of the iodine test for starch an accurate qualitative means of identifying this substance was provided. The accumulation of starch has proved to be one of the simplest means of following in a qualitative manner the photosynthetic process.

The iodine test for starch has undergone many minor modifications. The principle is, however, essentially the same as that used by Sachs. The leaves are placed in boiling water for about ten minutes. They are then transferred to 95 per cent alcohol at 50-60° for about one-half hour until all the chlorophyll and other pigments are removed. With some plant material the last traces of chlorophyll are removed with difficulty, and therefore require a longer time and more alcohol. The colorless leaves are placed in an iodine reagent made by dissolving one gram of iodine in 100 cc. of a 5 per cent potassium iodide solution. Usually the starch-iodine color appears immediately but in some cases requires slight heating. The excess iodine is washed off with water. For the microchemical detection of starch it is advantageous to add 5 grams of chloralhydrate to the iodine reagent which greatly clarifies the section. By means of this method the localization of the starch can be clearly followed. Only those portions of the leaves which have been illuminated form starch. In fact, under the proper circumstances, the leaf is so sensitive to light of different intensity in its starch formation that photographic positive images can be produced by means of this method.[75] The iodine method has been applied both to a study of the formation of starch in the light and to its disappearance when the leaves are kept in the dark. The great mass of observational and experimental results which have been gathered by means of this method indicates quite clearly, that while starch is a very common product of photosynthesis, it cannot be considered as the first product formed. Some plants form no starch at all though they carry on active photosynthesis and starch may also be produced in leaves which are artificially fed glucose, sucrose, and a variety of other organic compounds. Also, the formation of starch in leaves during photosynthesis depends very much upon conditions of temperature and water-content. In considering starch as a product of photosynthesis, as is the case with all other carbo-

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some source of error and special precautions must be taken to remove these substances. In the presence of large quantities of phosphatides it is impossible to obtain the blue coloration of starch when iodine is added, until the absorption capacity of the fatty acids for iodine has been satisfied.

[74] Grimbert, *Jour. pharm. chim.* (6), **17**, 225.

[75] Molisch, *Sitzb. Akad. Wiss. Wien.*, Abt. I, **123**, 923 (1914).

hydrates, due regard must be given the transformations which these compounds undergo quite independently of the photosynthetic process.

The quantitative determination of starch in leaves has usually been carried out by first hydrolyzing the starch by means of an enzyme and then determining the resulting reducing sugars with a cuproalkaline solution. The method of O'Sullivan,[76] which makes use of diastase for the hydrolysis of starch and which was used by Brown and Morris has the disadvantage that the dextrin which is formed is carried down by the subsequent treatment with lead acetate and the determinations are consequently low. Davis and Daish [77] used takadiastase which converts the starch entirely into maltose and glucose; these are then determined by measurement of the copper reducing power and optical rotation. In the absence of fructose and sucrose, the maltose and glucose mixture resulting from the action of the takadiastase on starch, can also be treated with I.N. solution of hydrochloric acid which converts all the maltose to glucose and the latter can then be determined by its reducing power.[78]

Inulin. An analogous position to starch in some plants is taken by inulin. These plants instead of storing starch accumulate inulin. On account of the fact that inulin is much more soluble in water than starch the circumstances effecting the accumulation of the former in different parts of the plant are different from that of starch. Inulin is usually found in the storage organs, but also occurs according to Grafe and Vouk [79] in the leaves of *Cichorium intybus*. These authors report a considerable increase of inulin in the leaves during photosynthesis and consider that inulin is directly produced in the photosynthetic process. Colin, on the other hand, considers that the inulin is not present in the leaves, but is condensed in the stem.

3. The Transformations of Carbohydrates in the Leaf

It is not our intention to undertake here an exhaustive discussion of the transformations which the carbohydrates undergo in the leaf. We wish merely to show the necessity of considering these transformations in attempting to arrive at any logical conclusions regarding the nature of the carbohydrates formed in the photosynthetic process. It will become evident that the various carbohydrates undergo changes quite independently of the photosynthetic process and hence due regard must be given the conditions effecting these changes in experiments designed to establish the nature of the photosynthetic products. These conditions include light intensity, duration of illumination, temperature, water-content, rate of respiration and the previous history of the plant. As a consequence of differences in these conditions the nature of the photosynthetic products

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[76] O'Sullivan, *Jour. Chem. Soc.*, **45**, 1 (1884).

[77] Davis and Daish, *Jour. Agri. Sci.*, **6**, 152 (1914).

[78] Tollenaar, Dissertation, Wageningen, 1925.

[79] Grafe and Vouk, *Biochem. Zeit.*, **43**, 424; **47**, 320 (1912); **56**, 249 (1913). Colin, *Rev. Gen. Bot.*, 31, 70, 179, 229, 277 (1919); *C. A.*, **14**, 1134 (1920).

have been found to vary considerably. On account of the neglect of giving due regard to these influences there exist in the literature of the subject many contradictory results. The fact must be kept in mind that a leaf is capable not only of photosynthesis, but that at the same time there are taking place migration of the substances formed, respiration and growth and that each of these functions may be influenced in a different manner by temperature, water-content, etc. Hence an increase or decrease of any particular carbohydrate (e.g. starch) or, for that matter, of the total carbohydrate-content, can give little exact information regarding the rate of photosynthetic activity.

Conclusions regarding the transformation of carbohydrates in the leaf have been based upon the analysis of the leaf components. It has proven to be a matter of considerable difficulty to devise methods for determining the carbohydrates in leaves which give reliable results. Although the sources of error are many there are two which are particularly troublesome. The first of these is due to enzyme action resulting in changes in the nature and amounts of carbohydrates after the leaves have been harvested. The second arises in the preparation of extracts and solutions for analysis. In this process it is possible that some of the carbohydrates are lost or other substances are included which behave in a manner similar to the carbohydrates and thus spurious results are obtained. The first of these difficulties must be met in the preparation of the leaf material for analysis. This is an exceedingly important step and the value of the analyses depends more upon the proper choice of a method for this procedure than has been commonly assumed. Various methods of killing and drying have recently been subjected to a critical study by Link and Tottingham [80] who have established valuable facts regarding the choice of methods for different types of leaf material. Attention has also been given to this phase of the subject by Parkin [81] who compared air dried leaves with those immersed in liquid air. Waterman and Tollenaar also have devoted attention to the effect of drying on the composition of vegetable material, particularly on the starch content. The choice of a method of killing and drying depends greatly upon the nature of the material to be analyzed.

The methods of analysis have also recently been critically discussed by Tollenaar, Gast, Link and Tottingham, Davis, and others.[82]

One limitation of the method of chemical analysis is that it gives no information regarding the cellular mechanism of carbohydrate transformation and movement. Different portions of a leaf exhibit different rates of carbohydrate accumulation as well as of transformation. The formation and hydrolysis of starch can be followed by microchemical tests

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[80] Link and Tottingham, *Jour. Amer. Chem. Soc.*, **45**, 439 (1923); **47**, 470 (1925).

[81] Parkin, *Biochem. Jour.*, **6**, 5 (1911). Waterman, *Chem. Weekblad.*, **12**, 48, 924 (1915). Tollenaar, Dissertation Wageningen, 1925.

[82] Gast, *Zeit. physiol. Chem.*, **99**, 1 (1917). Ahrens, *Bot. Archiv.*, **5**, 234 (1924). Davis and Daish, *Jour. Agri. Sci.*, **5**, 437 (1913); **6**, 152 (1914). Spoehr, Carnegie Inst. of Washington, Pub. No. 287 (1919).

and considerable information regarding these processes has been gained in this manner. These methods are, however, only of a qualitative value.

In general, the carbohydrates which are of importance in relation to the photosynthetic process, as far as our present knowledge goes, are: d-glucose, d-fructose, pentoses (probably l-arabinose and l-xylose) sucrose, maltose, starch and pentosans. Not all leaves contain all of these carbohydrates; some contain no maltose nor free pentoses, in some starch is replaced by inulin and in some neither of these polysaccharides are normally present. From the fact that the amounts of these carbohydrates in leaves show very decided changes when the latter are exposed to light in an atmosphere containing carbon dioxide (i.e. during photosynthesis) or when they are kept in the dark, it has been concluded that these sugars are of importance in the photosynthetic and respiratory processes of the leaves. Under these conditions, it should be stated, the pentoses and pentosans are normally but slightly affected.

a. Starch.

Probably the most easily detected product of photosynthesis is starch; this is accomplished by means of the iodine reaction. That there is a certain relationship between the soluble carbohydrates of the leaf and the starch follows from the familiar fact that starch is formed in the dark when leaves are placed in sugar solutions. The amount of starch thus formed is, within certain limits, proportional to the concentration of the sugar solution. Under natural conditions many leaves accumulate relatively large quantities of starch during days of bright illumination and sufficiently high temperature. In many cases this starch disappears entirely again during the night. This accumulation of starch during illumination has been interpreted as being a case of the formation of reserve material during photosynthesis which is consumed during the period the plant is in darkness. The questions to be discussed are what are the conditions which influence the formation and resorption of starch and in what manner does the starch disappear.

Different species of plants vary greatly in the rate at which starch is formed and disappears from the leaves. From the compilation of Miss Eckerson[83] an idea can be gained of these rates which are given in Table 28.

The quantity of starch found in leaves varies widely with the species, the time of day and conditions of temperature, water-content, etc. Brown and Morris, in *Tropaeolum* found 1.23 per cent of the dry leaves as starch when picked at 5 A.M. and 4.59 per cent at 5 P. M. Table 29 is compiled from the results of Gast.[84]

Brown and Morris[85] have shown that although leaves may produce large amounts of carbohydrates in the photosynthetic process only a small

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[83] Eckerson, *Bot. Gaz.*, **48**, 224 (1909).
[84] Gast, *Zeit. physiol. Chem.*, **99**, 1 (1917).
[85] Brown and Morris, *Jour. Chem. Soc.*, **63**, 669 (1893).

TABLE 28

The Formation and Disappearance of Starch in Leaves of Different Plants as Determined by the Iodine Test. (From Eckerson.)

Name of Plant	Disappearance of Starch in Darkness (18–22°)		Formation of Starch in Light (20–25°)		Iodine Test
	Nights and Days		Perceptible Figure Minutes	Good Figure Minutes	Minutes
Abutilon	2	1	30	120	5–10
Begonia coccinea	3	3	60	240	10
Brassica oleracea	1	0	20	50	5
Cineraria cruenta (young ls.)	5	4	45	180	30
Coleus blumei	2	1	30	50	3–8
Cucurbita pepo	1	0	15	50	4–15
Euphorbia pulcherrima	2	1	60	240	20
Fuchsia speciosa	1	0	45	90	15–25
Helianthus annuus	1	0	30	120	5
Heliotropium peruvianum	1	0	45	120	5–10
Impatiens sultani	1	0	30	120	5
Lupinus albus	1	0	60	240	3
Oxalis bowiei	1	0	45	240	10–30
Pelargonium domesticum	2	1	50	240	40
Pelargonium peltatum	3	3	50	270	60
Pelargonium hortorum zonale	2	1	20	50	10–15
Phaseolus vulgaris	1	0	20	90	5
Primula obconica (young ls.)	5	4	120	240	5–10
Primula sinensis (young ls.)	4	3	45	120	10
Raphanus sativus	1	0	35	60	3
Ricinus communis	1	0	20	60	5–15
Salvia involucrata	2	1	90	120	4
Salvia splendens	3	2	30	60	1–5
Senecio mikanioides	1	0	20	50	5
Senecio petasitis	3	2	30	180	25
Tropæolum majus	2	1	50	90	1
Vicia faba	1	0	60	240	10
Zea Mais	3	2	30	120	5

portion of this may remain in the leaf as starch. Thus leaves of *Helianthus annuus* produced about 12 grams of material per square meter in 12 hours, but of this amount only 1.40 gram was observed as an increase in starch in the leaves. A considerable proportion of the material synthesized in a

TABLE 29

Starch-content of Various Leaves at Different Times of the Day.

Species	Time	Per Cent Starch	Time	Per Cent Starch
Tropæolum majus L.	July 24, 2 P.M.	6.44	July 25, 3 A.M.	5.62
Cucurbita ficifolia Bché	Aug. 16, 3 P.M.	6.06	Aug. 24, 4 A.M.	1.50
Vitis vinifera L.	Aug. 24, 2 P.M.	5.34	Aug. 25, 6 A.M.	1.76
Musa ensete Gmel.	Aug. 24, 2 P.M.	1.27	Aug. 25, 6 A.M.	0.86

leaf, under normal conditions passes from the leaf to other portions of the plant, or is contained in the leaf as other carbohydrates besides starch. This is illustrated by another experiment of Brown and Morris. Analyses were made of the following three sets of leaves of Tropaeolum:

A, Leaves picked from plant at 5 A.M.
B, Leaves cut from plant, but insolated till 5 P.M.
C, Leaves picked at 5 P.M.

Results of analyses in percentages of the dry leaves:

	A	*B*	*C*
Starch	1.23	3.91	4.59
Sucrose	4.65	8.85	3.86
Glucose	0.97	1.20	0.00
Fructose	2.99	6.44	0.39
Maltose	1.18	0.69	5.33
Total sugars	9.69	17.18	9.58

The cut leaves, *B,* were deprived of the capacity of having the synthesized material move out into other portions of the plant. There is therefore a striking accumulation of material. Although there is here a large increase in the amount of material gained over *A,* the amount of starch has not reached that of the leaves, *C.* If the total amount of material synthesized is taken as that obtained in *B,* it is very apparent that neither starch nor any other sugar as determined in leaves attached to the plant, as in *C*, give a true measure of the rate of photosynthesis.

Saposchnikoff [86] endeavored to determine the maximum starch accumulation in leaves. For excised leaves of *Vitis vinifera* he regards that 27.5 per cent of the dry material of the leaf as starch represents the limit, while for *Vitis labrusca* the limit ranged between 17 and 25 per cent. The amounts of starch can be considerably increased if the leaves are placed in an atmosphere enriched in carbon dioxide; photosynthesis under these conditions produces starch amounting to 30-35 per cent of the dry leaves.

Of greatest importance in the production and transformation of starch in leaves are the conditions of temperature and water content. In general, low temperatures result in the disappearance of starch from leaves. Lidforss [87] showed that evergreen leaves in temperate latitudes are quite free from starch during the winter months. With the higher temperatures of spring starch again appears in the chloroplasts. It has been assumed that the low eutectic point of the monosaccharide solutions in the cell sap affords protection against freezing. It has been demonstrated that temperature is actually the determining factor in the transformation of starch in leaves by the fact that when evergreen leaves are brought to a higher temperature during any time of the winter, starch formation takes place very readily. Maximow [88] has demonstrated that an increased concentra-

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[86] Saposchnikoff, *Ber. bot. Ges.*, **9**, 293 (1891); **11**, 391 (1893).

[87] Lidforss, *Bot. Centralbl.*, **68**, 33 (1896).

[88] Maximow, *Ber. bot. Ges.*, **30**, 52 (1912). Miyake, *Bot. Gaz.*, **33**, 321 (1902). Mer, *Bull. Soc. Bot. France*, **23**, 231 (1876). Haberlandt, *Jahrb. wiss. Bot.*, **13**, 74

tion of soluble carbohydrates in the cell sap greatly increases the resistance of leaves to freezing. Miyake reports that in the winter many evergreen leaves in the middle and southern parts of Japan contain more or less starch, while in the colder regions the leaves are found to be starch-free. Henrici has demonstrated the effect of low temperatures on starch formation during photosynthesis. She has shown that below 0°, with the alpine plants used, starch is never formed. With super-optimal illumination there takes place an increase in the internal temperature of the plant which results in some starch formation. Henrici's investigations have been discussed in the section dealing with the effect of temperature on photosynthesis.

Czapek[89] has shown that, at low temperatures the sugar concentration in the cells of leaves must be very much higher in order that starch formation may take place, than at higher temperatures. In none of the leaves used by Czapek did starch formation occur at low temperatures with sucrose concentrations under 7 per cent, while at ordinary temperatures a 1 per cent solution produced starch. Tollenaar[90] found that tobacco leaves placed on sucrose solutions formed starch (detected with iodine), in one day at 28°, about the same amount in two days at 17°, and in three days at 12°. He finds that the minimum sucrose concentration which will produce starch is $\frac{1}{40}$ per cent at 28°.

In considering the photosynthetic activity of leaves not only is it necessary to regard the rate of formation of any one carbohydrate but at least three other factors are of importance, namely the rate of respiration, that of transformation into other carbohydrates and the rate of migration of the carbohydrates from the leaf to other portions of the plant. It is impossible to determine all of these factors with any degree of accuracy in a living plant. It must be apparent, therefore, that it is impossible to obtain even an approximation of the rate of photosynthesis from determinations of the changes in the starch or carbohydrate content of leaves. Starch is a transitory reserve material in the leaf; it appears when the supply of mono-and disaccharides is in excess of the metabolic and translocating power of the leaf or when the concentration of these sugars has reached a certain degree.

That the disappearance of starch in leaves is due to the action of diastase was demonstrated by Brown and Morris. They found diastase in leaves of many different species of plants. The amount of the enzyme varies greatly in different plants. It is interesting that those plants which ordinarily form no or very little starch also contain little diastase. The tannin in the leaf apparently inhibits the activity of the diastase. Brown and Morris also come to the conclusion that leaves contain "far more diastase than is requisite to transform within a moderate amount of time

(1882). Schultz, *Flora,* 71, 223 (1888). Henrici, *Verhand. Naturf. Ges. Basel.,* 32, 107 (1921).

[89] Czapek, *Ber. bot. Ges.,* 19, 120 (1911).

[90] Tollenaar, Dissertation, Wageningen, 1925, p. 42.

all the starch which the leaf can ever contain." They also noted that there exist decided periodic variations in the diastatic activity of leaves. This they explained on the basis of their "starvation hypothesis" by which they meant that the cell protoplasm elaborates the enzyme according to the needs of the cells. Based upon the observations of Sherman,[91] that amino acids exert a stimulating action on diastatic activity Spoehr and McGee[92] have suggested that the variation of the activity of this enzyme may be associated with variations in amino acid content of the leaves.

Mention has already been made of the influence of temperature on the rate of starch formation in leaves. Tollenaar, in the work previously cited, has also studied the influence of temperature on the disappearance of starch in leaves in the dark. Tobacco leaves, for example, were exposed to an illumination of 24,000 M.C. for 4.5 hours and were then placed in the dark at different temperatures. The time required for the leaves to lose their starch at the different temperatures was as follows: 28°, 3 hours; 17°, 5 hours; 10°, 12 hours; and 1.5°, 25 hours.

Of equal importance with temperature in the transformation of carbohydrates in leaves is the water-content. It has been observed that leaves lose starch more slowly in an atmosphere saturated with water vapor than under conditions in which the leaves wilt. The greater rate of loss of starch in the drier atmosphere is apparently directly related to the water-content of the leaves and has little to do with the rate of transpiration.[93] Lundegårdh also observed that in certain seeds rich in oil, the starch was converted into oil, with soluble sugar as an intermediate product, when the seeds were desiccated. Tollenaar is of the opinion that the relatively rapid conversion of starch into soluble sugars on desiccation of leaves is due to the fact, that under these conditions the enzyme which synthesizes starch is inactivated while the hydrolyzing enzyme is more resistant. The carbohydrate equilibrium, starch $\rightleftarrows$ sugar is therefore shifted in the direction of soluble sugars, sucroses and hexoses. Schroeder and Horn, on the other hand, consider that there is no such thing as a starch $\rightleftarrows$ sugar equilibrium. Their reason for this opinion, that an equilibrium between an insoluble substance such as starch and a soluble one such as sucrose cannot exist, is however, not tenable.

It is probably of considerable importance in considering these facts, that in the experiments in which ordinary thin leaves were subjected to drying, the desiccation was carried to an extreme point, so that in many cases the leaves were wilted, though probably still living. In organisms which are adapted to such extreme desiccation, as the cacti, Spoehr[94] found that the ratio, polysaccharides; total sugars, was greater in the

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[91] Sherman and Walker, *Jour. Amer. Chem. Soc.*, **41**, 1866 (1919); **43**, 2461 (1921).

[92] Spoehr and McGee, Carnegie Inst. of Washington, Pub. No. 325, p. 74 (1923).

[93] Molisch, *Ber. bot. Ges.*, **39**, 339 (1921). Schroeder and Horn, *Biochem. Zeit.*, **130**, 165 (1922). Tollenaar, *l. c.*, 80. Lundegårdh, *Jahrb. wiss. Bot.*, **53**, 421 (1914). Ahrens, *Bot. Arch.*, **5**, 234 (1924).

[94] Spoehr, Carnegie Inst. of Washington, Pub. No. 287, p. 57 (1919).

desiccated plants. The same was the case with pentosans; starch was not determined separately.

b. Disaccharides and Monosaccharides. The First Sugar Formed.

Although it has been very easy to demonstrate that starch accumulates in the leaves as a result of photosynthesis, the question as to what is the first carbohydrate formed in the process has been a subject of much dispute. As Sachs showed, starch is the "first visible product of photosynthesis," but such a conclusion naturally is unsatisfactory when it is desired to gain a clearer conception of the kinetics of the chemical reactions which evidently start with such simple substances as carbon dioxide and water and end with such a highly complex molecule as starch. In view of the fact that starch yields d-glucose on hydrolysis, the suggestion was made long ago that this sugar was the first product. This view gained support from the observations of Böhm and of Meyer that the hexoses, glucose and fructose, constitute the best material for starch formation, when leaves are floated on solutions of various organic substances, and the rate of starch formation in the leaves in the dark is followed. It should be stated, however, that Brown and Morris [95] in their experiments upon artificial nutrition of leaves by solutions of carbohydrates found that sucrose surpassed all other sugars in the formation of starch.[96] The fact that the emission of oxygen commences almost immediately on illumination of a green leaf and that the $O_2 : CO_2$ ratio is very close to unity, while some time is always required for the appearance of starch, gave further support to the view that glucose is a precursor in the formation of starch. This view was also favored by the experimental evidence which showed that starch was formed only when the concentration of glucose in the cell was above a certain concentration.[97]

The theory that glucose is the first carbohydrate formed in photosynthesis certainly has the advantage of relative simplicity and doubtless the development of this idea was to some extent influenced by the conceptions of carbohydrate chemistry which were being developed at the time. Boussingault [98] was probably the first to make a definite statement that the first product elaborated from carbon dioxide and water by the leaves is glucose. In 1870 Baeyer had proposed that formaldehyde was the reduction product of carbonic acid, and, based upon the discovery of Butlerow, that formaldehyde is condensed to a hexose by means of alkali, a method of synthesizing hexoses was developed by Fischer and Loew. Whether these chemical reactions can find direct application to the synthesis of carbohydrate in the leaf we shall leave for a later discussion. They have, nevertheless, found wide application among chemists and plant physiolo-

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[95] Brown and Morris, *Jour. Chem. Soc.*, **57**, 484 (1890).

[96] Böhm, *Bot. Zeitg.*, **41**, 33, 49 (1883). Meyer, *ibid.*, **44**, 81, 105, 129, 145 (1886); **43**, 417, 433, 449, 465, 481, 497 (1885).

[97] Schimper, *Bot. Zeitg.*, **43**, 737 (1885).

[98] Boussingault, *Agronomie*, etc., **4**, 399 (1868).

gists in endeavoring to explain the synthesis of glucose and the further synthesis of starch.

The first to subject the problem as to what is the first sugar formed to quantitative experimental investigation were Brown and Morris.[99] On the basis of chemical analyses of leaves they came to the conclusion that, not glucose, but sucrose is the first sugar formed in photosynthesis. Their argument is based upon analyses of *Tropaeolum* (already quoted). These analyses show a decided increase in the amounts of sucrose and fructose after the leaves have been illuminated. There is no appreciable increase in the amount of glucose. The accumulation of fructose is explained on the basis that this sugar results from the inversion of sucrose; the equivalent quantity of glucose, which is simultaneously produced in the inversion is used up in the process of respiration. Separate experiments of Brown and Morris would go to show that in the dark there is a decided loss of sucrose and that glucose is more rapidly used in respiration than fructose, so that there is an accumulation of fructose. According to this view sucrose is the first sugar formed and serves as a temporary reserve material; when the concentration exceeds a certain limit starch commences to be formed. The fact, observed by Brown and Morris, that, although the leaves had been illuminated for a long period and the amount of glucose did not increase, is taken as evidence that glucose is not the first sugar formed.

The great weakness in the experiments of Brown and Morris is the fact that the determinations of glucose and fructose are of very doubtful value. It is extremely difficult to determine these two sugars accurately when they are in a mixture such as is obtained from a leaf extract and, as Davis [100] has shown, small admixtures of non-carbohydrates, showing optical activity, can introduce very great errors. Also, as we know now, the system studied by Brown and Morris, containing glucose, fructose, sucrose, maltose and starch is too complex to permit accurate results to be obtained.

A simpler condition was presented by the snowdrop (*Galanthus nivalis*, L.), which was studied by Parkin.[101] The leaves of this plant contain only glucose, fructose, and sucrose. Parkin also favors the view that sucrose is the first sugar formed. He concludes: "During any single day of the spring the *percentage* of hexose sugars in the leaf remains fairly constant, no matter at what hour of the twenty-four the leaves may be examined. That of the sucrose, on the other hand, fluctuates greatly. It increases during the day and diminishes during the night. Further, leaves detached and insolated contain decidedly more sucrose than their controls, but the quantity of hexose sugars remains much the same."

With improved methods of analysis Davis [102] and his collaborators have

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[99] Brown and Morris, *Jour. chem. Soc.*, 63, 604 (1893).

[100] Davis, *Jour. Agri. Sci.*, 7, 327 (1916).

[101] Parkin, *Biochem. Jour.*, 6, 1 (1911).

[102] Davis, *et al.*, *Jour. Agr. Sci.*, 5, 437 (1913); 6, 152, 406 (1914); 7, 255, 327, 352 (1916).

made a careful study of the carbohydrates in the leaves of the mangold at different times of the day and night and at different seasons. The analyses made during the stage of early growth (August 26-27) show that the hexoses and sucrose increase rapidly after sunrise, reach a maximum at about noon, and decrease gradually until the following sunrise. In this stage the amount of sucrose is greater than that of the hexoses; also the variation in the amount of sucrose is smaller than that of the hexoses. In the second stage (September 10-11) the results of the analyses show very different conditions. Here the amount of hexoses is greater than that of sucrose, the increase is irregular, reaching a maximum at sunset and decreasing irregularly to the following sunrise. In the third stage of the development of the plant (October 11-12) the hexoses are still in excess of sucrose, both show very irregular increases and decreases. Analyses were also made of the carbohydrates of the leaves of potato plants at two hourly intervals. Here the sucrose is in excess of the hexoses and the former shows a gradual increase from sunrise to 2 P.M. and then a decrease till sunset, while the hexoses show great variation during the day and night.

The investigations of Brown and Morris, of Parkin and of Davis and his collaborators have been cited here to illustrate the nature of the evidence on which has been based the conclusion that sucrose and not glucose is the first sugar formed in photosynthesis. The same conclusion has been reached by Went,[103] Perry,[104] Girard[105] and others.[106]

The objections which may be raised to this conclusion are varied. It has been repeatedly emphasized that the difficulties associated with the preparation of leaf material for analysis and the execution of the analyses themselves are numerous, and that small errors, as for instance in polariscope readings, may result in very erroneous conclusions. For this reason the amounts of carbohydrates reported are not always reliable, especially in those cases in which conclusions were drawn from polarimetric readings. No doubt the contradictory results obtained by different investigators are to be ascribed in part to differences in the methods employed and in part to differences in light intensity, temperature and water content to which the experimental plants were subjected. There is, moreover, the very serious question as to whether it is justifiable to draw conclusions as to the kinetics of a series of reactions from the amounts of different substances which are found at any time to be present in the mixture. In so complex a series of reactions as that of photosynthesis, in which the total reaction runs from carbon dioxide and water to starch, the number of steps involved is, of course, great. Also, some of the steps comprising this total reaction are reversible; the polysaccharides may be hydrolyzed to monosaccharides for instance. The conditions under

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[103] Went, *Jahrb. wiss. Bot.*, 31, 323 (1898).
[104] Perry, *Compt. rend.*, **94**, 1124 (1882).
[105] Girard, *ibid.*, **97**, 1305 (1883).
[106] Marcacci, *Just Jahresb.*, **1**, 26 (1889). Colin, *Bull. assoc. chim. sucr. dist.*, **38**, 331 (1821).

which the latter occurs are not fully known. In a reaction which proceeds by steps, the rate is determined by the step which progresses with the lowest speed. The accumulation of a certain substance may tell nothing concerning in which step in the reaction this substance is produced. In fact, if the first product formed is very reactive, under the particular conditions existing, this product may not be detected at all by means of the methods of chemical analysis, or it may be found only in very small amounts.

Virtually the same point has recently been made by Priestley.[107] He states: "All these investigators, Brown and Morris, Parkin, and Davis, Daish and Sawyer, conclude that the evidence suggests that cane sugar plays this rôle and in general upon the same ground, namely, that if the sugars in the leaf are estimated at different times then the fluctuations in amount, with the passage of the leaf through alternating periods of light and darkness, are greater in the case of cane sugar than of the hexoses present, whilst maltose is not found in some leaves (as the snowdrop) and is perhaps only present in traces in other leaves. From the experimental data, this theoretical conclusion does not seem to the present writer to follow, because it must be remembered that in most green leaves sugars are formed as intermediate steps in the synthesis of more complicated bodies, such as starch, and it is a necessity of the case that for the prompt formation of these complex anhydrides the intermediate steps must be rapidly and smoothly passed through. It is to be expected then that the intermediate stages in the process, including the sugars first formed in photosynthesis, instead of accumulating in the light, and therefore fluctuating in amount should pass rapidly into other substances in the complex chain of metabolic changes so that little if any change in their concentration can be detected, and in any case no such phenomenon would occur as a local accumulation such as is characteristic of a storage product."

For reasons similar to those just given the view that sucrose is the first sugar formed in photosynthesis has not been very generally accepted by writers on the subject. Rather has the older view been retained that glucose is the first carbohydrate, though it must be admitted that this has little direct experimental evidence to support it. Weevers [108] has obtained results, which agree with those of Campbell,[109] to the effect that monosaccharides are the first sugars synthesized. Weevers worked with variegated leaves and found that in 10 out of 12 subjects examined the variegated portions contain sucrose only, while the green parts contain both sucrose and hexoses. In the green portions monosaccharides were the first sugars to appear when leaves, which had been freed by placing them in the dark, were illuminated. Weevers found invertase in the variegated as well as the green portion of the leaves.

As was mentioned in an earlier portion of this section the weak point

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[107] Priestley, *New Phytologist*, **23**, 255 (1924).

[108] Weevers, *Proc. Kon. Akad. Wetens, Amsterdam*, **27**, 46 (1923).

[109] Campbell, *Jour. Agri. Sci.*, **4**, 248 (1912).

in the entire method of attempting to draw conclusions regarding the first sugar formed in photosynthesis from analyses of leaves is that we have not sufficient information concerning the transformations which the sugars undergo independently of the photosynthetic process. We do know that such changes take place, that temperature, intensity of illumination and water-content affect these transformations. The manner in which they are brought about we are not as yet well informed on. It is clear that if such changes occur while the leaf is being illuminated (and there is little reason to suppose that they do not) the results of analyses may mean nothing as to the sequence of formation of the sugars. For example, in the experiments of Brown and Morris, it will be recalled that they regard the accumulation of fructose as resulting from the hydrolysis of sucrose, the equivalent quantity of glucose having been used in respiration. How could the accumulation of fructose be explained, however, if we accepted the idea of mutual conversion of glucose into fructose? That a conversion of glucose into fructose and *vice versa* actually takes place in plants there can be little doubt. We need but recall that when starch disappears under conditions of wilting of the leaves, the starch is converted into sucrose.[110] In leaves containing starch the sucrose content increases both in the light and in the dark when water content of the leaves decreases. From what we know of the chemistry of starch, this is a glucose anhydride. In order that sucrose be formed from starch some of the glucose must be converted into fructose. Similarly in the artichoke tuber inulin is transformed into reducing sugars; there is evidence that in the process there is produced not only fructose, but glucose is apparently also formed.[111] Inulin is a condensation product of fructose. If, in the hydrolysis of inulin in the plant, glucose and fructose are formed, can it not be concluded that the fructose has in part been converted into glucose? That these two sugars can be converted into each other by means of caustic alkalies, lead hydroxide and calcium hydroxide, has become evident from the work of Lobry de Bruyn[112] and van Ekenstein and of Nef; recently Spoehr and Wilbur have shown that a similar mutual interconversion takes place in solutions of disodium phosphate and in neutral mixtures of this salt and sodium dihydrogen phosphate. In the mammalian body fructose is converted into glucose.[113] Whether a similar action takes place in plants has not been demonstrated with certainty.

The point which we wish to stress is that in a complex system such as that with which we are dealing here, in which the synthesis and hydrolysis of complex compounds are affected by a variety of conditions, the quantity of any particular substance gives little information regarding the rôle of that substance in a series of reactions. All of the carbohydrates, monosaccharides, disaccharides and starch are capable of conversion into each

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[110] Ahrens, *Bot. Arch.*, **5**, 234 (1924). Tollenaar, Diss., Wageningen, 1925, p. 80.

[111] Colin, *Bull. assoc. chim. sucr. dist.*, **37**, 121 (1919).

[112] Lobry de Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 203 (1895); **15**, 92 (1896); **16**, 257 (1897). Nef, *Ann. chem.*, **376**, 1 (1910); **403**, 204 (1914).

[113] Lusk, "The Science of Nutrition," 1917, p. 258.

other quite independently of the photosynthetic process and even the identity of the hexoses is not fixed, but they may undergo stereoisomeric change. Unquestionably it would be very helpful if it were known just what the first sugar is that is produced in photosynthesis. In the present state of our knowledge glucose fits the theoretical requirements most adequately. Yet the fact cannot be entirely disregarded that the demonstration of glucose actually being the first sugar formed is still wanting.

4. Are Other Substances Besides Carbohydrates Formed in Photosynthesis?

The fact that the photosynthetic quotient, $\frac{CO_2}{O_2}$, is very close to unity has been very generally accepted as evidence that the product of the reduction of carbon dioxide is a carbohydrate. There are, however, some experimental observations, which, while not in direct contradiction to the fact just stated are not in entire accord therewith and may nevertheless be of considerable importance in throwing some light on the chemical steps involved in the process.

Saposchnikoff [114] endeavored to determine experimentally whether the amount of carbohydrate formed in a leaf corresponds to the amount of carbon dioxide which the leaf absorbs during the time it is illuminated. This was undertaken with the following equation as expressing the course of the photosynthetic reaction:

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

Leaves of *Helianthus annuus* were cut into two portions: one portion was used for the determination of the gaseous exchange, the other for analysis of the carbohydrate content. In this manner Saposchnikoff found that only 64-87 per cent of the carbon dioxide absorbed appears in the leaves in the form of carbohydrates, the latter being calculated as glucose. These results are apparently not of the highest degree of accuracy; the experiments were carried out over 35 years ago, before the methods of analysis of leaf components had received any critical study. Too much weight can therefore not be placed upon the numerical values given. If, however, we accept the results in principle, it follows that other products besides carbohydrates are formed simultaneously. These are probably fats and proteins. There is nothing surprising in this conclusion; it has long been known that proteins are formed in the leaf when there is an adequate supply of carbohydrates and there is some evidence that the rate of protein synthesis in leaves is greater in light than in the dark.[115] The results of Saposchnikoff further demonstrate the error of endeavoring to determine the rate of photosynthesis from the amount of carbohydrate formed in the leaves of a plant during illumination.[116]

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[114] Saposchnikoff, *Ber. bot. Ges.*, **8**, 240 (1890).

[115] Czapek, "Biochemie der Pflanzen," 2nd Edition, II, 298, 1920.

[116] Mazé, *Compt. rend.*, **180**, 306 (1925).

If the carbohydrate which is formed in photosynthesis has the empirical formula $C_nH_{2n}O_n$ as, for example, a hexose, then the absorption by the leaf of one part by weight of carbon dioxide corresponds to the formation of 0.681 parts of the carbohydrate. If sucrose were formed, with the formula $C_{12}H_{22}O_{11}$, one part by weight of carbon dioxide would result in 0.647 parts of this carbohydrate. A similar calculation for starch or inulin with the formula $C_6H_{10}O_5$, shows that each part of carbon dioxide yields 0.613 parts of either of these carbohydrates.

A more detailed study of the relation of the amount of carbon dioxide reduced to that of carbohydrates formed has been made by Kraschenninikoff.[117] The results, unfortunately, have appeared in a rather obscure publication in the Russian language. The question was approached in different ways. Five different plants were studied and the relations of the amount of carbon dioxide absorbed to the amount of dry matter formed and to the quantity of carbohydrate formed were determined. Thus, in the following table, *A* represents the ratio of the increase in dry weight to the amount of carbon dioxide decomposed, *B* the ratio of the carbohydrates formed to the amount of carbon dioxide decomposed, and *C* the ratio of the amount of carbohydrates formed to the increase in dry weight.

	A	*B*	*C*
Bamboo	0.60	0.45	0.75
Cherry-laurel	0.60	0.31	0.51
Sugar-cane	0.67	0.50	0.75
Linden	0.75	0.56	0.75
Tobacco	0.65	0.37	0.57

From these results it is apparent that when all the increase in dry weight is taken as carbohydrate a value is obtained which is close to the one demanded by the theory that the products of photosynthesis are carbohydrates. It is, however, experimentally impossible from determinations of this nature to differentiate whether the carbohydrate formed is a hexose or starch; the method is not sufficiently accurate. The values under *B*, would go to show that only a portion of the carbon dioxide absorbed by the plants was recovered as carbohydrate. Similarly, the values under *C*, would indicate that not all of the gain in dry material during photosynthesis can be ascribed to carbohydrates.

Kraschenninikoff also endeavored to gain further information regarding the nature of the material which is formed in photosynthesis from the heat of combustion of the leaf material. As in the previous experiments the half-leaf method was used. Thus, per one square meter of leaf surface the following values were obtained:

Increase in heat of combustion of the dry matter	15.4 Kg. cal.
Heat of combustion calculated from the amount of CO_2 absorbed	14.5 " "
Heat of combustion when increase in dry material is calculated as consisting of carbohydrate	14.0 " "

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[117] Kraschenninikoff, "Accumulation of Solar Energy in Plants" (Russian), Moscow, 1901.

It was found that there was an increase of from 2.2 to 3.6 calories in the heat of combustion of the leaf material for each gram of carbon dioxide absorbed. The calculated amount for the formation of carbohydrate from this amount of carbon dioxide is 2.6 calories. From these results it appears that while there are probably some substances besides carbohydrates formed in the photosynthetic process, the amount is variable and, considering the uncertainties of the experimental determination, not very large.

Bose has endeavored to determine the relation of the amount of oxygen evolved to the increase in weight of the plant during photosynthesis. He employed the aquatic plant *Hydrilla* in his experiments and determined the difference in weight of the plant by weighing it in water on a torsion balance and taking the gas emitted from the plant during photosynthesis as being oxygen. This should give a factor on the assumption that the oxygen-carbohydrate factor will be according to:

$$\frac{\text{Oxygen}}{\text{Glucose}} = \frac{C_6H_{12}O_6}{6O_2} = \frac{180}{192} = 0.9375.$$

In no case was this value realized, but in "normal" specimens a factor of 0.8906 was obtained. The difference between the observed and calculated values Bose attributes to the formation of substances other than glucose and if starch is assumed to be a product of photosynthesis a lower carbohydrate-oxygen factor would be expected. It is essential to bear in mind that the gas emitted from a submerged plant during photosynthesis is not pure oxygen but a mixture of oxygen, nitrogen and carbon dioxide. As is discussed in the chapter of this book on Methods of Measuring Photosynthesis the proportion of oxygen in the gas emitted varies with the rate of photosynthesis. In order to arrive at a true carbohydrate: oxygen value it would be necessary to determine the amount of oxygen in the emitted gas. As far as can be judged from Bose's [118] observations if this were done, the results would be close to the calculated values.

That starch or other carbohydrates are not the only products of photosynthesis has been frequently reported. In some of the algae oil droplets appear instead of starch when the plants are illuminated. These observations have usually been made by the aid of the microscope and the composition of the oil and its relation to photosynthesis have not been definitely established. On account of the small quantities and the fact that substances of a fatty nature may result from the metabolic activity of the plant it has been difficult to determine the nature of the oil and its manner of formation. It is a well-known fact that in the plant under certain circumstances, carbohydrates are converted into fats. It is therefore very difficult to determine definitely whether the oil observed in the cells is a direct product of photosynthesis or whether it is a con-

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[118] Bose, "The Physiology of Photosynthesis," 1924, p. 198.

version product of the carbohydrates. Both views have been defended by various investigators, but it is evident that considerably more work will be necessary in order to determine the nature of the chemical reactions involved.[119]

Meyer,[120] on the basis of microchemical examination comes to the conclusion that the oil droplets which appear in the chloroplasts of *Vaucheria* are a direct product of photosynthesis. The accumulation of the oil, according to Meyer, depends upon the rate at which it moves into the cytoplasm and possibly also on its consumption in respiration. He considers that the oil is not a fat, though the evidence on this point does not seem to be altogether clear from the chemical viewpoint.

There are many plants the photosynthetic activity of which has not been studied at all and others in which this process has been followed only in a very crude manner. Undoubtedly many very important observations regarding the process could be made by using plants in which the course of the photosynthetic reactions is apparently different from those usually studied. The diatoms, which also form oil, and many of the plants of the sea offer very interesting material for further study.

The formation of hydrogen peroxide has for theoretical reasons been a matter of much interest. The rôle of hydrogen peroxide in the oxidative processes of the plant in accordance with the theories of Traube has received a great deal of attention, though, largely through the studies of Pfeffer, the presence of hydrogen peroxide in the living cell seemed unlikely. In the theoretical treatments of the photosynthetic process also, the intermediate formation of hydrogen peroxide has been assumed, as, for example, Nef[121] suggested that the reduction of carbonic acid to formaldehyde was brought about by the dissociation of the carbonic acid:

$$\mathrm{OC(OH)_2} \longrightarrow \mathrm{OC}\!\!\begin{array}{l}\diagup\\ \diagdown\end{array} + \mathrm{H_2O_2} \longrightarrow \mathrm{OCH_2} + \mathrm{O_2}.$$

Thunberg[122] has also formulated a theory in which hydrogen peroxide should reduce the carbonic acid; though it has since been found that the theory is based upon an erroneous observation.

Kleinstück[123] reported that hydrogen peroxide could be detected as a result of the photosynthetic activity of *Elodea*. His experiments were repeated by Molisch with negative results. Tanaka, on the other hand, has found that *Chlorella* in Knop's solution free of phosphate, when

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[119] Briosi, *Bot. Zeitg.*, **31**, 529 (1873). Holle, *Flora*, **35**, 113, 154, 161, 184 (1877). Godlewski, *ibid.*, **35**, 215 (1877). Schimper, *Jahrb. wiss. Bot.*, **16**, 178 (1885).

[120] Meyer, *Ber. bot. Ges.*, **35**, 586 (1917); **36**, 235 (1918).

[121] Nef, *Ann. Chem.*, **357**, 253 (1907).

[122] Thunberg, *Zeit. phys. Chem.*, **106**, 305 (1923). Weigert, *ibid.*, **106**, 313 (1923). Bach and Monosson, *C. A.*, **18**, 2910 (1924).

[123] Kleinstück, *Ber. chem. Ges.*, **51**, 108 (1918). Molisch, *Biochem. Zeit.*, **125**, 257 (1921). Tanaka, *ibid.*, **157**, 433 (1925).

illuminated, does produce hydrogen peroxide. The latter accumulated to a concentration of about N. 0.001 when there was present in the nutrient solution hydrocyanic acid in N. 0.01 concentration. It was found that the hydrogen peroxide was not the result of photosynthetic activity, however, but was produced by the photo-oxidation of the chlorophyll.

Chapter 4

The Methods of Measuring Photosynthetic Activity

1. General Considerations

"When you can measure what you are speaking about and express it in numbers, you know something about it, and when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind. It may be the beginning of knowledge, but you have scarcely in your thought advanced to the stage of a science." [1]

As was pointed out in Chapter 2, our knowledge of the phenomenon of photosynthesis is most intimately associated with the development of information concerning the gases of the atmosphere. Practical experimental work as well as the broader conceptions of the process were entirely dependent upon an understanding of the chemical composition of the air. With the discovery of oxygen and its relation to oxidative processes the cosmical function of green plants was quickly elaborated by the genius of de Saussure. Furthermore, improvements in the technique of constructing and manipulating apparatus for the analysis of gases had direct application to the problem of photosynthesis and were reflected in more precise knowledge.

The modern biologist has a doubly difficult task. Not only must he be familiar with physical and chemical methods and manipulation, but he must also be able to apply these methods to his particular problems and be sufficiently conversant with the principles and theory of such physical and chemical methods to be able to apply them to his demands. Just as the practical application of some chemical discovery often requires far more labor and ingenuity than was entailed in the purely scientific work, so the application of physical and chemical methods to specialized biological investigation demands particular scrutiny and cross-checking. It is easy enough to set up a piece of physical apparatus and get numberless readings and numerical results. Whether these mean anything in the biological process under investigation is another question. There is probably no branch of science, with the possible exception of medicine, which is so susceptible to fads in methods and concepts as biology. This is undoubtedly due to the fact that there is still an enormous amount of groping in the dark; the method of trial and error is still the prevalent one. The introduction of a physical or chemical method is all too often

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[1] From Lord Kelvin, quoted by Millekan, R. A., "The Electron," University of Chicago Press, p. 4.

undertaken without the slightest scrutiny of the special work it is supposed to perform.

Furthermore, it is not infrequent that the biologist "strains out gnats and swallows camels" in that the most refined methods of physical measurement are used while some other manipulation of equal importance does not approach the former in accuracy.

The methods which have been used to detect and measure the photosynthetic activity of illuminated chlorophyllous leaves and other parts of plants are based upon three different principles. These are:

1. The liberation of oxygen.
2. The absorption of carbon dioxide.
3. The formation of organic substance—the gain in dry weight.

It is essential to bear in mind that these three methods do not always yield the same results; that the three methods are based upon different chemical reactions, each of which represents a step or a series of steps in a long chain of reactions which are, of course, more or less dependent upon each other, but nevertheless fall in the class of step-reactions. We are evidently not dealing with a single reaction, but with a long series of reactions involving the manufacture of highly complex organic substances from the most stable carbon compound. We are able to observe the raw materials, carbon dioxide and water, which enter the factory. Also we note the end products, oxygen and carbohydrates, and have some idea of the power or energy necessary to run the factory. So far all attempts to establish the intermediate steps and thus determine the course of the various reactions have failed. At present we are then confined to determining the nature and course of the process by inference from careful quantitative investigation of the relation of the factors: intake of raw material, carbon dioxide and water, formation of synthesized product, carbohydrate, "waste product," oxygen, and energy consumed.

Furthermore, as has been repeatedly emphasized, respiration always accompanies photosynthesis. At best we can measure only the difference between two reactions which are in general running in opposite directions. As yet it has not been possible to separate clearly the two processes. The intensity or rate of the respiratory activity can, of course, be established by parallel experiments, or still better with the same plant during periods of darkness. Here the assumption must be made that the rate of respiration does not change in the light. In such experiments of alternate periods of light and darkness it is also essential that these periods be not too long. The question of the separation of the two processes of respiration and photosynthesis has been already dealt with.

A very large number of qualitative methods have been devised to detect photosynthetic activity; most of these are based upon tests for oxygen. However, as the development of the subject is dependent upon a more intense and exact study, the emphasis is here laid upon those methods which are applicable to quantitative investigation.

It should be stated at the outset that none of the methods thus far developed is entirely satisfactory, and undoubtedly several of these could be greatly improved. As almost the entire interpretation of the process of photosynthesis is based upon these methods and the literature of the subject is filled with many erroneous assumptions and explanations which have been often repeated, it seems not amiss to subject these methods to a critical examination in the light of the experiences of various workers. In general, the determination of the amount of carbon dioxide absorbed has been the most satisfactory method. This is due first of all to the relative ease with which carbon dioxide can be determined quantitatively, but also because the carbon dioxide absorbed bears a directer relation to the material synthesized than does the amount of oxygen liberated. Oxygen is utilized in subsequent respiratory oxidation of organic material and may be held in varying amounts combined with a large variety of organic acids, thus being, as it were, drawn out of the direct path of the photosynthetic process. This fact is most clearly brought out through a study of the $\frac{CO_2}{O_2}$ ratio. In using the principle of the amount of oxygen liberated the fact has very frequently been overlooked that this merely represents the difference between the amount of oxygen produced in the photosynthetic process and that used by respiration.

It has not been possible as yet to establish a satisfactory standard of photosynthetic activity. The amount of carbon dioxide absorbed, of oxygen emitted or of dry material formed have been calculated on the basis of area illuminated, fresh or dry weight of the plant and on the amount of light absorbed by the plant under investigation. For leaves the area standard gives a fairly satisfactory means of comparison. But this method cannot be applied to aquatic plants or only with great difficulty and lack of precision and, of course, not at all to algae; and it is with the latter that some of the best work on measuring the rate of photosynthesis under different conditions has been done. The standard of weight is most unsatisfactory, for relatively slight changes in either the fresh or dry weight of the leaves, changes which may in no manner be connected with the photosynthetic process, may alter the results very materially. From the point of view of the energy changes involved, the method of calculating photosynthetic activity on the basis of radiant energy absorbed by the plant offers many advantages, though this entails rather elaborate apparatus and a number of corrections.

2. The Liberation of Oxygen

A variety of methods have been employed to detect and determine the oxygen liberated in the photosynthetic process. They include (A) the use of the leucobases, (B) luminous bacteria, (C) motile bacteria, (D) the optical properties of hemoglobin, (E) counting the bubbles of liberated oxygen from a plant submerged in water and (F) gas analytical methods. Most of these are of a qualitative nature rather than methods of quantitative measurement. However, by every one of these methods some knowledge has been gained if it has been only to serve as a check on some previous observation. Nevertheless, only the last two can be considered as at all possessing the qualities of exactness and in the strictest sense this applies only to the last, the analytical gas method.

In order to demonstrate the formation of oxygen Boussingault[2] introduced the use of phosphorus, the presence of oxygen being demonstrated by formation of a fog of P_2O_5, the luminescence of the phosphorus in the dark and the reduction of the total volume of the gas. The method is of limited value on account of the poisonous character of the phosphorus and of the acids formed.

a. The Use of the Leucobases

When certain dyestuffs in solution are reduced, the colorless leucobases are formed. These leucobases in turn react very readily with oxygen reforming the colored compound and thus are very sensitive reagents for this gas. One of the best dyes for this purpose is indigo or indigo-carmine. This fact has been used for the detection of the evolution of oxygen by algae by Beijerinck[3] and has found extensive use in bacteriology for anaerobic cultures. A good method of preparing the indicator is to shake a concentrated solution of sodium bisulphite with zinc dust for about five minutes, keeping the mixture cool all the time. Neutralize exactly with a very thin paste of $Ca(OH)_2$. This is allowed to settle, and the clear solution is used to decolorize a solution of indigo-carmine, adding the reducing solution drop by drop. The blue dye solution turns a light yellow; naturally air must be kept away from this solution as much as possible and it is best kept in a vessel which the solution fills entirely. If a few filaments of an alga are placed in the solution and the whole exposed to light, the blue color will appear in the solution immediately surrounding the plants. It is essential that the water used for making up the solutions has stood in the air so as to have dissolved some carbon dioxide and also that not too much $Ca(OH)_2$ is added. The method is applicable for demonstration rather than for purposes of investigation. Unless the indicator is very carefully made it will turn blue in the light even in the absence of oxygen. Furthermore, it is now quite well established that in complete absence of oxygen photosynthesis does not take place even in intense light. It is therefore not desirable to produce strictly anaerobic conditions in experiments on photosynthesis. If an indicator for such conditions is desired the method given by Fuhrmann[4] for the preparation of an indigo solution is very serviceable.

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[2] Boussingault, *Ann. Sci. nat.* V., **10**, 331 (1869).

[3] Beijerinck, *Bot. Zeitung*, **48**, 741 (1890). Linsbauer, L. and K., "Vorschule der Pflanzenphysiologie," Vienna (1906).

[4] Fuhrmann, F., in Abderhalden, E., "Handbuch der Biochemischen Arbeitsmethoden," Vol. III-2, p. 1241, Berlin, 1910.

b. Luminous Bacteria.

Another method of detecting the oxygen produced in photosynthesis was devised by Beijerinck. The method depends upon the phenomenon of luminescence which is produced in certain bacteria in the presence of oxygen; in the absence of oxygen the bacteria emit no light. The method is best adapted to the use of plate cultures of luminous bacteria (Micrococcus phosphoreus Cohn), in seawater containing marine algae. The culture containing an alga can also be sealed in a tube; when all the oxygen in the tube has been consumed by respiration, the bacteria cease to luminesce. After illumination of but a few minutes if the tube is then taken into the dark, the luminescence is plainly visible. Molisch[5] claims that such a tube which has been allowed to become dark, becomes clearly luminescent if it is illuminated for only a few seconds with a match. The amount of oxygen produced by photosynthesis by so feeble and brief an illumination must be exceedingly small and is less than can be detected by any chemical means or probably even by any physical method. Harvey and Morrison[6] have determined the concentration of oxygen which causes "just perceptible" luminescence of luminous bacteria. This they find to be at a pressure of oxygen of about 0.005 mm. of mercury or 1 part by weight of oxygen in 3,700,000,000 cc. of sea water.

c. Motile Bacteria Method.

By means of this method, which was introduced by Englemann, many of the earlier observations on photosynthesis were made. The method depends upon the fact that certain aerobic bacteria are motile in the presence of oxygen and quite inactive in the absence thereof. It is essential that the appropriate bacteria be employed. Those which have been most generally used are the forms usually grouped under the general term Bacterium termo (Proteus vulgaris, Hauser). Certain Spirillum forms may also be employed, but these are exceedingly sensitive, so that great care must be used in order to avoid experimental error. Care must also be exercised to ensure absolutely pure cultures which are free from anaerobic forms. Slope or stab cultures may be made on bullion-agar, which are allowed to develop at 25°. The cultures should not be more than one to two weeks old and the bacteria taken from the edges of the growth in order to obtain the motile forms.

The plant material, the photosynthetic activity of which is to be observed, is of course confined to small objects; and some experience in the use of the microscope is essential. Algal filaments, sections of leaves or single cells are placed on a slide in a fluid containing the bacteria. It is desirable to add sufficient bacteria so that a drop shows slight turbidity. The solutions to be used should contain a small percentage

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[5] Molisch, "Lichtentwickelung in den Pflanzen," Leipzig, 1905, p. 15. "Leuchtende Pflanzen," Jena, 1904.

[6] Harvey and Morrison, *Jour. Gen. Physiol.*, 6, 13 (1924).

of salts, as in ordinary tap water, and but very little nutriment, as sucrose. In neutral meat extract of 0.2 to 0.5 per cent, in time products may be formed which exercise an injurious effect on the bacteria or the plant cells. The preparation is covered with a coverglass and is sealed with a mixture of vaseline and paraffin. In the dark the oxygen which was enclosed in the preparation is soon exhausted by the respiration of the bacteria and the green plant and the bacteria soon come to rest. If oxygen is admitted or generated in the preparation the bacteria begin to move and are chemotactically attracted by a bubble of air or oxygen. If the sealed preparation is illuminated the bacteria congregate and move in the neighborhood of the green plant. When the preparations are kept in the dark for about 24 hours the bacteria become very sensitive to minute traces of oxygen. The best temperatures for these tests are between 20° and 30°. Since the bacteria are sensitive to exceedingly small traces of oxygen (Pfeffer states they react to a billionth part of a milligram) the photosynthetic activity of a single cell and even of an isolated chloroplast can be detected. In the dark and consequent stopping of photosynthesis, the movement of the bacteria gradually ceases and they are again distributed in the field. Subsequent illumination again produces the movement and congregation of the bacteria.

The method is essentially a test for oxygen, and while it has been applied in a number of ways it has distinct limitations in the study of the factors influencing photosynthesis and its use must be subjected to strict control. A number of classical investigations have been carried out by means of this method and its interest is probably rather an historical one. Thus Englemann [7] endeavored to demonstrate what portions of the spectrum are most effective in producing photosynthesis and was also able to demonstrate that photosynthetic activity was confined to those portions of the plant bearing chloroplasts, and takes place only in illuminated chloroplasts.

d. Optical Properties of Hemoglobin.

The use of hemoglobin as a test for the oxygen emitted by plants during photosynthesis was introduced by Hoppe-Seyler.[8] Small plants or a portion of a plant such as *Elodea* are sealed into a tube with nutrient solution and some dilute hemoglobin. After remaining in the dark for a while the tube, when examined spectroscopically, shows the characteristic absorption bands of hemoglobin. If the tube is brought into the light, the oxygen produced by the photosynthesis of the plant converts the hemoglobin into oxyhemoglobin and the formation of the latter can be detected by the change in the absorption spectrum.

[7] Englemann, *Arch. ges. Physiol. Pflüger's*, **57**, 375 (1894); *Bot. Zeitg.*, **40**, 419 (1882); **42**, 81, 97 (1884). Pfeffer, *Untersuchungen aus dem bot. Inst. Tübingen*, **2**, 589 (1888). Ewart, *Jour. Linnéan Soc. Bot.*, **31**, 364 (1897). Kny, *Ber. bot. Ges.*, **15**, 388 (1897).

[8] Hoppe-Seyler, *Zeit. physiol. Chem.*, **2**, 325 (1879).

Englemann[9] adapted this method to microscopical demonstration of the production of oxygen. A filament of an alga, as *spirogyra,* is sealed under a coverglass with a drop of hemoglobin solution which has previously been reduced. On illumination of the preparation the formation of the bright scarlet oxyhemoglobin surrounding the plant can be clearly followed under the microscope. In the dark hemoglobin is formed again and the transition of the oxyhemoglobin into hemoglobin can be followed in the immediate neighborhood of the plant. With the aid of a spectralocular it is possible to detect these changes taking place within a few seconds after illumination. If the preparation is illuminated with a microspectrum differences in the rate of change of color can be followed in different portions of the spectrum.

e. The Bubble Counting Method.

This method depends upon the fact that when cut leaves or other parts of aquatic plants are placed in water containing dissolved carbon dioxide, on illumination gas bubbles are liberated at the cut ends or surfaces. The method does not yield satisfactory results with land plants. The solubility of carbon dioxide in water is relatively high; during photosynthesis oxygen is formed, the water is already saturated with this gas which is very sparingly soluble in water and the oxygen escapes in the form of small bubbles. Sachs[10] showed that the rate at which these bubbles are liberated may be taken as a measure of the relative rate of photosynthesis. With the proper cut surface such plants as *Elodea, potamogeton, ceratophyllum, hydrilla* liberate bubbles at a rate which can be easily counted. On account of its simplicity and ease of manipulation this method has been used not only for demonstration purposes but also to investigate the rate of photosynthesis under various conditions. The method possesses the following advantages: (1) the execution of a large number of observations of short duration (a few minutes). As against the determination of gas volumes this may have some distinct advantages, for such small volumes of gas as are liberated in a period of observation of gas bubbles can hardly be determined accurately by volumetric analysis. (2) Natural illumination can be used and can be taken as of constant intensity for such short periods. (3) The short duration of the periods of observation largely excludes the errors caused by changes within the plant. It is, of course, essential that comparative experiments be made with one and the same plant, as it is impossible to get two parts of a plant that are absolutely alike. It is also of importance that the relative position of the plant to the light should not change, as otherwise the amount of illuminated surface may change. The cut surface should not be submerged too deeply in the water and the distance

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[9] Englemann, *Biol. Zentralbl.,* XX. 8, 33 (1888).
[10] Sachs, J., *Botan. Zeitung.,* 22, 363 (1864).

between the place where the gas is being emitted and the surface of the water must be constant.

Now the principle of the method lies essentially herein: that the rate of bubble emission is taken as a measure of the rate at which the plant is reducing carbon dioxide and liberating oxygen. In order that bubble formation may take place it is necessary that there be an increase in pressure in the intercellular spaces. This pressure must overcome (1) the frictional resistance of the gas in the minute intercellular canals, (2) the capillary forces of the water in these canals, (3) the weight of the capillary water column, and (4) the pressure of the water from the cut surface to the level of the water. A direct relationship between the rates of bubble emission and photosynthesis will exist only if the composition of the gas is pure oxygen or contains only negligible impurities or if the gas contains always a definite percentage of oxygen independent of the rate of emission. The method has been subjected to considerable experimental criticism, particularly by Pfeffer and by Kniep,[11] although little attention has been paid to their findings and many mistakes have been repeated. Pfeffer on the basis of theoretical considerations pointed out that the oxygen content of the emitted bubbles varies in the same direction as the rate at which the bubbles are emitted. This very important question has been thoroughly investigated by Kniep. His results are discussed below. It is very important that the CO_2-tension in the water should be constant or nearly so during the entire course of the experiments which are to be compared. Sachs ran carbon dioxide into the water. An excess of carbon dioxide is to be avoided, however, as the gas will pass through the plant and be emitted, quite independently of photosynthesis. This gas stream may continue for a long time even in the dark.[12]

The best medium is a large supply of tap water to which the plant has free access or better, as Anglestein[13] has found, a freshly prepared solution of one per cent potassium bicarbonate.

Kniep,[14] by using the very accurate methods of gas analysis of Krogh in which about 3-6 cmm. of emitted gas were analyzed, investigated the relation between the composition of the gas and the rate at which it was emitted. It was found that the oxygen-content of the gas was higher the greater the rate of bubble emission. The bubble rate was regulated by means of altering the light intensity. Thus it developed that with changing light intensity the rate of bubble emission increases and de-

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[11] Daubney, H., *Phil. Trans. London*, **126**, 149 (1836). Devaux, H., *Ann. scien. nat. Bot.*, Serie 7, **9**, 35 (1889). Reinke, J., *Bot. Zeitg.*, **41**, 697 (1883). Cloez, S., and Gratiolet, *Ann. sci. nat. Bot.*, Serie 3, **32**, 41 (1851). Pfeffer, W., *Arb. d. bot. Inst. Würzburg*, **1**, 1 (1871). Kniep, H., *Jahrb. wiss. Bot.*, **56**, 460 (1915).

[12] Van Tieghem, P., *Compt. rend.*, **69**, 482 (1869).

[13] Anglestein, V., *Cohns Beitr. z. Biol. der Pfl.*, **10**, 87 (1910).

[14] Kniep, H., and Minder, F., *Zeit. f. Botan.*, **1**, 619 (1909). Pantanelli, E., *Jahrb. wiss. Bot.*, **39**, 167 (1903).

creases more slowly than the actual rate of photosynthesis. Thus for example the following analyses are given by Kniep:

Cabomba caroliniana.

12:30. 20 bubbles in 8.8 seconds, gas occupying 79.2 scale divisions in 2 minutes. The percentages of O_2 and N_2 are based upon the total volume after deduction of the CO_2.

CO_2	2.2	Per cent
O_2	40.2	" "
N_2	59.8	" "

Photosynthesis was allowed to continue uninterruptedly.

3:40. 20 bubbles in 11 seconds. Gas occupying 63.1 scale divisions in 2 minutes.

CO_2	1.9	Per cent
O_2	35.5	" "
N_2	64.5	" "

4:15. Further reduction in light intensity.

4:45. 20 bubbles in 30 seconds. Gas occupying 72.3 scale divisions in 8 minutes.

CO_2	1.5	Per cent
O_2	22.8	" "
N_2	77.2	" "

The emission of oxygen is made possible because of an increase of pressure thereof in the intercellular spaces, a pressure greater than can be taken care of by the diffusion of the gas into the surrounding water which is already saturated with oxygen. The gas bubbles at first contain a per cent of oxygen not much greater than is contained in the intercellular air before photosynthesis started. Nitrogen and carbon dioxide thereafter are drawn out with the oxygen. If these two gases were not again replaced, the escaping gas would soon be composed of pure oxygen. But both the nitrogen and carbon dioxide are replaced as soon as their percentage in the intercellular spaces drops below their respective tensions in the water surrounding the plant. In the case of the carbon dioxide, which is very low, the conditions are somewhat different from nitrogen, both on account of its being used in the photosynthetic process and because of its relatively high solubility in water. When the rate of bubble emission is high, relatively large quantities of nitrogen are carried away in a given time interval. The composition of the emitted gas will thus decrease in nitrogen and increase in oxygen. But the less nitrogen there is in the intercellular spaces, the greater is the tendency of this gas to diffuse in from the surrounding water. A condition is then arrived at in which the relation of N_2 to O_2 remains constant at a constant rate of bubble emission. This represents a state where the rate at which diffusion of nitrogen into the intercellular spaces is equal to the rate at which it is removed by the passage out with the oxygen. During very active photosynthesis the *absolute* amount of nitrogen emitted is greater than during a low rate of photosynthesis, although the per cent of nitrogen liberated under the former conditions is lower than during the latter.

Another factor which must receive consideration in the discussion of this method is the solution of oxygen from the plant to the surrounding water. Kniep has shown experimentally that this is not a negligible factor. According to Henry's law the solubility of a gas in a given volume of water is proportional to the pressure of that gas. Assuming then that the pressure of the gas in the intercellular system is equal to that of the atmosphere, even in weak photosynthesis there would be the conditions for oxygen going into solution, for solution depends upon the partial pressure of the intercellular gas and this for oxygen during photosynthesis exceeds that of the atmosphere which is in equilibrium with the water. As Kniep furthermore showed, water which has been covered with mineral oil and saturated by running a stream of oxygen through it increases in oxygen content when *Elodea* is permitted to carry on photosynthetic activity. A similar increase in O_2-content is obtained when water which is in equilibrium with the atmosphere is placed over photosynthetically active plants.

Movement of the water surrounding the plants has a decided effect on the rate at which the bubbles are given off. The contradictory results of Darwin and Pertz[15] and of Nathanson[16] have been explained by Kniep in the paper referred to above. The difficulty lies primarily in the interfering of the physical stream of diffusion through the plant.

The rate of bubble emission is decreased in moving water when this is not saturated with the atmospheric gases. The oxygen which is formed in photosynthesis escapes partly as bubbles and partly it is dissolved in the water surrounding the plant. Thus in quiet water an area about the plant becomes saturated with oxygen and more oxygen escapes as bubbles. If now the water about the plant is in motion this saturated area is removed, a larger proportion of the oxygen is dissolved and less gas escapes as bubbles.

The rate of bubble emission is increased in moving water when this is supersaturated with the gases of the atmosphere. Under these circumstances there is a pure physical stream of diffusion through the plant, long ago described by Van Tieghem, and already referred to. This stream is augmented either by movement of the water or by slight jarring of the plant.

There is another important phenomenon of bubble emission which has been described by Kniep. When the bubble stream from a plant is interrupted by withdrawing the light, and the plant is then reilluminated,

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[15] Darwin, F., and Pertz, *Proc. Cambridge Philos. Soc.*, **9**, II-76 (1896).
[16] Nathanson, A., *Ber. d. Verh. d. Kgl. Saechs. Ges. d. Wiss.*, **59**, 711 (1907).

the bubble stream does not commence at once. In fact, the longer the duration of the dark period the longer does it take for the bubbles to appear. Thus, for example, Kniep found for *Elodea canadensis:*

20 Bubbles in 5.4 seconds.

Time plant was kept in dark	20	20	40	40	80	300	300	600
Time required for bubble stream to start after reillumination	8.0	8.2	13.2	13.0	18.2	60.0	61.0	93.5

The explanation for this phenomenon given by Kniep is that when photosynthesis ceases there is a reduction of gas pressure in the intercellular spaces. This permits the further entrance of water into these spaces. When photosynthesis recommences a certain gas pressure must be reestablished to force out the water-column before the bubbles can be forced through the intercellular canals. The time required for the bubble stream to start after reillumination is the time during which this gas pressure is being developed.

Kohl[17] has also called attention to the fact that an error is introduced on account of the varying size of the gas bubbles. With a rapid bubble stream the individual bubbles are smaller than when the rate is slower and each bubble clings to the plant longer. Kohl devised therefore his volumetric bubble counting method. He used a single *Elodea* leaf which is cut in such a manner that a small portion of the stem is attached to the leaf. The leaf is held in the bottom of a shallow dish of water by means of a small glass plate. The bubbles emitted in the light from the cut surface take on a practically spherical form so that by measuring their diameter with the microscope micrometer the volume of the gas emitted can be easily calculated. In this way an advantage is gained in that the angle of the light falling on the leaf can be controlled. However, the method has found very little application.

It is evident then that the bubble counting method can rarely be used as a strictly quantitative measure of photosynthetic activity. For purposes of demonstration and for comparative observations it has certain advantages. It is possible that for studying the effects of substances in solution, different frequencies of light, of varying light intensity and of temperature the method may be put to use. However, it is exceedingly important to remember that the relative position of the plant to the source of light must be maintained and that after changing any of the external conditions some time must elapse before a constant rate of bubble emission is attained. It is certain that under strictly constant external conditions an increase or decrease in the rate of bubble emission represents respectively an increase or decrease in the rate of photosynthesis. This relation is not directly proportional however. In order to determine the absolute rate of photosynthesis it is necessary to deter-

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[17] Kohl, F. G., *Ber. bot. Ges.*, 15, 111 (1897).

mine the volume of the gas liberated in unit time and to determine the oxygen content of this. Also it is important to observe the increase of oxygen in the water. Finally it must be realized that the temperature of the water surrounding the plants must remain constant, for a variation of 0.2° affects the size of the bubble very appreciably.

Bose [18] has devised an apparatus in which the bubbles escaping from the cut end of the plant are collected under an oil valve. When the pressure of the gas attains a certain pressure, it lifts the oil valve and escapes. The apparatus is devised so that successive escapes from the valve represent equal volumes of gas which are independent of the frequency and the size of the bubbles which are emitted from the plant. With this apparatus Bose has also constructed an electrical device which records by a dot on the drum of a clock each time the gas passes the valve. By means of this apparatus the rate of photosynthesis is recorded directly on the drum by the frequency of the dots in a period of time. Undoubtedly this ingenious piece of apparatus can serve to demonstrate approximately the rate of photosynthesis under different conditions. It has, however, the same objection as the original method—namely, that it does not measure the amount of oxygen which is emitted, but only the total gas, and, as has been pointed out, the composition of this gas is not constant, but the amount of oxygen contained therein varies with the rate of photosynthesis.

f. Gas Analytical Methods.

Oxygen Absorbents. The methods of gas analysis for general chemical analytical work have been repeatedly discussed and compiled and several excellent treatises have been prepared.[19] While, of course, the general principles are the same for biological work and especially for investigations in plant respiration and photosynthesis, little has been done to collect the required information. In applying these general methods it has been found that biological work requires many alterations both as to procedure and accuracy demanded. For this reason biological gas analysis is to be regarded as a special and advanced chapter of chemical analysis and sets as a prerequisite a thorough understanding of the fundamental chemical principles involved. This includes the precautions and treatment of glass ware, stop-cocks, mercury, rubber, the calibration of apparatus, the taking and handling of gas samples, reading of burettes, etc., as well as, of course, the fundamental gas laws.

The composition of the atmosphere and especially its oxygen-content has been a subject of keen interest for a very long time. In fact, as has been stated, Ingen-Housz, the discoverer of photosynthesis, was pri-

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[18] Bose, "The Physiology of Photosynthesis," Longmans, Green & Co., 1924, p. 17.

[19] Hempel, W., translated by G. L. M. Dennis, "Methods of Gas Analysis," The Macmillan Company. Foster, C., Le Nève and Haldane, J. S., "The Investigation of Mine Air," Charles Griffin & Co., London. Haldane, J. S., "Methods of Air-Analysis," London, 1912. Geppert, I., "Die Gasanalyse und ihre physiologische Anwendung," Berlin.

marily interested in the influence of plants on air. It was recognized early that no single chemical element enters so intimately into the existence of man on the earth as does oxygen. The manifold phases of this fact have led to the investigation of the relations of oxygen in many branches of science, in technology and the arts. After the discovery of oxygen by Priestley and Scheele the question of the composition of the atmosphere became a popular subject with scientists and the scientific dilettantes. From then on a variety of methods was devised and their virtues and faults widely discussed. The development of the methods of air analysis forms a most interesting chapter in the history of chemistry which has been admirably compiled by Kreusler[20] and more recently by Benedict.[21] Both of these works are rich sources of information on physiological gas analysis.

With the development of more rapid and accurate methods of air analysis there has been a very general trend away from gravimetric methods toward volumetric methods of oxygen determination, so that at the present time only the latter principle demands consideration. For practical and accurate determinations of oxygen in work on respiration and photosynthesis it is desirable that these can be carried out without consuming too much time and that successive determinations can be made easily. It is essential to have an efficient absorbent for oxygen which means that: (1) the oxygen should be completely absorbed; (2) in the chemical reaction between the oxygen and the absorbent no gases should be given off into the residual gas and thereby increase its volume; (3) any temperature changes produced by the absorption must be completely and readily compensated. Besides these requirements it is, of course, essential that the oxygen absorbent does not affect any other gas in the gas mixture, i.e. that the contraction in volume as measured should be due only to the absorption of oxygen. Finally, as is general in gas analysis, changes in barometric pressure taking place during an analysis must be fully compensated and the tension of aqueous vapor in the gas must be the same in the original and in the final measurement after absorbing the oxygen.

A very large number of oxygen absorbents have been suggested and used in various apparatus. Those which demand consideration for our purpose are briefly discussed below.

The use of a heated copper spiral for the absorption of oxygen was introduced by von Jolly[22] and has been extensively used. Kreusler improved the method so that it is now one of the most exact methods known.[23]

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[20] Kreusler, U., *Landw. Jahrb.*, **14**, 305 (1885).

[21] Benedict, F. G., "The Composition of the Atmosphere, with Special Reference to Its Oxygen Content," Carnegie Institution of Washington, Pub. No. 166 (1912).

[22] Jolly, Ph. von, *Wiedemann's Ann. N. F.*, **6**, 520 (1879). Kreusler, U., *Landw. Jahrb.*, **14**, 305 (1885).

[23] Durig, A., *Biochem. Zeit.*, **4**, 65 (1907). For very accurate work, it must be remembered that copper oxide loses oxygen at high temperatures; *cf.*, Gmelin-

On account of the many precautions which must be taken and the time consumed in the various steps of the process it is too complex and tedious for making a large number of determinations.

The absorption of oxygen takes place much more slowly and finally ceases when considerable quantities of oxide are formed. It is possible to dissolve this coating of oxide in a solution of ammonium carbonate. Oxygen is rapidly and completely absorbed when brought in contact with copper and a solution of ammonium carbonate; however, in this process considerable carbon dioxide is given off. This latter difficulty can be avoided by the use of metallic copper in contact with solution (containing undissolved pieces) of ammonium sesquicarbonate in a saturated solution of ammonium hydroxide specific gravity 0.90. Such a combination has an absorption capacity about 24 times its volume of oxygen. It is essential that only bright copper be used. The combination can be used in a pipette as designed for phosphorus. It has a distinct advantage over phosphorus of absorbing oxygen completely even at —7 , and has also a much higher absorption coefficient than potassium pyrogallate. The mixture also absorbs carbon monoxide and acetylene so that it cannot be used for analytical purposes in the presence of these gases. In gas mixtures containing more than 30 per cent oxygen the absorption is not quantitative. The tension of ammonia in this solution is not great but for accurate analytical work it is advisable to wash the residual gas with water or dilute sulfuric acid before making readings.

A higher absorption, fifty to sixty times its volume of oxygen, can be obtained by using a solution made by saturating with ammonium chloride a mixture of one part concentrated ammonia and one part of water.[24]

Sodium hyposulfite is an excellent absorbent of oxygen: $Na_2S_2O_4 + H_2O + O \longrightarrow 2NaHSO_3$. Durig has used the following solution with success: 50 grams of $Na_2S_2O_4$ are dissolved in 250 cc. of water and before using mixed, excluding air, with a solution of 30 grams NaOH in 50 cc. of water. The absorption pipette must be protected against the air by a bulb containing water and mineral oil. On account of the corrosive action of this reagent on glass a special technique is necessary which requires some skill; also it is advisable to coat the inside of the absorption pipette with a thin covering of gutta percha. The reagent has an extremely high absorption coefficient, about 40 air analyses can be made with 100 cc. solution. It is advisable to pass the gas mixture into the absorption pipette at least twice.

An improvement in the sodium hyposulfite method has been devised by Fieser[25] by the use of anthraquinone-β-sulfonate. For use in a shaking pipette the following solution is recommended: 16 grams $Na_2S_2O_4$ (86.7 per cent), 6.6 grams NaOH (sticks), 2 grams anthraquinone-β-

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Kraut's "Handbuch der Anorganischen Chemie," 7th Ed., Vol. **5**, Part I, pp. 736, 741, 1564. It is advisable therefore to pass the gas finally over iron filings.

[24] Badger, W. L., *Jour. Ind. Eng. Chem.*, **12**, 161 (1920).

[25] Fieser, *Jour. Amer. Chem. Soc.*, **46**, 2639 (1924).

sulfonate (95 per cent), and for a bubbling pipette: 16 grams $Na_2S_2O_4$, 13.3 grams NaOH, and 4 grams anthraquinone-β-sulfonate; both the solutions represent the amounts of the materials dissolved in 100 cc. of water.

One of the commonest and best absorbents for oxygen is yellow phosphorus. It reacts very readily with oxygen and since the various reaction products are all soluble in water, all that is necessary is to renew the water surrounding the phosphorus from time to time in order to keep the surface of the phosphorus fresh. Thus the phosphorus can be used for a very large number of analyses, especially if it is protected from the light. For this purpose there are special pipettes of brown glass, or the ordinary glass pipette can be covered with paint or a light-tight box. Furthermore, the reaction products of phosphorus and oxygen are solids or liquids having a very low tension so that no error is caused should any of these products be present in gas residue after absorption. Freshly prepared sticks of phosphorus do not absorb oxygen quantitatively. It is advisable to run air through the pipette a few times before making air analysis. Gas mixtures containing more than 50 per cent oxygen should be diluted with an equal volume of nitrogen, as with such high concentration of oxygen the phosphorus may melt or ignite. To dilute the oxygen, air which has previously been passed over phosphorus can be used. There are a number of substances which hinder the oxygen absorption, e.g. ammonia, hydrocarbons, etherial oils and alcohol.

It is essential to watch the temperature at which analyses are being made when phosphorus is used as an absorbent. At 20° the reaction proceeds normally, i.e. it requires less than 10 minutes; below 14° at least 15 minutes is required to absorb the oxygen; while it is not advisable to use phosphorus below 10°. Under such conditions it is necessary to have the absorbing bulb surrounded by a bath of warm water.

It is best to prepare the phosphorus which is to be used shortly before placing it into the absorption pipette. For this purpose yellow phosphorus is melted under water in a small vessel in a water bath at 50°. The operation should be carried out in as weak illumination as possible. The molten phosphorus is then carefully drawn into tubes of about 2 mm. diameter; the upper end of the tube is closed and at once placed in cold water. The phosphorus undergoes a decided contraction on cooling so that the sticks of phosphorus can be easily pushed out of the glass tubes with a rod. In working with phosphorus, of course, proper precautions must always be taken on account of its great toxicity and inflammability.

Potassium pyrogallate has in recent years found greatest favor as an oxygen absorbent. However, there are a number of precautions which are absolutely essential to get accurate results. After Liebig announced his discovery that potassium pyrogallate can be used to absorb oxygen quantitatively it was found that in the interaction of oxygen with an alkaline solution of pyrogallol a certain amount of carbon monoxide is formed.[26] Hempel[27] also called attention to the fact that it is essential that potassium hydroxide purified from alcohol should not be used. Considerable difficulty has been encountered by various workers to find just the right solution of potassium pyrogallate. Haldane recommends a fully saturated solution of potassium hydroxide of specific gravity of 1.55. The solution is made up of 1 gram of pyrogallic acid to 10 cc. of the potassium hydroxide specific gravity 1.55. However, at lower temperatures this solution is apt to solidify which may cause a great deal of trouble in the apparatus. The solution is the most efficient oxygen absorbent thus far recommended. The directions given by Benedict[28] for a slightly more dilute solution have proven very trustworthy: "A solution of potassium hydroxide was prepared by dissolving 500 grams of stick potassium hydroxide, not purified by alcohol, in 250 cc. of water. Usually the specific gravity of the resulting solution was 1.55. During the progress of this research, several shipments of stick potassium hydroxide were used, and the varying water-content of the chemical is shown by the fact that it was frequently necessary to add more potassium hydroxide to bring the solution to the desired density. To 135 cc. of this saturated solution was added a solution of 15 grams of pyrogallic acid in 15 cc. of distilled water. . . .

"This solution takes up oxygen rapidly and has a high absorptive capacity. It has been assumed that since the solution was so much more concentrated than Hempel's, his assertion that no carbon monoxide was developed with his weaker solutions held true in this case also, particularly as Haldane states that with his extremely concentrated solution no traces of carbon monoxide are found. Furthermore, certain evidence here presented seems to support this view. When a known sample of air is analyzed a number of times, the percentage of oxygen at the beginning of the series does not differ from that found at the end even when as many as 30 analyses are made with the one charge of potassium pyrogallate. It seems reasonable to suppose that if carbon monoxide were formed, a somewhat different amount would be produced after the first, second or third analysis than after the twenty-eighth or twenty-ninth. On the other hand, it is not impossible that in the production of carbon monoxide there may be an extremely small quantitative relationship between the oxygen absorbed and the disintegration of the pyrogallic acid, so that the carbon monoxide given off may remain strictly proportional to the amount of oxygen consumed. Since in each of these analyses exactly the same amount of oxygen is absorbed, there still may be a slight constant factor present; consequently, it is necessary to take into consideration the fact that in all of these analyses there may be traces of carbon monoxide produced. In that case the tendency

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[26] Boussingault, J. B., *Compt. rend.*, **57**, 885 (1863).

[27] Hempel, W., *Ber. chem. Ges.*, **20**, 1865 (1887). Tacke, B., *Archiv. fuer die Gesammte Physiol.*, **38**, 40 (1886).

[28] Benedict, F. G., Carnegie Inst. of Washington, Pub. No. 166, p. 80 (1914).

would be to make the percentage of oxygen slightly too small. Although throughout the whole research a slightly modified Haldane solution was used, subsequent experiments with the Haldane formula show a somewhat larger oxygen percentage. This increased percentage may be due to two causes: (1) the actual absorption of more oxygen, or what is more probable (2) the formation of less or no carbon monoxide. It remains a fact, nevertheless, that the solution as used is without question suitable for a comparative study of the oxygen percentage of the atmosphere."

The Haldane potassium pyrogallate solution is more concentrated than the one of Benedict; the solution recommended by Hempel is more dilute than Benedict's.

Thus Benedict used two solutions made up according to Hempel: (1) 120 grams of KOH in 80 cc. of water to which was added 5 grams of pyrogallic acid in 15 cc. of water, i.e. 120 grams KOH, 95 cc. H_2O, 5 grams $C_6H_6O_3$, (2) 130 grams KOH, 70 cc. H_2O, 5 grams $C_6H_6O_3$. Benedict gives analyses of outdoor air and cylinder air made with these four solutions of different concentration of the same method. The averages of his analyses are given in Table 30.

TABLE 30

Average Results of Comparative Study of Oxygen Absorption by Potassium Pyrogallate of Varying Concentration (Benedict).

Outdoor Air

Hempel Solution 1	Hempel Solution 2	Benedict Solution	Haldane Solution
20.851	20.913	20.938	20.956

Cylinder Air

Hempel Solution 1	Hempel Solution 2	Benedict Solution	Haldane Solution
20.766	20.830	20.863	20.877

It is obvious then that by using the more dilute potassium pyrogallate solutions low values of the oxygen-content of a gas mixture will result. That this is due to small quantities of carbon monoxide seems most likely. Whether even with Haldane's solution traces of this gas are formed is not absolutely certain, though comparison with results obtained by other methods would indicate that this could be the case only for exceedingly small amounts. In making up the potassium pyrogallate solution, difficulty may arise from the varying amount of water in the potassium hydroxide; in different grades this ranges from 5 to 25 per cent. The safest way is to make the solution to a specific gravity of 1.55.

Temperature also may play an important rôle in the absorption of oxygen by pyrogallate solution: Below 15° the absorption is slow, but above this temperature there is little difference in the rate of the absorption of oxygen.

Chromous chloride has been used as an absorbent of oxygen when it is desired to determine this gas in the presence of hydrogen sulfide or carbon dioxide; the latter gases are not affected by the absorbent. The preparation of the chromous chloride reagent is associated with considerable inconvenience, and it is doubtful whether the oxygen is completely absorbed.[29]

Gas Samples. It is sometimes desirable or necessary to take a number of gas samples which cannot be analyzed at once, but must be preserved before the analysis can be made. Such samples can serve, of course, not only for the oxygen-content but carbon dioxide and other gases as well. It has been found absolutely essential that such containers should be dry and clean, for even very small quantities of dust or grease will decrease appreciably the amount of oxygen and increase the carbon dioxide-content. Also the alkali of the glass dissolves slowly in water and absorbs CO_2. It is therefore necessary that all glass containers should be thoroughly treated with a chromic acid cleaning mixture, carefully washed and dried in such a manner that no dust enters the vessel (but not with alcohol and ether).

The work of recent years on photosynthesis and respiration indicates that it is far more satisfactory to arrange the experiments in such a way that the plants are subjected to a stream of air rather than confine them and determine the composition of the atmosphere at intervals. The former method necessitates either the analysis of all the gas which has passed over the plant for a definite period or the taking of samples of the gas stream from time to time.

g. The Determination of Oxygen in Water.

Although in aquatic plants, just as in land plants, photosynthesis can be determined far more satisfactorily by differential determination of the carbon dioxide, it is nevertheless often desirable to determine the rate of photosynthesis by means of changes in the oxygen-content of the water. On account of the slight solubility of oxygen in water a differential method for oxygen is not satisfactory, i.e. in which the oxygen-content of a stream of water is determined before and after it has passed over a photosynthetically active plant.[30]

A large number of methods have been devised for the determination of oxygen in water.[31] The method suggested by Winkler has been very generally used and with quite satisfactory results. The principle of the method is as follows: the water containing dissolved oxygen is treated with a solution of sodium hydroxide and one of manganous chloride. Thereby the heavy precipitate of manganous hydroxide is formed; this

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[29] Anderson, R. P., and Riffe, J., *Jour. Ind. Eng. Chem.*, **8**, 24 (1916).

[30] Gail, F. W., Pubs. Puget Sound Biological Station, 1922, p. 177. Fromageot, *Compt. rend.*, **177**, 779, 892 (1923). Harder, *Zeit. Bot.*, **15**, 305 (1923).

[31] Cronheim, W., *Zeit. angew. Chem.*, **20**, 1939 (1907). Winterstein, H., *Biochem. Zeit.*, **19**, 425 (1909). Report of the Metropolitan Sewerage Commission, New York, 1910, p. 401. Boige and Juday, Wisconsin Survey Bulletin **22**, 11, 12 (1911). Soerensen and Anderson, *Zeit. anal. Chem.*, **47**, 279 (1908).

is oxidized to manganic hydroxide. By the addition of hydrochloric acid, the latter hydroxide is converted into the unstable manganic chloride. The latter compound breaks down to manganous chloride and free chlorine; this is then determined by means of titration with potassium iodide and potassium thiosulfate in the usual manner.

Sometimes in water containing organic substances it may be necessary to apply a correction because a portion of the chlorine may be absorbed. Also in the presence of organic impurities some of the iodine will be used up after acidulation. Under such circumstances it is advisable to run a blank on the amount of chlorine which is absorbed.

If nitrites are present in the water in quantities above 0.2 p.p.m. it is necessary to remove these before proceeding with the analysis. This can be accomplished by treating the water samples with 1 cc. of a 10 per cent solution of urea and 1 cc. 25 per cent sulfuric acid and allowing to stand for 3-4 hours. This is based on the common reaction: $2HNO_2 + CO(NH_2)_2 \longrightarrow CO_2 + 3H_2O$.

The effect of nitrites has been investigated by Hale and Melia [32] who recommended the addition of 2 cc. potassium acetate solution (1000 grams per liter) to the bottom of the bottles containing the water after the hydrochloric acid has been added in the regular course of the analysis.

For sea water the Winkler method causes some difficulty, because the heavy precipitate which is formed does not permit complete oxidation of the $Mn(OH)_2$. It is necessary therefore to dilute the sea water before making the analysis.[33] A variety of modifications of the original Winkler method have been proposed for various purposes.[34]

h. Apparatus for Gas Analysis.

A detailed description of various types of apparatus for gas analysis or a discussion of the principles used in different methods would go beyond the domain of this book. During recent years a number of very satisfactory instruments for gas analysis have been devised with special consideration for use in investigations of the gaseous exchange of living organisms. Most of these have been applied to studies of the oxygen-carbon dioxide ratio in the respiration of animals. Some of these instruments are well suited for investigations of photosynthesis and respiration in plants, though they have found little application for this purpose.

In endeavoring to obtain a true measure of the rate of photosynthesis it is absolutely essential that a number of external conditions such as temperature and intensity of illumination be experimentally controlled.

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[32] Hale, F. E., and Melia, T. W., *Jour. Ind. Eng. Chem.*, **5**, 976 (1913).

[33] Jorissen, W. P., *Zeit. anal. Chem.*, **49**, 424 (1910).

[34] Van Eck, J. J., *Chem. Weekblad.*, **10**, 455 (1913); *C. A.*, **7**, 2982 (1913); *C. A.*, **8**, 540 (1914). Winkler, L. W., *Zeit. anal. Chem.*, **53**, 665 (1914). Bruhns, G., *Chem. Ztg.*, **39**, 845 (1915). Clarke, F. W., "The Data of Geochemistry," Bull. 770, U. S. Geological Survey, Washington, 1924, p. 143. Krümmel, O., "Handbuch der Ozeanographie," Stuttgart, 1907, p. 292. Osterhout, W. J. V., and Haas, A. R. C., *Jour. Biol. Chem.*, **32**, 141 (1917).

Without such control, determinations of the quantities of carbon dioxide absorbed or of oxygen emitted are of very little value. In order to maintain these controlled conditions the amount of plant material or the size of the plant that can be used is naturally limited. It has, for instance, been found impractical thus far to determine the rate of gaseous exchange of a small tree though such determinations would undoubtedly be very desirable. To maintain a truly equable temperature in an enclosed space sufficiently large to contain a tree, while at the same time it is being illuminated, requires rather elaborate apparatus. Moreover, under such conditions the position of the leaves is apt to change so that it is impossible to obtain the rate of photosynthesis per unit of illuminated leaf surface. To this may be added the difficulty that in a large space changes in composition of the atmosphere are relatively slight which by the ordinary methods of gas analysis can be detected only with uncertainty. Finally, if it is desired to obtain information regarding the photosynthetic process under natural conditions, i.e. using atmospheric air with a carbon dioxide content of approximately 0.03 per cent by volume, large volumes of air must be passed over the plant. If this is not done, the carbon dioxide content of the air will be so greatly reduced that the rate of photosynthetic activity will be greatly impaired and moreover, natural conditions will not be maintained. Thus for example McLean [35] had to use a flow of nearly 19 liters per hour to determine the rate of photosynthesis of a single sugar-cane leaf. The differences in the carbon dioxide-content of the air stream before and after passing over the leaf are very small. For this reason only the most exact methods of gas analysis can be used.

In much of the earlier work on photosynthesis, the plant was enclosed in a vessel in an atmosphere containing a known quantity of carbon dioxide mixed with air. After exposure to light for a time, the gas in the container was again analyzed; the reduction in the amount of carbon dioxide and the increase in that of oxygen was ascribed to photosynthesis. This principle has its decided limitations especially when it is desired to determine rates of photosynthesis, for the rate is greatly influenced by the partial pressure of the gas, which is by this method constantly being reduced in the course of the experiment. For this and other reasons the method just mentioned has been replaced by that of keeping the plant in a stream of air, the composition of which is determined before and after passing over the plant. The simplest method of making such determinations is to absorb all the carbon dioxide in the air stream by means of barium hydroxide or sodium hydroxide; but it is frequently desirable to determine the oxygen as well as the carbon dioxide. Where gas samples are taken from an air stream the best method is to make accurate gas analyses.

One of the most convenient forms of gas analysis apparatus is the

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[35] McLean, F. T., *Ann. Bot.*, **34**, 367 (1920).

ordinary form of the Haldane[36] apparatus. It is compact and can be manipulated quite rapidly. The limits of accuracy are, however, such that it does not serve where very small differences must be determined. The apparatus has been somewhat simplified by Henderson.[37]

A gas analysis apparatus for carbon dioxide and oxygen which should be of considerable value for experiments on photosynthesis has been recently devised by Carpenter.[38] It is based upon the Haldane principle, but has a number of valuable refinements. It has a capacity of 40.04 cc. In its present form it is somewhat limited in the per cent of carbon dioxide which can be determined; gases containing up to 1.7 per cent of carbon dioxide and from 78.4 to 79.6 per cent of nitrogen can be analyzed. Readings can be made to 0.001 per cent. Naturally, in every apparatus the measuring pipette must first be calibrated. In this apparatus oxygen is absorbed by potassium pyrogallate and carbon dioxide by potassium hydroxide.[39]

An apparatus for gas analysis of a high order of precision has been developed by Sondén.[40] In this apparatus the two measuring pipettes, the reagent reservoirs and the manometer, are connected by glass tubing and glass stop-cocks so that the gas to be analyzed when once in the apparatus does not come in contact with any rubber connections. The percentage of carbon dioxide that can be analyzed is limited to one per cent. With this apparatus undoubtedly results of a high degree of precision can be obtained. It is, however, rather difficult of manipulation and transportation, quite fragile and can be repaired only by the most expert glassblower. Recently Krogh[41] has devised an apparatus of great accuracy though also of considerable complexity.

Another apparatus[42] for gas analysis which may be mentioned and which permits the determination of higher percentages of carbon dioxide is that of Polowzow-Richter. This has been used by the Russian school of plant physiologists. The apparatus of Bonnier and Mangin is so well known that it needs no comment.[43] The two forms of apparatus just mentioned are especially suitable for the determination of the respiratory and photosynthetic quotients. For determinations of this nature Maquenne and Demoussy[44] have also devised methods which are now well known.

Warburg's Method. In his extended series of investigations on various phases of the photosynthesis problem Warburg[45] has used a unicellular green alga *Chlorella.* In order to determine the rate of gas interchange

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[36] Haldane, "Methods of Air Analysis," London, 2nd ed., 1918.

[37] Henderson, *Jour. Biol. Chem.*, **33**, 31 (1918).

[38] Carpenter, T. M., *Jour. Metabolic Research*, **4**, 1 (1923).

[39] Benedict, Carnegie Inst. of Washington, Pub. 166, 75 (1912).

[40] Sondén, *Zeit. Instrumentenk.*, **9**, 472 (1889).

[41] Krogh, *Biochemical Jour.*, **14**, 267 (1920).

[42] Richter, *Trav. soc. imp. nat. St. Petersburg*, **33**, 311 (1902).

[43] Aubert, *Rev. gén. Bot.*, **3**, 97 (1891). Thoday, *Ann. Bot.*, **27**, 565 (1913).

[44] Maquenne and Demoussy, "Nouvelles recherches sur les échanges gazeux des plantes vertes avec l'atmosphère," Paris, 1913.

[45] Warburg, *Biochem. Zeit.*, **100**, 230 (1919).

a modified form of the Haldane-Barcroft blood gas analysis apparatus was employed. The method has the great advantage that determinations of the amount of photosynthesis can be made in relatively short periods of time and thus the course of the photosynthetic activity followed with considerable exactness.

A fundamental assumption in this method is that the photosynthetic quotient equals unity, that the volume of carbon dioxide absorbed equals that of oxygen emitted. This implies that when a plant is enclosed in an atmosphere of air containing carbon dioxide there will be no change in pressure when the plant is illuminated. A change in pressure does occur if an aquatic plant is employed, and the volume of water is not small as compared to that of the gas volume. Under these conditions the difference in the absorption coefficient of oxygen and of carbon dioxide is noticeable. Also a change in gas pressure in the illuminated vessel containing the plant is produced when the carbon dioxide used by the plant is taken from a compound dissolved in water.

Both of these principles have been applied by Warburg. In one the differences in the absorption coefficient of carbon dioxide and oxygen for water at known temperatures and pressures are taken as the basis of calculation. In the other the carbon dioxide is derived from mixtures of sodium carbonate and sodium bicarbonate, the CO_2-content of which is known for certain temperatures. When the amount of carbon dioxide used is small in comparison to the total amount of salt the effect of the change produced in concentration of the latter is very small and the carbonate mixtures play the rôle of buffers. The change in gas pressure is very nearly entirely due to alteration in the partial pressure of the oxygen, the amount of change in pressure due to alteration in the partial pressure of the carbon dioxide being relatively very small.

One form of apparatus which was used by Warburg is shown in Figure 15. The entire apparatus was immersed in a thermostat and the changes in pressure read on the manometer. The latter was filled with a solution made by Brodie for this purpose with which 10,000 mm. = 760 mm. of mercury. The algae were introduced into the containers in the form of a suspension in water. As neither the weight nor the surface of cells could be determined, it was not possible to obtain absolute values of photosynthetic activity by this method. Warburg has also developed several modifications of the apparatus shown in Figure 15 for work with suspensions of unicellular algae.

3. The Absorption of Carbon Dioxide

For the determination of the rate of photosynthesis of land plants the method which has found most general application is the one based upon the estimation of the amount of carbon dioxide withdrawn by the leaves (or usually a single leaf) from a current of air containing carbon dioxide. The carbon dioxide-content of the air stream is first determined. The

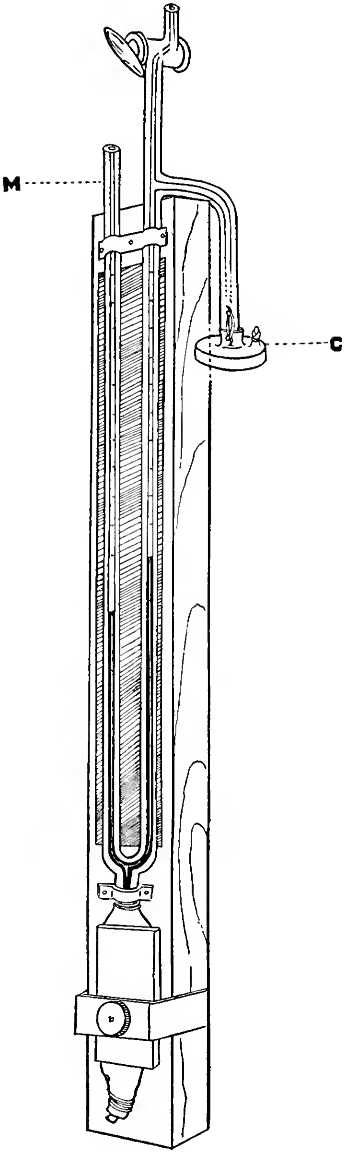

Fig. 15.—Apparatus for determining the rate of photosynthesis used by Warburg. This is a modified form of the Haldane-Barcroft bloodgas analysis apparatus. M is the manometer, and C the container for the unicellular alga which is illuminated from below.

current of air is made to pass over the leaf at a constant rate, and the amount of carbon dioxide in the air stream after passing over the leaf in the dark is established. This value is higher than that for the amount of carbon dioxide originally in the air current, for through the process of respiration the plant emits carbon dioxide to the air passing over it. The difference between the amount of carbon dioxide originally in the air current and that found after the air has passed over the leaf in the dark gives a value for the rate of respiration. If the leaf is now illuminated, the air current continuing at a constant rate, the carbon dioxide-content of the air stream after passing over the leaf is lower than was the case before illumination. This decrease is due to the photosynthetic appropriation of carbon dioxide by the leaf. The value of this decrease in unit time and per unit area of leaf surface is taken as the rate of photosynthesis.

This method permits the plant to be under very nearly normal conditions, it prevents the accumulation of gases which may be injurious to the leaf as well as large decreases in the amount of carbon dioxide in the atmosphere surrounding the leaf and it is relatively easily manipulated. The method was first applied by Kreusler [46] and has been modified and improved by various workers.

While the method just referred to is very simple in principle there are many details connected with the regulation of the air-stream, the temperature control, the illumination and the determination of the quantity of carbon dioxide which require a great deal of attention both in construction of the apparatus and in its manipulation if reliable results are to be attained. The construction of the apparatus is, of course, determined by the nature of the investigation and the problem to be solved. For the detailed discussion of matters relating to construction and manipulation the publications of the investigations here cited must be referred to.

The air-stream has been produced in several ways by different workers. An aspirator of the Mariot bottle type can be used and the rate of flow measured by an accurate gas meter. (It is very important that the gas meters be kept in a constant temperature bath.) The whole air-stream system may also be kept in a chamber of constant temperature. It is often desirable that the air which is used should contain more carbon dioxide than that which is normally present in the atmosphere. For this purpose the air may be drawn from a gasometer to which carbon dioxide has been added or from a gas mixture previously compressed into cylinders. In the latter case the air is allowed to escape from the cylinders and the rate determined by means of a bubble counter and flow meter.

In much of the work which has been done by means of this method leaves which have been detached from the plant have been used. While

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[46] Kreusler, *Landw. Jahrb.*, **14**, 915 (1885); **16**, 711 (1887); **17**, 161 (1888); **19**, 649 (1890). Blackman, *Phil. Trans. Roy. Soc.*, B **186**, 485 (1895). Brown and Escombe, *Proc. Roy. Soc.*, B **76**, 29 (1905). Willstätter and Stoll, "Untersuchungen Über die Assimilation der Kohlensäure," Berlin, 1918, p. 62.

experiments with excised leaves apparently represent highly artificial conditions, the method possesses many distinct advantages. An entire plant requires so much space that it is exceedingly difficult to construct apparatus which permits adequate control of temperature during illumination and the change of position of the leaves relative to the direction of the light and various other conditions are impossible to control. By using excised leaves or at least single leaves the amount of light which is absorbed by the leaf as well as the gas exchange can be more accurately determined.

The fact must not be overlooked, however, that when excised leaves are used rate of photosynthesis is quite different from that obtaining in attached leaves. In excised leaves the factor of translocation of materials formed in the leaf to other parts of the plant has been eliminated. It would be expected that with continued photosynthetic activity and consequent accumulation of starch and sugars there would occur a diminution in the rate of photosynthesis. As a matter of fact Brown and Escombe [47] found that contrary to expectations the leaf attached to the plant shows a lower rate than the excised leaf. The latter is in the mean about 44 per cent higher than the former. Brown and Escombe ascribe this difference in the behavior of the two sets of leaves to difference in the stomatal openings in the two cases. The petiole of the excised leaf, in these experiments, was in water, while in the attached leaf the water was supplied to the leaf through the roots. It is conceivable that in the former case conditions were more favorable to a wide opening of the stomata and consequent freer access of carbon dioxide. Whatever the explanation of the difference in behavior of the two sets of leaves may be, it is essential to bear these facts in mind before drawing too wide conclusions from observations made with excised leaves.

Various types of leaf containers have been devised which permit the leaves to be held at right angles to the source of illumination, to be submerged in a constant temperature bath and through which the air-stream may be passed. Willstätter and Stoll have described such a chamber to contain a number of leaves and in Figure 16 is shown a leaf frame designed by Spoehr for a single leaf.

In this method the estimation of the rate of photosynthesis depends upon the determination of small differences in the amount of carbon dioxide. In order that the carbon dioxide in the air-stream be not too greatly reduced, and the rate of photosynthesis consequently also affected, it is usually advisable to enrich the air-stream with carbon dioxide. Under certain experimental conditions, to make sure that the carbon dioxide is not the limiting factor, it is necessary to add considerable quantities of carbon dioxide. In this way relatively large quantities of carbon dioxide are contained in the air-stream whether the leaves are illuminated or not. As a consequence the accuracy of this method depends upon determining small differences between two large values in the amount of carbon dioxide in the air-stream.

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[47] Brown and Escombe, *l. c.*, p. 51.

For this purpose various methods have been used. These have included the absorption of the carbon dioxide by barium hydroxide solutions in Pettenkofer tubes, Meyer's bulb tubes and the Reiset apparatus. The change in strength of the barium hydroxide solution due to the formation of barium carbonate is then determined by means of titration. If this method is used it is essential to add some barium chloride to the barium hydroxide solution when it is prepared. The latter solution is usually prepared as a tenth normal solution with one gram of barium chloride added for each liter. Barium carbonate is slightly soluble in barium hydroxide solution and from the work of Vesterburg[48] and of Weisenberger[49] it is evident that the addition of barium chloride suppresses the hydrolysis of barium carbonate.

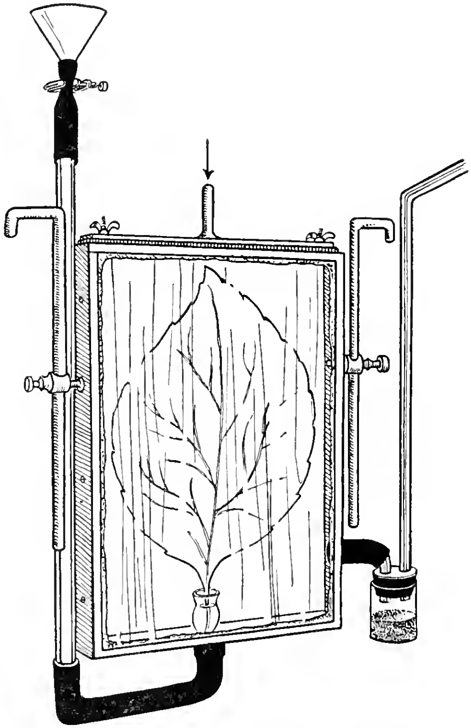

FIG. 16.—Container for excised leaf used in experiments on photosynthesis and respiration. The petiole of the leaf is in a nutrient solution the level of which can be adjusted. The air-stream enters at the top, leaves at the lower edge of the frame, and is dried over P_2O_5 before passing to the absorption tubes.

The carbon dioxide can, of course, also be absorbed in soda-lime or in Liebig absorption bulbs and determined gravimetrically, though this method is probably not as accurate as the volumetric one.

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[48] Vesterburg, *Zeit. phy. Chem.*, **70**, 550 (1910).
[49] Weisenberger, *ibid.*, **88**, 257 (1914).

A gas analysis apparatus for carbon dioxide which can be used in the field has been devised by Lundegårdh.[50] The air sample is drawn into a gasometer and the amount of carbon dioxide is determined from the decrease in volume after this gas has been absorbed in barium hydroxide.

Brown and Escombe [51] used a pure 4 per cent caustic soda (made from sodium) solution for the absorption of the carbon dioxide. This solution was then subjected to double titration with two indicators, one of them, phenolphthalein, sensitive to free carbon dioxide and the other, methyl orange, reacting only with the excess mineral acid used in the titration. These titrations are carried out in flasks provided with a cap and tubulures for the delivery tubes of the burettes to prevent access of the carbon dioxide of the air.

An electrometric method has been devised by Spoehr and McGee [52] based upon the absorption of carbon dioxide in barium hydroxide solution and the determination of the change in concentration of the latter through electrical resistance measurements. The method permits the determination of relatively large quantities of carbon dioxide in an air-stream with a high degree of accuracy so that changes in a stream containing over twenty times the amount of carbon dioxide normally in the atmosphere can be determined to 0.002 per cent. The type of cell used depends very much upon the amount of carbon dioxide which it is necessary to measure and the accuracy demanded.

The fact that hot barium hydroxide absorbs carbon dioxide more rapidly than a cool solution has been used by Warburg [53] for the determination of small quantities of this gas. Thus weaker solutions of barium hydroxide and a more compact apparatus can be used.

It has long been known that aquatic plants are capable of using bicarbonates as a source of carbon dioxide, and that with the abstraction of carbon dioxide from the solution by the plant the former takes on an alkaline reaction. Osterhout and Haas [54] have made use of this fact, and have developed a method for estimating the rate of photosynthesis based upon the change in color of an indicator added to the water. Phenolphthalein is used as the indicator, and a comparison is made of the time required to produce the same change in color. Within a restricted range of the pH., the amount of photosynthesis as indicated by the evolution of oxygen is approximately a linear function. Under these conditions it is permissible to measure the amount of photosynthesis by determining the change in pH. It is, of course, essential to use glass vessels which do not give an alkaline reaction, and in the case any nutrient substance is added to the solution which may change the buffer value, this must be allowed

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[50] Lundegårdh, *Biochem. Zeit.*, **131**, 109 (1922).

[51] Brown and Escombe, *Phil. Trans. Roy. Soc.*, **193**, 289 (1900).

[52] Spoehr and McGee, *Ind. and Eng. Chem.*, **16**, 128 (1924); Carnegie Inst. of Washington, Pub. No. 325, pp. 28, 89 (1923).

[53] Warburg, *Zeit. physiol. Chem.*, **61**, 261 (1909).

[54] Osterhout and Haas, *Jour. Gen. Physiol.*, **1**, 1 (1918). Osterhout, *Jour. Bio. Chem.*, **35**, 237 (1918).

for as has previously been described by Osterhout. The effect of the respiration of the plant on the pH of the solution is in the opposite direction to that of photosynthesis and must be allowed for.

It is also essential to determine that the plant does not produce an alkaline reaction in the water in which it is contained, independently of its photosynthetic activity, for it has been reported that certain aquatic plants in pure water when exposed to light develop an alkaline reaction in the water.[55] No entirely satisfactory explanation of this phenomenon has as yet been obtained. It is, none the less, of considerable importance when the photosynthetic activity is to be determined from changes in the pH of the water. This fact is further emphasized by the discovery of Ulehla [56] who has found that certain cultures of *Oedogonium* increase the alkalinity of the water not only in the light but also in the dark, although in the dark the amount of carbon dioxide in the water is increased due to the respiratory activity of the alga. This remarkable situation has its explanation in the fact that the *Oedogonium* filaments are associated with an incrustation known to the algologists as *Psichohormia*, in fact an iron bacterium which surrounds the alga filament. The incrustation contains iron and calcium carbonates and acts as a buffer system. As the carbon dioxide-concentration of the water increases, a corresponding amount of carbonate is dissolved as bicarbonate and depresses the hydrogen ions through the formation of the hydroxyl ions formed by hydrolysis. Because of the buffer effect of the incrustation, which virtually constitutes a symbiotic relationship between the alga and the bacterium, the former can exist in water which undergoes relatively large changes in pH. Without this buffer the alga can exist in only a relatively narrow range of hydrogen ion concentration.

4. Formation of Organic Matter

The first extensive attempts to determine the amount of material formed in photosynthesis were made by Sachs.[57] His method is well known and in spite of inherent inaccuracies, attempts have been made to modify it and it still continues in use. The method consists essentially of determining the dry weight of a unit area of one half or a smaller portion of a leaf at the beginning of the experiment and comparing this with the dry weight per unit area of the other half of the leaf after it has been exposed to the light for a time. From the difference in dry weight, with certain corrections, the weight of material formed in photosynthesis per unit area and time are calculated. In the original method the leaves were cut along the mid-rib, the cut half being used for the determination of the weight of the leaf in its original condition. After a period of insolation the other half of the leaf was cut from the mid-rib and the gain in weight

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[55] Klebs, *Untersuchungen Bot. Inst. Tübingen*, **2**, 340 (1888). Hassack, *ibid.*, **2**, 465 (1888). Anglestein, *Cohn's Beiträge zur Biol. der Pflanzen*, **10**, 87 (1911).

[56] Ulehla, *Ber. bot. Ges.*, **41** (20) (1924). Studia Mendeliana, Brno (1923).

[57] Sachs, *Arb. bot. Inst. Würzburg*, **3**, 1 (1884).

determined. This gain in weight does not, of course, represent the total amount of material synthesized; an important correction must be applied. Sachs found that there was a greater gain in dry weight in leaves which had been detached from the plant and illuminated than in leaves which were still attached to the plant and he attributed the lower gain in the latter case to the migration of the products of photosynthesis from the leaf into the stem of the plant. Therefore, he figured, that by adding the rate of loss in weight of leaves during the period of darkness to the rate of gain during periods of insolation he would obtain a value which would represent a close approximation of the true rate of photosynthesis during illumination.

Brown and Escombe[58] subjected the method of Sachs to a careful investigation. They compared values of the rate of photosynthesis obtained by the half leaf method with those obtained by the measurement of the actual intake of carbon dioxide by the leaf. The two methods gave widely different results. First of all they found that the rate of photosynthesis of leaves attached to the plant as measured by the absorption of carbon dioxide is about 45 per cent less than that of detached leaves. Sachs was therefore not justified in making a correction of the diurnal depletion of material synthesized. According to the results of Brown and Escombe the method of Sachs gives values which are far in excess of that indicated by the direct measurement of the absorption of carbon dioxide. This fact is illustrated in the results tabulated in Table 31 taken from the work of Brown and Escombe of the rate of photosynthesis of *Catalpa bignonioides*. The results by the two methods were obtained on the same leaves.

TABLE 31

RATE OF PHOTOSYNTHESIS AS DETERMINED BY THE HALF LEAF METHOD AND BY ESTIMATION OF THE AMOUNT OF CARBON DIOXIDE ABSORBED. THE VALUES ARE CALCULATED PER SQUARE DECIMETER PER HOUR.
(From Brown and Escombe.)

No.	Observed Increase in Dry Weight in mg.	CO_2 Absorbed by Leaf, cc.	Carbohydrate Formed Calculated from CO_2 Absorbed, mg.
1	9.83	1.41	1.76
2	7.14	1.43	1.79
3	2.60	2.35	2.94
4	7.22	2.33	2.92
Mean	6.69		2.35

From Table 31 it is evident that the method of determining the rate of photosynthesis from the increase in dry weight yields results which are very much higher than does the direct method of determining the amount of carbon dioxide absorbed. Brown and Escombe suggest that this discrepancy may in part be due to "changes in the power of retention

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[58] Brown and Escombe, *Proc. Roy. Soc.*, B **76**, 49 (1905). Thoday, *ibid.*, B **82**, 1 (1909).

of water at 100° C. by the colloidal elements of the cell contents." The discrepancy may also be partially due to differences in the symmetry in venation and the thickness of the two halves of the leaf. Brown and Escombe's studies show that such differences show a mean of ± 2.2 per cent. They also found that leaves of *Catalpa bignonioides* undergo changes in area as the result of insolation, amounting to as much as 3 per cent and Thoday has obtained similar results with *Helianthus annuus* in which there was a diminution in area of more than 5 per cent when conditions favor rapid transpiration.

Both these sources of error are of considerable magnitude, for if a leaf underwent a reduction in area during insolation so that its area as measured after illumination was less than at the beginning of the experiment the dry weight per unit area would be increased. On the basis of their studies of the half leaf method, Brown and Escombe conclude: "The average error observed due to differences in symmetry amounts to 2.2 per cent and that due to change of area to 1.1 per cent. Assuming that the accumulated errors from all sources in a Sachs experiment amounted to 2.0 per cent with a leaf having a dry weight of 0.50 gram per square decimeter, this would lead to an over- or under-estimate of the matter assimilated of 0.010 gram per square decimeter for the total time of the experiment, and if the duration of the experiment were five hours, to an apparent assimilation or depletion at the rate of 0.002 gram per decimeter of leaf per hour. But this is about the average amount of true assimilation observed for the leaf of *Catalpa bignonioides* by the direct method of determining the carbon dioxide assimilated, so that an under-estimate of the area of only 2 per cent in the insolated half leaves would on the Sachs weighing method give an over-estimate of the assimilation of 100 per cent of the true value, whereas it would only affect the results obtained from our method of carbon dioxide absorption to the extent of a 2 per cent."

Ganong[59] has devised a leaf area cutter by means of which discs of one square centimeter can be cut from the leaves. This to a measure avoids the errors due to lack of symmetry of the two halves of the leaf. It is, however, only a partial improvement on the original method.

The most convenient method of determining leaf areas is to place the leaves or portions thereof which are used on blue print paper in a printing frame and thus make a direct print of the leaf. The area of the photographic image of the leaf can then be determined by means of a planimeter.

In conclusion it may be stated that while the method of determining the rate of photosynthesis from the amount of dry material present in the leaves offers the simplest means of estimating this activity and that for many forms of investigation this simplicity is highly desirable, in its present form it is not reliable. Aside from the sources of error arising from differences of symmetry and area of the leaves the two very important factors of respiration and translocation of synthesized material offer difficulties which we have at present no means of overcoming. In order

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[59] Ganong, *Bot. Gaz.*, 39, 150 (1905).

to obtain sufficiently large increases in weight it is necessary that the leaves be illuminated at least one to two hours. It is impossible to establish any definite rates of respiration or translocation of materials at the same time. These two factors cannot be disregarded.

To take the difference in dry weight between leaves removed in the morning and at sunset as a measure of the amount of photosynthetic work done is obviously erroneous. The gain in dry weight of a leaf during a period of time is no more a measure of the amount of synthetic work done than the amount of money which a manufacturer deposits in a bank at the end of a day is a measure of the amount of work which has been accomplished in his plant. We shall probably have a true measure of photosynthesis only when we are capable of making a determination of the total energy changes taking place in the plant.

Chapter 5

The Chemistry of Photosynthesis

From the chemical viewpoint the first definite observation of value regarding the phenomenon of photosynthesis was made by Priestley in the discovery that plants are capable of forming oxygen. This fact was further elaborated by Ingen-Housz who showed that plants absorb carbon dioxide and emit oxygen in the light and by Senebier that only those portions of plants bearing chlorophyll have this capacity. It was realized already at this time that in the dark the gaseous exchange of plants goes in the opposite direction. Through the combined investigations of Ingen-Housz and of Senebier the rôle which photosynthesis plays in the development of plants and that of plants in the economy of all living organisms was greatly advanced. Based upon the newer conceptions of Lavoisier, de Saussure established quantitative relations between the amounts of carbon dioxide absorbed and of oxygen emitted in photosynthesis and demonstrated the importance of water in the photosynthetic reactions. To these facts were added the observations of von Mohl regarding the rôle of the chloroplasts and about twenty years later those of Sachs relating to the formation of starch as the first visible product of photosynthesis. At about the same time Boussingault had expressed the idea that glucose was the first product formed in the photosynthetic process. These were roughly the facts with which the chemists in about the middle of the nineteenth century were familiar.

With the development of organic chemistry under Liebig, Dumas, Laurent, Gerhardt, Woehler and others, it became possible to formulate theories and chemical conceptions of the mechanism of the molecular reactions involved in the reduction of carbonic acid to carbohydrates. Something was known of the chemistry of the materials which enter the reaction as well as of the products which are formed. It seemed possible to subject the phenomenon of photosynthesis to the same method of reasoning which had been employed in interpreting other reactions of organic compounds.

The literature on the chemistry of photosynthesis which has accumulated during the past seventy-five years is quite voluminous. A careful perusal of these writings clearly reveals certain facts concerning the manner in which the problem of photosynthesis has been attacked as well as of the nature of the problem itself. Considering the importance of the phenomenon of photosynthesis, its great economic significance, the fact that it is daily before the eyes of every one, it is a rather remarkable fact that there have been no chemical investigations embodying a sustained and persistent effort. There have been no long continued investigations such as characterize the work of Fischer on the carbohydrates and the proteins, of the Curies on radioactivity or Richards on atomic weights. The reason for this may lie in the fact that the problem is an exceedingly difficult one, and that the research work did not yield results as readily as in other less complicated fields.

Among the features of the photosynthesis problem which make a careful theoretical analysis of the reactions a difficult task are the following. First of all, it is in part at least a photochemical reaction. There is as yet very little known regarding the mechanism of photochemical reactions. We have no law for the effect of radiant energy corresponding to Faraday's law for the effect of electrical energy. Moreover, photosynthesis is an endothermal reaction. There are no analogous reactions known, reactions in which there is a storing of energy approaching in amount that taking place in photosynthesis. The rôle of chlorophyll is still not clearly understood.

There is in photosynthesis a great increase in potential energy between carbonic acid and glucose. It is possible to conceive of a number of ways in which this change can take place and the efforts of the chemists have to a large extent been devoted to devising theories to account for the steps between carbonic acid and glucose. One difficulty has been that some of these theories are not amenable to test by means of experimentation on the living plant or only to a very limited degree. Moreover, many of the theories have been formulated with almost complete disregard of well founded physiological facts.

Probably the most difficult point is that photosynthesis is closely associated with, in fact, is apparently dependent upon the life activities of the plant. Attempts to define more closely the relationship between photosynthesis and the activity of the plasma have not been very successful. The idea that there exists such a relationship is based upon the inactivation experiments of Ewart, the fatigue phenomena of Pantanelli and similar experimental observations which have already been discussed. Similarly, Willstätter and Stoll, after greatly advancing our knowledge regarding the rôle of chlorophyll, come to the conclusion that the rate of photosynthesis is to a large measure controlled by the activity of an enzyme. This idea was not new, but has found considerable support in the experimental results of Willstätter and Stoll. These are based largely upon differences in the temperature coefficient with changes in external conditions and in chlorophyll content. Direct evidence of the participation of an enzyme is still wanting.

The complexity of the chloroplasts, which are undoubtedly the centers of photosynthetic activity, adds to the difficulty of the problem. Whether we are dealing here with surface phenomena and pressures is a problem regarding which we have hardly any information. The failure to accomplish photosynthesis outside of the cell should therefore not seem surprising even to those who do not believe that plasmatic or enzymatic activity plays a rôle in the first steps. The difficulties confronting an extracellular photosynthesis may, in fact, lie not so much in the first steps, the reduction of the carbon dioxide, but in the removal of the reduction products, i.e., in transforming these into more stable compounds. An accumulation of such intermediate products, even in concentrations which escape our methods of analysis, may be sufficient to inhibit completely the course of the reaction. Were this the case, in the normal course of the reaction, we could expect a condition of steady state in which the concentration of the intermediate products was such that it would escape our notice when analytical methods are employed. That such conditions exist in the normal photosynthetic process has been postulated in a number of theoretical considerations of the problem. The treatment of a problem in this manner may lead to valuable conclusions; the danger lies in accepting the assumption of the existence and nature of such intermediate substances, as proven.

When the theories which have been advanced are studied critically, the conclusion cannot be escaped that, up to the present time, none of these theories has found substantiation on the basis of experimental facts. It is true that substantiation has been repeatedly claimed for one theory or another. But on account of the complex nature of the reactions which go to make up the photosynthetic process, special care must be exercised to avoid drawing conclusions from limited data, and more especially, generalizing from observations gained under limited or particular conditions. A perusal of the publications which have appeared during the past 75 years also shows that the train of thought has been strongly influenced by the type of investigation which was "in fashion" at the time the particular theory was advanced. The entire subject of the chemical reactions constituting the reduction of carbon dioxide, the liberation of oxygen and the formation of carbohydrates is still in a purely hypothetical stage as far as the explanation for this process is concerned.

In the various attempts that have been made to describe the steps involved in photosynthesis the course of reasoning has been very similar. This has usually included one or more of the following forms:

(1) A formulation of the theoretical steps from carbon dioxide and water to glucose.

(2) Attempts to find in the plant substances which form the intermediate steps demanded by the theory.

(3) The feeding to the plant of these intermediate products with the object of determining whether the plant is capable of using these substances for the synthesis of sugars or starch.

(4) Attempts to effect a photochemical reduction of carbon dioxide *in vitro*, including in some cases the use of chlorophyll preparations.

In endeavoring to exercise critical judgment on the various theories suggested to describe the chemical steps involved in the reduction of carbon dioxide and water to sugar, it is well to bear in mind the old familiar warning of Pfeffer. This runs to the effect that it is a very confusing error to presume that an organism must, in its metabolic economy, follow a course which seems to man, under the influence of existing chemical and physical knowledge, the most plausible course. All of these theories are still dealing with probabilities drawn largely from experiences gained from laboratory experiments carried out *in vitro*. How far one is justified in applying such experiences to reactions taking place in the living organism, physiological experimentation only can determine.

Regarding the second mode of formulating theories of the photosynthetic process, that of discovering in the plant the substances which the theory demands are intermediate products in the process, it must be borne in mind that the number of compounds found in plants is very great and many different types are represented. It is very difficult to associate directly the presence of any compound in a green leaf with the photosynthetic process. Thus, for example, the presence of formaldehyde in illuminated leaves is evidence of only limited value in favor of the theory that formaldehyde is an intermediate product in the reduction of carbon dioxide to sugars, for formaldehyde is formed in the photolysis of a number of substances which are almost constant components of leaves, so that the presence of formaldehyde may be ascribed to more than one cause. Moreover, the concentration of highly reactive intermediate products is probably very low and such products would consequently be very difficult to detect.

The third argument, that of drawing conclusions regarding intermediate products from the fact that the feeding to plants of such products results in the synthesis of sugar or starch, must also be applied with great caution. Experience has taught us that plants are capable of forming starch from a large variety of compounds some of which have never been found as normal components of leaves.

Finally, the fourth argument, that of drawing conclusions regarding the photochemical reduction of carbon dioxide from *in vitro* experiments will be dealt with at greater length where these reactions are discussed more in detail.

It should perhaps be emphasized that some of the schemes which have been proposed to describe the chemical steps in the conversion of carbon dioxide to carbohydrates cannot be dignified with the term theory, though they have been spoken of as such. In some cases they are mentioned quite "en passant," as mere suggestions in the discussions of subjects which have little application to the problem of photosynthesis.

1. Theories Regarding the Reduction of Carbon Dioxide and Water to Carbohydrates

a. Organic Acids.

Probably the first attempt to picture the course of the reaction leading from carbon dioxide and water to starch was made by Liebig.[1] He re-

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[1] Liebig, *Ann. Chem.*, 46, 62 (1843).

garded that this reaction took place in a series of steps in which the compounds formed showed a gradual decrease in the amount of oxygen they contain and an increase in the amount of hydrogen. This was, of course, an hypothesis drawn in very general terms. Liebig, it will be recalled, had devoted much attention to the elementary analysis of a large number of organic compounds by means of combustion. He now emphasized that the synthesis of carbohydrates from carbon dioxide and water was a step-reaction, starting with a reduction of carbonic acid to oxalic acid, then to tartaric, malic and other aliphatic carboxylic acids. Liebig's conclusions as to which acids thus formed the theoretical intermediate steps were arrived at entirely from a consideration of their composition as to C : H : O.

Liebig sought substantiation of his theory in the fact that in ripening fruits the acidity decreases and the carbohydrate content increases. Unfortunately, Liebig had entirely misconstrued the phenomenon of oxidative respiration in plants. As a consequence he also misinterpreted the presence of organic acids in fruits and fleshy plants, and considered that these acids could disappear from the plant only through reduction.

It was established through a number of extensive investigations [2] that although decrease in the acid content of succulents was accompanied by increased carbohydrate content, this was not a direct conversion of organic acids into carbohydrates. Rather, the carboxylic acids are broken down by a process of photolysis with the liberation of carbon dioxide and the latter is used in photosynthesis to form carbohydrates. The acids accumulate in the night and disappear during the day. On account of the complex morphological conditions in succulent plants it required considerable time to arrive at this conclusion. At the same time the fact was established that similar conditions, only to a lesser degree, prevail in most leaves. The accumulation of acids is apparently the result of incomplete oxidation of carbohydrates; in the light the acids disappear due partly to the better supply of oxygen furnished by photosynthesis and partly due to a direct photolysis of the acids.

The principal idea of Liebig's theory, that the organic acids are intermediate products in the synthesis of carbohydrates has in the course of years reappeared periodically with slight modification of Liebig's original ideas. At this time may be mentioned the theories with formic acid as a first reduction product. An early suggestion of this nature was made by Rochleder,[3] though the limited chemical knowledge of the time did not permit a very clear statement. This scheme is as follows: carbon dioxide

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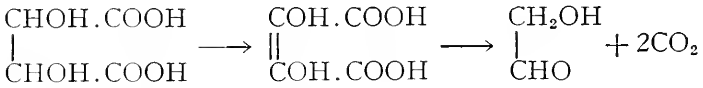
[2] De Saussure, "Récherches chimiques sur la végétation," 1804, p. 64. Mayer, *Landw. Versuchstat.*, **18**, 428 (1875); **21**, 277 (1878); **30**, 217 (1884); **34**, 127 (1887). Kraus, *Abh. Natur. Ges. Halle*, **16**, 143 (1883). De Vries, *Bot. Zeitg.*, **42**, 336 (1884). Warburg, *Unters. bot. Inst. Tübingen*, **2**, 53 (1886). Aubert, *Rev. gén. Bot.*, **2**, 369 (1890); **4**, 203 (1892); *Ann. Sci. Nat. Bot.* (7), **16**, 1 (1892). Purjewiecz, *Bot. Cent.*, **58**, 368 (1894). Gerber, *Ann. Sci. nat. Bot.* (8), **4**, 153 (1896). Astruc, *Ann. Sci. nat. Bot.* (8), **17**, 1 (1903). Spoehr, *Biochem. Zeit.*, **57**, 93 (1913). Richards, Carnegie Inst. Washington, Pub. No. 209 (1915). Hempel, *Compt. rend. trav. Lab. Carlsberg*, **13**, 1 (1917).

[3] Rochleder, "Phytochemie" (1854), p. 321.

is reduced to carbon monoxide, the latter is converted to formaldehyde which in turn is oxidized to formic acid; this unites with carbon dioxide to form oxalic acid by a reversal of the familiar reaction $C_2O_4H_2 \rightarrow CO_2 + HCOOH$.

The carboxylic acids found in plants which might come under consideration as intermediate products in the conversion of carbonic acid to carbohydrates under the Liebig theory are: formic, oxalic, glycollic, glyoxylic, tartaric, lactic, malonic, succinic, malic and citric acids. The chemical evidence in support of the principle of Liebig's theory, as has been stated, is rather meagre. In this connection may be mentioned Fenton's [4] reaction of the formation of glycollic aldehyde from tartaric acid. The latter with hydrogen peroxide and ferrous sulfate yields dioxymaleic acid which on warming in water solution loses carbon dioxide and yields glycollic aldehyde.[5]



$$\begin{array}{c}\mathrm{CHOH.COOH}\\ |\\ \mathrm{CHOH.COOH}\end{array} \longrightarrow \begin{array}{c}\mathrm{COH.COOH}\\ \|\\ \mathrm{COH.COOH}\end{array} \longrightarrow \begin{array}{c}\mathrm{CH_2OH}\\ |\\ \mathrm{CHO}\end{array} + 2CO_2$$

Tartaric acid Dioxymaleic acid Glycollic aldehyde

Glycollic aldehyde is easily converted into hexoses. Neuberg and Schwenk [6] have found that dioxymaleic acid is converted into glycollic aldehyde and carbon dioxide by the action of yeast. Neuberg and Kerb [7] have also found that hydroxypyruvic acid, $CH_2OH \,.\, CO \,.\, COOH$, by the action of yeast yields glycollic aldehyde and carbon dioxide. Similar reactions, involving the splitting out of carbon dioxide from the carboxyl group, have been obtained by the action of light.[8] Thus, for example, lactic acid yields acetaldehyde and tartaric acid yields glyoxal, pyruvic acid, acetaldehyde, etc.

While reactions of this nature may play a rôle in the ripening of fruits and in the deacidification of plants in general, it is difficult to apply them directly to the photosynthetic process. The formation of compounds such as malic or glycollic acids from carbonic acid is difficult to picture. It is more probable that such acids arise from the incomplete oxidation of carbohydrates and are decarboxylated, leaving a residue which can possibly be converted into carbohydrates by the plant.

Based upon better chemical experience was the suggestion of Erlenmeyer.[9] He had found that α-hydroxy-carboxylic acids, RCH(OH)

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[4] Fenton, *Jour. Chem. Soc.*, **65**, 899 (1894); **67**, 48, 774 (1895); **69**, 546 (1896); **71**, 375 (1897); **73**, 71 (1898); **81**, 426 (1902); **87**, 817 (1905).

[5] Fischer and Landsteiner, *Ber. chem. Ges.*, **25**, 2549 (1892). Meldola, *Jour. Chem. Soc.*, **89**, 756 (1906).

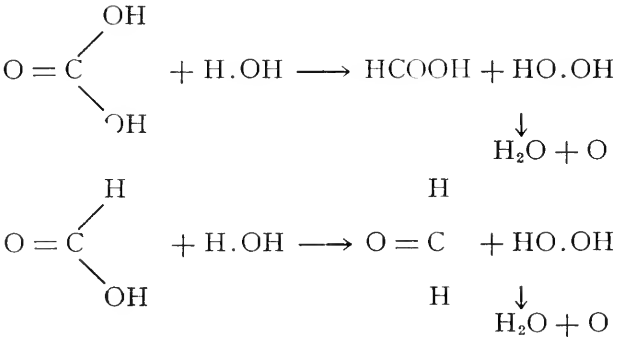
[6] Neuberg and Schwenk, *Biochem. Zeit.*, **71**, 104 (1915).

[7] Neuberg and Kerb, *ibid.*, **53**, 406 (1913).

[8] Neuberg and Peterson, *ibid.*, **67**, 63, 71 (1914). Spoehr, *ibid.*, **57**, 93 (1913). Nef, *Ann. Chem.*, **357**, 291 (1907). Locke, *Jour. Am. Chem. Soc.*, **46**, 1246 (1924).

[9] Erlenmeyer, *Ber. chem. Ges.*, **10**, 634 (1877).

COOH, yield aldehydes and formic acid and he thought that the same reaction might be applied to carbonic acid. The carboxyl group (-COOH) would be reduced to formic acid (H-COOH) and the remaining two free hydroxyl groups united to form hydrogen peroxide. Finally the formic might undergo a similar reaction with the formation of formaldehyde and another molecule of hydrogen peroxide. These reactions may be written as follows:



According to Erlenmeyer's view, formaldehyde is obtained from carbonic acid by way of formic acid. This is the first proposal which assumes the formation of a peroxide. The scheme also implies that it is not carbon dioxide but carbonic acid, H_2CO_3, which is reduced and it is essentially a formaldehyde theory.

There have been a number of variations to the ideas of Liebig and of Erlenmeyer; most of these have had very little experimental work as a basis.[10]

Thus Ballo,[11] in a measure, united the views of Liebig and of Erlenmeyer. He assumes that formic acid is the first reduction product, this is oxidized to oxalic acid which in turn yields tartronic → tartaric → saccharic acids.

Bach [12] has proposed a mechanism which supposes a reaction analogous to the oxidation of sulfurous acid to sulfuric acid and sulfur; thus carbonic acid would yield percarbonic acid:

$$3H_2CO_3 = 2H_2CO_4 + \underset{\text{formaldehyde}}{\boxed{H_2O + C}}$$

$$\begin{array}{c} 2H_2CO_4 = 2CO_2 + 2H_2O_2 = 2CO_2 + 2H_2O + O_2 \\ \hline 3H_2CO_3 = 2H_2CO_4 + H_2CO = 2CO_2 + 2H_2O + O_2 \end{array}$$

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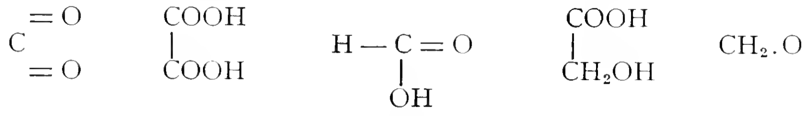
[10] Brunner and Brandenburg, *Ber. chem. Ges.*, **9**, 984 (1876). Reinke, *Ber. chem. Ges.*, **14**, 2149 (1881). Brunner and Chuard, *ibid.*, **19**, 595 (1886). Königs, *ibid.*, **25**, 800 (1892).

[11] Ballo, *Ber. chem. Ges.*, **17**, 10 (1884).

[12] Bach, *Compt. rend.*, **116**, 1145 (1893).

The criteria upon which Bach bases his reactions have, however, been found to be erroneous by Euler.[13]

Another system involving the formation of organic acids as intermediate products in the reduction of carbon dioxide to formaldehyde has been formulated by Baur.[14] He assumes the following steps:



$$C\begin{matrix}=O\\=O\end{matrix}\qquad \begin{matrix}COOH\\|\\COOH\end{matrix}\qquad \begin{matrix}H-C=O\\|\\OH\end{matrix}\qquad \begin{matrix}COOH\\|\\CH_2OH\end{matrix}\qquad CH_2.O$$

carbon dioxide — oxalic acid — formic acid — glycollic acid — formaldehyde

$$H_2C_2O_4 \longrightarrow H_2CO_2 + CO_2$$

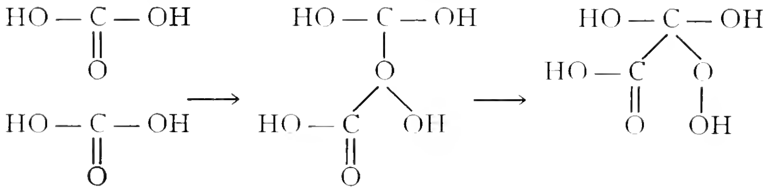
$$H_2C_2O_4 + 2H_2CO_2 \longrightarrow CH_2OH.COOH + H_2O + 2CO_2$$

$$\underline{CH_2OH.COOH \longrightarrow CH_2O + H_2CO_3}$$

$$2H_2C_2O_4 \longrightarrow CH_2O + 3CO_2 + H_2O$$

Bauer considers that oxalic acid is the first reduction product of carbon dioxide and figures that the formation of this reduction product requires ¼ to ⅓ less energy than the direct reduction to formaldehyde; in alkaline solution, i.e. with the salts of the acids, even less energy is required. The oxalic acid is supposedly reduced by means of the formic acid. The theory has been critically examined by Euler.

Tschelinzeff[15] has also suggested that a condensation of carbonic acid and the subsequent splitting out of hydrogen peroxide may be obtained through the secondary valencies of the carbonyl group by the formation of oxonium compounds. This theory he developed for the aldol condensation and has attempted to apply it to other compounds containing a C = O group. Thus he considers the following steps as offering a means of passing from carbonic acid to compounds containing less oxygen:



The peroxide thus formed decomposes with the elimination of oxygen and water and the formation of oxalic acid.

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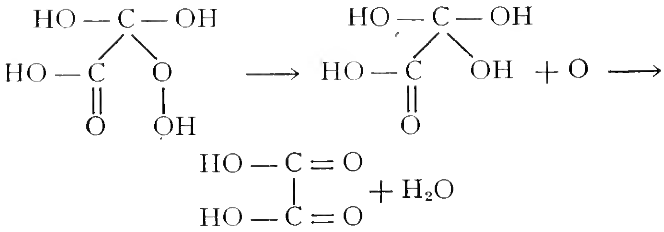
[13] Euler, *Ber. chem. Ges.*, 37, 3411 (1904).

[14] Baur, *Naturwissenschaften*, 1, 474 (1913). Parnas, *ibid.*, 1, 819 (1913). Euler, *Zeit. physiol. Chem.*, 59, 122 (1909).

[15] Tschelinzeff, *Bull. soc. chim.*, 37, 181 (1925).



The oxalic acid may in turn condense in the same manner with carbonic acid to form mesoxalic acid, this by decarboxylation yielding glyoxylic acid. Condensation of this with carbonic acid and decarboxylation yielding glyceric acid and in this manner successively to hexonic acids and hexoses.

b. Formaldehyde. The Baeyer Theory.

As a matter of fact most of the theories of the mechanism of photosynthesis, with the exception of those of Liebig, Ballo and of Tschelinzeff, assume formaldehyde to be the final stage of the reduction. Difference of opinion centers largely around the question as to whether the reaction, carbon dioxide (or carbonic acid) → formaldehyde is accomplished in one step or whether there are intermediate products. The formaldehyde theory has been so very much in the center of the discussions of the mechanism of photosynthesis that some analysis of the facts regarding the development of this theory seems in place, though this can be done here only in the briefest form.

Already in 1863 Berthelot[16] expressed the idea that glucose was an alcohol-aldehyde, that it was the first aldehyde derived from mannitol. Berthelot also regarded as possible the dissociation of carbon dioxide into carbon monoxide and oxygen and of water into hydrogen and oxygen, that the carbon monoxide and hydrogen in the "nascent" condition were capable of reacting and that the product, CHO (old atomic weights) now written CH_2O, was the beginning of the synthetic process. This idea was adopted by Boussingault[17] who emphasized that with the formation of carbon monoxide and hydrogen the proportion of carbon, hydrogen and oxygen as existing in glucose was given. Thus Berthelot and Boussingault may be considered the first proponents of the formaldehyde theory.

At the same time it must be realized that the chemistry of the carbohydrates was at this period only imperfectly worked out. Schorlemmer[18] in 1868 showed that the hydrocarbon obtained from the reduction of mannitol was normal hexane and a few years later established that dipropyl obtained from normal propyl iodide was identical with the hexane obtained from mannitol. The relation of glucose to normal hexane was later defi-

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[16] Berthelot, "Sur les principes sucrés," Supplement, 1862. "Leçons sur les methodes général de synthèse en chimie organique," Paris, 1864, p. 180.

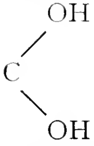
[17] Boussingault, *Agronomie*, **4**, 301, 399 (1868).

[18] Schorlemmer, *Ann. Chem.*, **147**, 220 (1868); **161**, 275 (1872).

nitely established by de la Motte [19] who obtained adipic acid by the reduction of saccharic acid with hydriodic acid.

Another contribution to the theory was made through the investigations of the Russian chemist Butlerow.[20] By the action of methyl iodide on silver oxalate he had obtained a white solid substance which he called dioxymethylene and to which he ascribed the formula $C_2H_4O_2$. By treating the substance with alkalies he obtained a sugarlike substance which he called methyléneitan, with the formula $C_6H_{12}O_6$ and which he considered a sugar: "C'est le premier exemple de la production synthétique d'une substance ayant les allures d'un corps sucré au moyen des composés les plus simples de la chimie organique." After Hofmann had discovered formaldehyde in 1869 and had shown that Butlerow's dioxymethylene was a polymerized form of his formaldehyde, which from vapor density determinations had the formula CH_2O, the question arose as to how six formaldehyde molecules could unite to form a hexose molecule and consequently what was the distribution of the hydroxyl groups in a hexose such as glucose.

A few years later this question was discussed by Baeyer [21] in his interesting paper entitled "Ueber die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung." He showed that more than one hydroxyl group was very rarely united to a single carbon atom, the group



$\mathrm{C}\genfrac{}{}{0pt}{}{\diagup \mathrm{OH}}{\diagdown \mathrm{OH}}$

in most cases spontaneously goes over into C= O. In glyoxylic and mesoxalic acids both forms are known as is also the case with chloralhydrate; the presence of negative elements as O and Cl seem to stabilize the double hydroxyl grouping. These views were applied to the formation of sugar from formaldehyde as indicated by Butlerow's experiments.

Baeyer's theoretical deductions on this subject have been so much quoted and have, in fact, been taken as the starting point of a great deal of work on photosynthesis that it may not be amiss to give here his own words:

"Ein Vorgang von grosser Wichtigkeit gehört aber jedenfalls hierhin, nämlich die Bildung von Methylenitan aus dem Aldehyd der Ameisensäure, Nach Butlerow ensteht ein zuckerartiger Körper, wenn man die wässrige Lösung des Formaldehyds mit Alkalien versetzt. Der Formaldehyd hat nach Hofmann's Untersuchungen in Gasform die Zusammensetzung COH_2, aber nights hindert, ihn in wässriger Lösung als $CH_2(OH)_2$ anzusehen. Wenn man nun annimmt, dass je ein OH eines Molecüls mit je einem H eines andern Wasser bildet, und dass die dadurch frei gewordenen C-Affinitäten sich mit einander verbinden, so bekommt man bei 6 Molecülen

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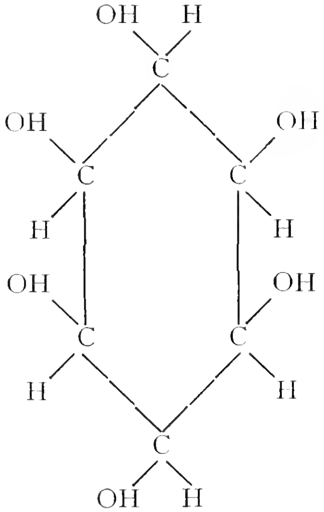
[19] De la Motte, *Ber. chem. Ges.*, **12**, 1572 (1879). Kiliani, *ibid.*, **19**, 1128 (1886).
[20] Butlerow, *Ann. Chem.*, **111**, 242 (1859); *Compt. rend.*, **53**, 145 (1861).
[21] Baeyer, *Ber. chem. Ges.*, **3**, 63 (1864).

folgende Gleichung: $6\ CH_2(OH)_2 - 5\ H_2O = C(OH)_2H.C(OH)H.C(OH)H.C(OH)H.C(OH)H.C(OH)H_2$. Nimmt man dann noch ein Wasser fort, indem man aus der Gruppe $C(OH)_2$ am linken End eins austreten lässt oder durch Condensation der beiden Endglieder einen Ring bildet, so bekommt man entweder:

$$COH.(C[OH])_4CH_2(OH)$$

oder



"Die Zusammensetzung des Traubenzuckers muss nach allen vorliegenden Erfahrungen entweder mit einer der beiden vorigen Formeln übereinstimmen oder wenigstens ihnen sehr nahe verwandt sein, und daher liegt die Vermuthung nahe, dass die Bildung des Zuckers im Pflanzenkörper mit der besprochenen Reaction in Zusammenhang steht. Die gewöhnliche Annahme über die Bildung des Zuckers und der verwandten Körper in der Pflanze ist die, dass die Kohlensäure in den gruenen Theilen unter Einwirkung des Lichtes reducirt und durch eine allmälige Synthese in Zucker übergefürt wird. Die Zwischenglieder hat man in den organischen Säuren gesucht, der Ameisen-Oxal-Wein-Säure u.s.w., die man allerdings als Reduktionsprodukte der Kohlensäure auffassen kann. Nach dieser Ansicht müsste also in den Momenten, wo die Pflanze am stärksten reducirt, d. h. bei der Einwirkung der Sonnenstrahlen auf grüne Blatttheile eine starke Anhäufung von Säuren stattfinden, die dann allmälig erst dem Zucker Platz machen könnten. Meines Wissens ist dies nicht beobachtet worden, und wenn man bedenkt, dass in der Pflanze unter allen Umständen Zucker und die Anhydride desselben entstehen, dass das Vorkommen der Säuren aber je nach den Arten der Pflanze, ihren besonderen Theilen und nach ihrem Alter ein verschiedenes ist, so gewinnt die schon öfters ausgesprochene Ansicht an Wahrscheinlichkeit, dass der Zucker sich direct aus der Kohlensäure bildet. Die Entdeckung von Butlerow giebt hierzu den Schlüssel, und es ist eigentlich zu verwundern, dass sie bisher von den Pflanzenphysiologen noch so wenig ausgebeutet ist. Man hat vielfach auf die Aehnlichkeit hingewiesen, welche zwischen dem Blutfarbstoff und dem Chlorophyll der Pflanzen existirt. Danach muss es auch als wahrscheinlich erscheinen, dass das Chlorophyll ebenso wie Hämoglobin CO bindet. Wenn nun Sonnenlicht Chlorophyll trifft, welches mit CO_2 umgeben ist, so scheint die Kohlensäure dieselbe Dissociation wie in hoher Temperatur zu erleiden, es entsteht Sauerstoff und das Kohlenoxyd bleibt mit dem Chlorophyll verbunden. Die einfachste Reduction des Kohlenoxyds ist die zum Aldehyd der Ameisensäure, es braucht nur Wasserstoff aufzunehmen:

$$CO + H_2 = COH_2$$

und dieser Aldehyd kann sich unter dem Einfluss des Zelleninhalts ebenso wie durch Alkalien in Zucker verwandeln. In der That, man hätte Mühe, nach der andern Ansicht durch allmäligen Aufbau so einfach zu dem Ziele zu gelangen! Das Glycerin konnte ferner durch Condensation von drei Molekülen und Reduktion des gebildeten Glycerinaldehyds entstehen.

"Die Bildung des Zuckers auf einem andern, umständlicheren Weg bleibt übrigens hierdurch nicht ausgeschlossen, und es könnte sehr wohl sein, dass auch die Pflanzensäuren unter Umständen in diese Substanz übergeführt werden, die in tausend wechselnden Formen den Körper der Pflanzen aufbauen hilft.

"In welcher Weise der Zellinhalt condensirend auf den Aldehyd der Ameisensäure einwirkt, lässt sich vor der Hand gewiss nicht verfolgen, indess kann man doch annehmen, dass der gebildete Zucker zunächst mit ihm verbunden bleibt und später erst je nach den Umständen als Cellulose, Stärke, Zucker oder Glukosid abgespalten wird. Dafür spricht wenigstens die Entwicklungsgeschichte der Schleimpilze, bei denen in einem gewissen Stadium aus der Zelleninhalt ähnlichen Masse plötzlich eine grosse Menge von Cellulose abgespalten wird. . . ."

This is Baeyer's own statement of the theory of the reduction of carbon dioxide and water to formaldehyde and the synthesis of hexose sugars. This theory has held the center of attention in investigations on photosynthesis for over half a century. Its apparent simplicity and no doubt also the fact that it was advanced by one of the most eminent organic chemists have largely contributed to its popularity. A careful analysis of Baeyer's statements will show that there are several assumptions which are vital to the validity of his theory. These assumptions are 1. that carbon dioxide is dissociated into carbon monoxide and oxygen in the light; 2. the analogy between hemoglobin and chlorophyll in the power to fix carbon monoxide; 3. the origin of hydrogen which reduces carbon monoxide to formaldehyde and 4. the mode of condensation of formaldehyde to hexose sugars under conditions existing in the plant.

Contributions have been made from various sides bearing directly on all of these points and a discussion of them will occur in different portions of this chapter. The attempts which have been made to determine whether there occurs a splitting of carbon dioxide into oxygen and carbon monoxide are described in section 4 of this chapter.

Baeyer's assumption of the close analogy between chlorophyll and hemoglobin and the property of the former to absorb carbon monoxide is erroneous. This in itself is, however, not a serious objection. Whether carbon monoxide is actually formed is still an open question. The fact that carbon monoxide cannot replace carbon dioxide in photosynthesis and that the former is poisonous to plants in larger concentrations cannot be taken as an argument that no carbon monoxide could be formed in photosynthesis as Krascheninnikoff[22] has pointed out.

A far more serious question in Baeyer's original theory is the mode of reduction of carbon monoxide to formaldehyde. Baeyer's statement is: "Es braucht nur Wasserstoff aufzunehmen." Undoubtedly formaldehyde can be formed in this manner, but is there actually hydrogen available and what is the source of the hydrogen? Many authors including Berthelot, Boussingault, Liebig, Baeyer, Brunner and Chuard make the tacit assumption of the availability of active or "nascent" hydrogen; few have gone into the question as to whether any support can be found for such an assumption.

It has been assumed that water is decomposed into hydrogen and oxygen by the action of light; the oxygen escapes and hydrogen is taken up by the carbon monoxide. Bredig[23] also suggested that the hydrogen is absorbed by a substance having properties in this respect similar to palladium which then catalytically reduces carbonic acid. This is based upon the observation of Bredig and Carter that formic acid is formed when alkali salts of carbonic acid are treated with hydrogen under pressure in the presence of "contact substances." As an example of the latter Bredig calls attention to the fact that platinum is capable of dehydrogenating many organic compounds in a manner which is similar to the action of certain enzymes. In the plant it is assumed there exist enzymes which are capable of absorbing hydrogen, resulting from the photochemical decomposition of water.

In most of the theories[24] the decomposition of water has been taken as the source of hydrogen, either directly or according to $CO + H_2O \rightarrow CO_2 + H_2$. These all assume that one of the complex of endothermal reactions comprising photosynthesis is the formation of hydrogen from water. We have no evidence that there actually occurs a splitting of water into hydrogen and oxygen in the plant under the influence of light. These assumptions are generally based upon the observations that in ultra-violet-light water is thus broken up, but it is exceedingly doubtful whether this has any application to the photosynthetic process.

[22] Krascheninnikoff, *Rev. gén. Bot.*, **21**, 177 (1909).

[23] Bredig, *Ber. chem. Ges.*, **47**, 541 (1914); *Die Umschau*, **18**, 362 (1914).

[24] Löb, *Landw. Jahrb.*, **35**, 541 (1906); *Zeit. Elektrochem.*, **12**, 282 (1906).

Pollacci's[25] views differ from these and are based upon his claim of having found hydrogen and methane in the gases emitted during photosynthesis. He assumes that the hydrogen arises from intramolecular rearrangement of organic compounds and is used in the following manner:

$$2\ H_2CO_3 + 2\ H_2 + \text{Light} = CH_2O + CH_4 + H_2O + 2\ O_2$$
$$2\ H_2CO_3 + 3\ H_2 + \text{Light} = CH_2O + CH_4 + H_2O + 2\ O_2 + H_2$$

Later he simplified these to:

$$H_2CO_3 + H_2 = CH_2O + H_2O + O$$

According to this author the function of the light is to produce electric currents within the plant which bring the hydrogen into a "nascent" condition. In the dark the hydrogen is not in this condition and is therefore not capable of reducing carbonic acid. The last equation can obviously not represent the photosynthetic reaction, for it would require a photosynthetic quotient ($CO_2 : O_2$) of 2 : 1 which is quite contrary to experience.

It is exceedingly doubtful whether assumptions such as these in which the hydrogen is produced independently of the action of light, can be upheld. Even if hydrogen were formed within the plant, and there can be little doubt that reducing actions take place, the amount of this when compared to the total oxidative reactions is small. In photosynthesis there is a lift in energy represented by carbon dioxide passing to carbon:

$$CO_2 \rightarrow C + O_2$$

or when water is added, of:

$$H_2CO_3 \rightarrow (H_2CO) + O_2$$

It is difficult to devise a rational scheme (other than the decomposition of water or the formation of hydrogen peroxide) by which hydrogen could be obtained from the (H_2CO) material of the plant in sufficient quantities to yield a total increase in carbohydrate material. Many of the attempts which have been made in this direction represent mere juggling of chemical formulae with disregard of the fact that the plant during photosynthesis increases in total carbohydrate content and that the ratio of the volume of carbon dioxide absorbed to that of oxygen formed is unity. In photosynthesis there is 10-30 times as much carbon dioxide absorbed as is produced in respiration, so that any scheme which endeavors to utilize the dehydrogenation in the oxidative processes of respiration as a source of reduction in photosynthesis must, under the most favorable assumptions, fall short by 10 to 30 times of the amount of hydrogen required for the reduction of all the carbon dioxide. The fact that hydrogen is actually produced in the respiratory consumption of glucose has, moreover, never been definitely established.

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[25] Pollacci, *Atti del. Istit. bot. Pavia*, 7, 97 (1902); 8, 1 (1902).

This criticism would apply to the theoretical speculations of Stoklasa and Zdobnicky[26] in their first schematic representation of the photosynthetic reactions. In a second publication (to be discussed later) hydrogen is not included in their equations. What becomes of the hydrogen, supposedly formed from glucose when the plant is in the dark or formed in chlorophyll-free cells is another question of much importance. Certainly it is not liberated as such in any quantity and one is obliged to assume that under these conditions it combines with oxygen to form water, and that in the light and in the presence of chlorophyll the active hydrogen, instead of combining with oxygen, reacts with carbon monoxide to form formaldehyde.

In the original theory of Baeyer the rôle of hydrogen was the reduction of carbon monoxide to formaldehyde; the carbon dioxide is split into carbon monoxide and oxygen by the action of light. Thus half of the oxygen which is emitted in the photosynthetic process is already accounted for, the question being how the other atom of oxygen is set free. There can be little doubt that this comes from water either directly or indirectly. That the carbonic acid is reduced directly by "active" hydrogen, as is assumed by Pollacci has little support. On the other hand, Zenghelis[27] has reported that when hydrogen is passed through filter paper (extraction thimbles) in water saturated with carbon dioxide, formaldehyde is produced. The assumption is that on passing through the fine pores of the filter paper or within the pores, the hydrogen is split into atomic hydrogen which reduces the carbonic acid. Besides formaldehyde, Zenghelis reports also having obtained sugar in this manner; the amount of the latter is greater when the action takes place in light. The author may be permitted to state that he has repeated the experiments of Zenghelis but obtained only negative results.

While there are, no doubt, these difficulties in the mechanism of the photosynthesis reaction as originally stated in the Baeyer theory, the assumption of formaldehyde as an intermediate step has appeared very plausible and gained many adherents resulting in various modifications of the original mechanism. Thus, Nef[28] on the basis of his theory of methylene dissociation assumes a splitting of carbonic acid into carbon monoxide and hydrogen peroxide.

$$OC(OH)_2 \rightleftarrows OC< + H_2O_2 \rightarrow OCH_2 + O_2$$

This implies a reduction of the carbon monoxide by the hydrogen peroxide. The formation of the latter substance in the photosynthetic reaction has also been assumed by Erlenmeyer and has been used by Usher and Priestley[29] in their schematic representation of the process as follows:

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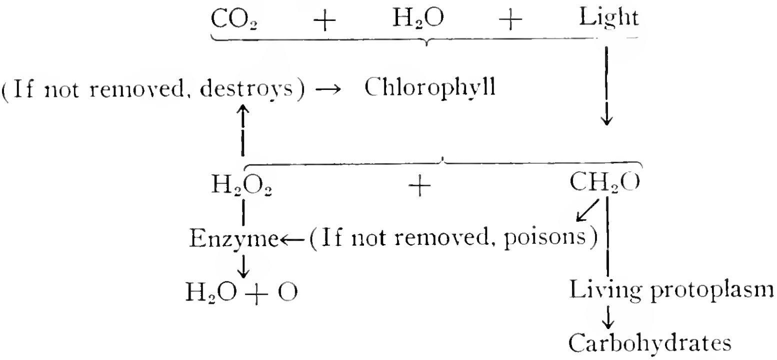
[26] Stoklasa and Zdobnicky, *Biochem. Zeit.*, **30**, 448 (1911).

[27] Zenghelis, *Compt. rend.*, **170**, 883 (1920); **171**, 167 (1920).

[28] Nef, *Ann. Chem.*, **357**, 253 (1907).

[29] Usher and Priestley, *Proc. Roy. Soc.*, **77** B, 369 (1905). Phipson, *Chem. News*, **50**, 37, 288 (1884).



Usher and Priestley point out that an accumulation of hydrogen peroxide would be harmful to the plant and a destruction of any excess is accomplished by the enzyme catalase. Similarly an accumulation of formaldehyde would poison the enzyme.

The reports of Thunberg [30] that salts of carbonic acid can be reduced to formaldehyde with hydrogen peroxide have been found to be erroneous.[31] Although Weigert [32] partially bases his theory on Thunberg's results, the fact that the latter have not been substantiated does not entirely annul Weigert's theory. According to this view chlorophyll is to be regarded as an optical sensitizer. The primary effect of the absorption of light by quanta results in an internal photoelectric effect, i.e. a shifting of an electron from one atom to another. The primary reaction in photosynthesis is a loss from the chlorophyll molecule of an electron which is then taken on by a molecule of oxygen, it being recognized that oxygen is essential for photosynthesis. The resulting negative oxygen ion transfers its charge to the water with the breaking up of the latter into hydrogen and hydroxyl ions. Whether it is the free hydrogen atoms or hydroxyl ions or the H_2O_2 which reacts with carbonic acid Weigert does not decide.

$$\begin{array}{l}
2\ \text{Chlorophyll} + 2\ h_\nu + 2\ O_2 = 2\ \text{Chl}^+ + 2\ O_2^- \\
2\ O_2^- + 2\ H_2O = 2\ H + 2\ OH^- + 2\ O_2 \\
2\ H + 2\ OH^- + 2\ \text{Chl}^+ = H_2 + H_2O_2 + 2\ \text{Chl} \\
\hline
2\ H_2O + 2\ h_\nu = H_2 + H_2O_2 \\
CO_2 + H_2 + H_2O_2 = HCOH + H_2O + O_2 \\
\hline
CO_2 + H_2O + 2\ h_\nu = HCOH + O_2
\end{array}$$

According to these equations two energy quanta suffice to reduce one molecule of carbon dioxide, i.e. it is necessary that only one electron be carried by the molecule of oxygen to each of the two water molecules. Weigert further bases his theory upon certain energy relations which are discussed in Chapter 6. The theory is clearly based upon the formal-

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[30] Thunberg, *Zeit. physik. Chem.*, **106**, 305 (1923).

[31] Bach and Monson, *Ber. chem. Ges.*, **57**, 735 (1924).

[32] Weigert, *Zeit. physik. Chem.*, **106**, 313 (1923); **109**, 79 (1924).

dehyde hypothesis and the supposition that carbon dioxide is reduced by hydrogen. Other modifications of Baeyer's theory and the attempts which have been made to verify this will be discussed later.

c. The Condensation of Formaldehyde by Means of Alkali.

Turning to the fourth assumption in Baeyer's theory, that the formaldehyde formed by reduction of carbon dioxide is condensed to sugars under conditions existing in the plant, it must be realized that this is an important feature of the theory and was based upon the discovery of Butlerow of the condensation of his dioxymethylene (trioxymethylene) to a sugar-like substance by means of alkalies. Shortly after Baeyer had suggested that the condensation of formaldehyde to a sugar was to be ascribed to the splitting out of water from $CH_2(OH)_2$ Würtz,[33] on the basis of his discovery of aldol, concluded that the condensation of formaldehyde was also an aldol condensation and thus modified Baeyer's theory. "On conçoit d'ailleurs que le plus simple des aldéhydes, l'aldéhyde formique puisse prendre naissance dans les procédés de la végétation, par la reduction partielle d'une molécule d'acide carbonique

$$CO^2 + H^2O - O^2 = CH^2O$$

et que la condensation de plusieurs molécules d'aldéhyde formique puisse donner naissance á des hydrates de carbon, á la fois alcools et aldéhydes au même titre et par le même procédé que la condensation de deux molécules d'aldéhyde ordinaire produit l'aldol." Würtz therefore provided the first rational conception of the reactions involved in the condensation of formaldehyde to hexose sugars.

Many modifications of Butlerow's method of condensing formaldehyde have been devised, but in principle these do not differ greatly from the original method; the modifications consist in the main in the use of weaker alkalies and lower temperatures than were used by Butlerow. Thus Loew [34] introduced the use of calcium hydroxide at 15 to 20° and called the product obtained formose. Lobry de Bruyn and van Ekenstein [35] found that an excellent condensing agent is lead hydroxide. There are many other substances which bring about the condensation: the hydroxides of potassium, sodium, magnesium and barium, finely divided zinc and lead, basic lead salts and sodium acetate. With weak alkaline solutions higher temperatures increase the condensation, although above about 70° the alkalies also decompose the condensation product.

Formose obtained by any of these methods is a mixture of a number of substances, the nature of which has for the most not been definitely determined. Of considerable significance in relation to the origin of sugars

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[33] Würtz, *Compt. rend.*, **74**, 1366 (1872). Kekulé, *Ann. Chem.*, **162**, 77 (1872).

[34] Loew, *Jour. prakt. Chem.*, **33**, 321, **34**, 51 (1886); **92**, 133 (1915). *Ber. chem. Ges.*, **22**, 470, 478 (1889).

[35] Lobry de Bruyn and van Ekenstein, *Rec. trav. chim.*, **18**, 309 (1899).

in nature was the discovery of Fischer and Passmore [36] that formose contains α-acrose, a synthetic sugar mixture, which Fischer and Tafel [37] showed to contain some of the naturally occurring sugars, i.e. the inactive forms of glucose and fructose. From Nef's studies of the products obtained by the action of alkalies on sugars he came to the conclusion that formose consists of a mixture of half pentoses and half hexoses. Of the hexoses there are 24 members present in the formose mixture, viz. 8 aldohexoses, 8 2-ketohexoses and 8 3-ketohexoses; the aldoses constituting about one-half of the mixture are primarily dl-glucose and dl-galactose. These definite statements of Nef will, however, require further substantiation.

The mechanism of the condensation of formaldehyde to hexose and pentose sugars has not yet been satisfactorily determined. Various theories have been proposed; these include:

1. The intermediate formation of glycollic aldehyde, $CH_2OH . CH:O$, and the condensation of this to tetroses and hexoses.
2. The intermediate formation of the trioses, glycerinealdehyde, $CH_2OH . CHOH . CH:O$, and dioxyaceton, $CH_2OH . C:O . CH_2OH$, which then condense to hexoses.
3. The stepwise condensation of one molecule of formaldehyde, with the successive formation of diose, triose, tetrose, pentose, and hexose.

The formation of glycollic aldehyde from formaldehyde has been reported by H. and A. Euler [38] by the action of calcium carbonate, though Nef [39] maintains that in the experiments of the former authors the calcium carbonate must have contained some calcium hydroxide,[40] for Nef could not obtain any sugar formation with either calcium carbonate or barium carbonate even after heating the mixture for days.

It is quite well established that glycollic aldehyde condenses to tetroses and hexoses and Neuberg [41] reports the formation also of pentoses in this condensation which implies a splitting of some kind. It is a question therefore whether the pentoses are derived directly from formaldehyde or through a degradation of the hexoses.

Fischer [42] regarded it as probable that glycerinealdehyde and dioxyacetone are intermediate products in the condensation of formaldehyde. This was largely based upon the fact that α-acrose is obtained in this manner and Fischer found some α-acrose in the formose mixture. H. and

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[36] Fischer and Passmore, *Ber. chem. Ges.*, **22**, 359 (1889).

[37] Fischer and Tafel, *ibid.*, **22**, 97 (1889). Fischer, *ibid.*, **23**, 2114 (1890).

[38] Euler, *Ber. chem. Ges.*, **39**, 39, 43 (1906).

[39] Nef, *Ann. Chem.*, **403**, 355 (1914).

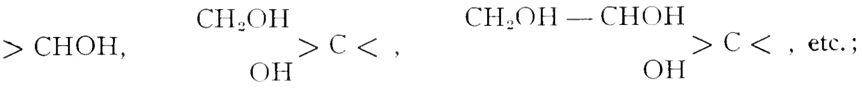
[40] Pechmann, *Ber. chem. Ges.*, **30**, 2460 (1897).

[41] Neuberg, *Biochem. Zeit.*, **12**, 339 (1908); *Ber. chem. Ges.*, **35**, 2630 (1902). Fischer and Landsteiner, *ibid.*, **25**, 2553 (1892). Fischer, *ibid.*, **27**, 3200 (1894). Nef, *l. c.*, 205.

[42] Fischer, *Ber. chem. Ges.*, **23**, 2128 (1890). Piloty, *ibid.*, **30**, 3168 (1897). Wohl and Neuberg, *ibid.*, **33**, 3108 (1900).

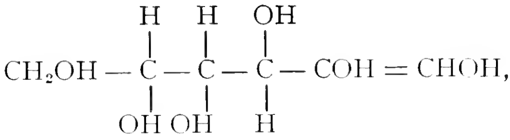
A. Euler report finding dioxyaceton in a mixture of formaldehyde treated with calcium carbonate.

The tremendous complexity of the reactions which occur when a solution of formaldehyde is treated with an alkali is made evident by the extensive investigations of Nef on the action of alkalies on various hexose and pentose sugars. In such a reaction Nef calculates there may take part no less than 116 different substances. These include (1) the 32 theoretically possible normal aldoses with one to six carbon atoms; (2) the 32 methylenenols which are formed from these as:



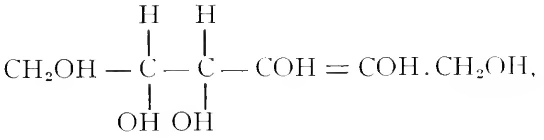
$$>\mathrm{CHOH},\qquad \begin{matrix}\mathrm{CH_2OH}\\ \mathrm{OH}\end{matrix}\!>\mathrm{C}<,\qquad \begin{matrix}\mathrm{CH_2OH-CHOH}\\ \mathrm{OH}\end{matrix}\!>\mathrm{C}<,\ \text{etc.;}$$

(3) all 26 ketoses with three to six carbon atoms in a straight chain and (4) the 26 endiols which may be formed from the different aldoses and ketoses. As a matter of experience Nef found the number of substances reacting is no more than 93. The endiols may undergo rearrangement, thus bringing about a transformation of aldoses into ketoses and vice versa as was first made evident by the discovery of Lobry de Bruyn and van Ekenstein that d-glucose, d-mannose, d-fructose, d-pseudo-fructose and α and β d-glutose are all interconvertible. Also the endiols may undergo a splitting at the double bond yielding aldoses with fewer carbon atoms. Thus, for example, d-glucose in alkaline solution may yield the following endiols:

1. 

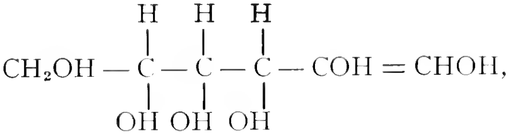
$$\mathrm{CH_2OH-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}-COH=CHOH}$$

α-1, 2-d-Glucose endiol.

2. 

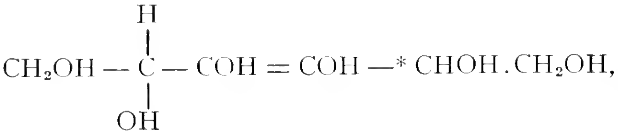
$$\mathrm{CH_2OH-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-COH=COH.CH_2OH}$$

2, 3-d-Glucose endiol.

3. 

$$\mathrm{CH_2OH-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-COH=CHOH}$$

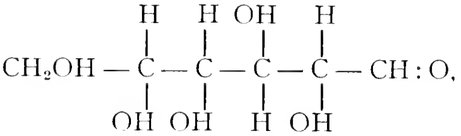
β-1, 2-d-Glucose endiol.

4. 

$$\mathrm{CH_2OH-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-COH=COH-{}^{*}CHOH.CH_2OH}$$

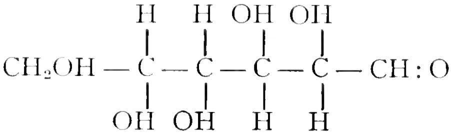
3, 4-d-Glucose endiol (2 forms)

From 1 there are formed by rearrangement of the endiol:



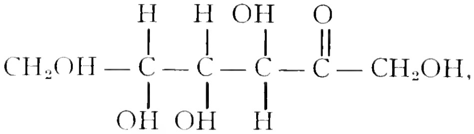
$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{H} & & \\ & & | & & | & & | & & | & & \\ \mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH{:}O}, \\ & & | & & | & & | & & | & & \\ & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{OH} & & \end{array}$$

d-glucose



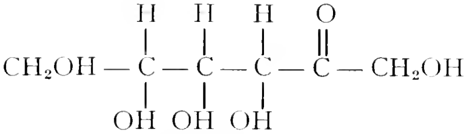
$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{OH} & & \\ & & | & & | & & | & & | & & \\ \mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH{:}O} \\ & & | & & | & & | & & | & & \\ & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{H} & & \end{array}$$

d-mannose



$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{O} & & \\ & & | & & | & & | & & \| & & \\ \mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH_2OH}, \\ & & | & & | & & | & & & & \\ & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & & & & \end{array}$$

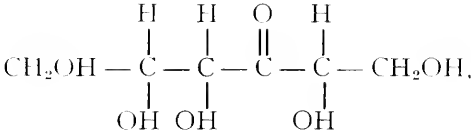
d-fructose



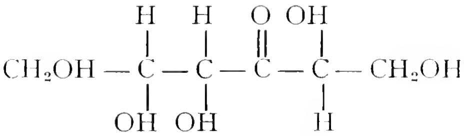
$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{H} & & \mathrm{H} & & \mathrm{O} & & \\ & & | & & | & & | & & \| & & \\ \mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH_2OH} \\ & & | & & | & & | & & & & \\ & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{OH} & & & & \end{array}$$

d-pseudofructose

From 2 by rearrangement,



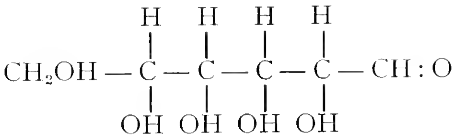
$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{H} & & \mathrm{O} & & \mathrm{H} & & \\ & & | & & | & & \| & & | & & \\ \mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH_2OH}, \\ & & | & & | & & & & | & & \\ & & \mathrm{OH} & & \mathrm{OH} & & & & \mathrm{OH} & & \end{array}$$



$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{H} & & \mathrm{O} & & \mathrm{OH} & & \\ & & | & & | & & \| & & | & & \\ \mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH_2OH} \\ & & | & & | & & & & | & & \\ & & \mathrm{OH} & & \mathrm{OH} & & & & \mathrm{H} & & \end{array}$$

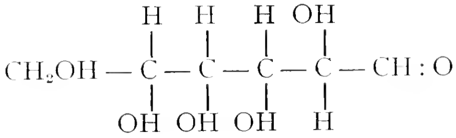
α and β-d-glutose

From 3 d-allose and d-latose:



$$\mathrm{CH_2OH}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{CH:O}$$

and



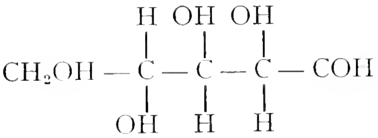
$$\mathrm{CH_2OH}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\mathrm{CH:O}$$

would be obtained, but these are not formed, so Nef concludes that d-pseudo-fructose forms the 2-3-d-glucosendiol (2) only. This is an important point in the theory.

From 4 by rearrangement a mixture of all the aldo-2-keto and 3-keto hexoses would be obtained; since this does not occur Nef concludes that this endiol is not formed from d-glucose, d-mannose or d-fructose.

An entirely analogous situation prevails in the d-galactose series. Here there are possible the following endiols:

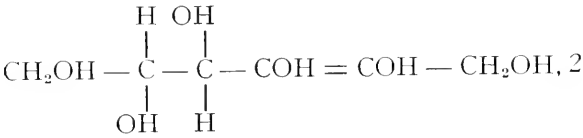
1.



$$\mathrm{CH_2OH}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\mathrm{COH}=\mathrm{CHOH}$$

α-1, 2-d-galactosendiol

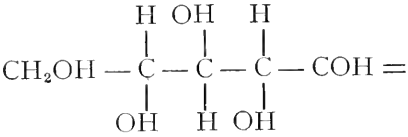
2.



$$\mathrm{CH_2OH}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\mathrm{COH}=\mathrm{COH}-\mathrm{CH_2OH}$$

2, 3-d-galactosendiol

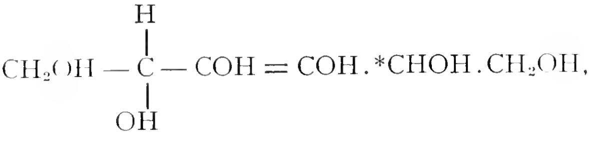
3.



$$\mathrm{CH_2OH}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{COH}=\mathrm{CHOH}$$

β-1, 2-d-galactosendiol,

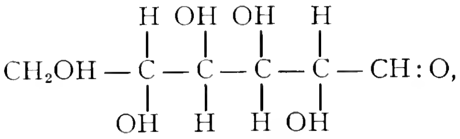
4.



$$\mathrm{CH_2OH}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\mathrm{COH}=\mathrm{COH.{}^{*}CHOH.CH_2OH}$$

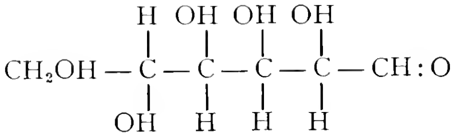
3, 4-d-galactosendiol (2 forms)

From 1 there could be formed by rearrangement:



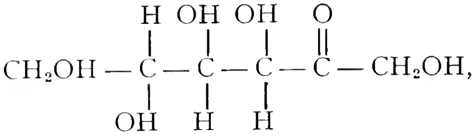
$$CH_2OH-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-CH:O,$$

d-galactose



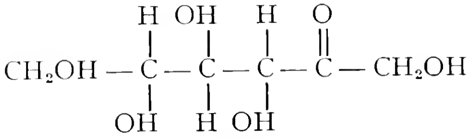
$$CH_2OH-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-CH:O$$

d-talose



$$CH_2OH-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\overset{\substack{O\\\|}}{C}-CH_2OH,$$

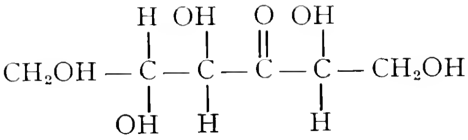
d-tagatose



$$CH_2OH-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\overset{\substack{O\\\|}}{C}-CH_2OH$$

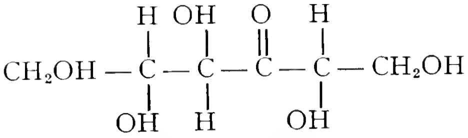
l-sorbose

From 2 by rearrangement:



$$CH_2OH-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\overset{\substack{O\\\|}}{C}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-CH_2OH$$

and



$$CH_2OH-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-\underset{\substack{|\\H}}{\overset{\substack{OH\\|}}{C}}-\overset{\substack{O\\\|}}{C}-\underset{\substack{|\\OH}}{\overset{\substack{H\\|}}{C}}-CH_2OH$$

α and β-d-galtose

From 3 l-gulose and l-idose would be formed. As these sugars are not formed when d-galactose is treated with alkalies Nef concludes that the β-1,2-d-galactosendiol is not formed. Similarly 4, which is identical with

3,4-glucosendiol, is in Nef's opinion probably not formed, for it could rearrange to a mixture of all the theoretically possible aldo, 2-keto and 3-keto hexoses, which is definitely proved not to be the case. On the other hand, to the writer the formation of the 3,4-endiols (both of the glucose and galactose series) is not entirely excluded owing to the fact that in all experiments with hexoses a considerable amount of tar is formed. This may arise from a splitting of the 3,4-endiols into two molecules of glycerine aldehyde which may polymerize to a tar. The writer has found that in the presence of a reducing agent (aluminium amalgam) no tar formation takes place, but considerable quantities of aceton are formed.

Confining our attention for the moment to the hexoses, it is very interesting that in the glucose series at equilibrium ketoses and aldoses are formed in about equal quantities; of the aldoses, glucose and mannose there is present five times as much of the former as of the latter. Similarly in the galactose series over 90 per cent of the sugars present is d-galactose. These facts are of direct bearing on the problem of the synthesis of different sugars in nature. It gives a clue as to why allose, latose and talose have not been found in nature; this presumably also applies to gulose and idose and makes the presence of these sugars in formose highly improbable. These investigations of Nef offer the first chemical evidence of the fact that certain of the hexose sugars are apparently more stable or that the tendency of formation of certain ones is greater than of others. Why this is so, why the space relationship of one sugar makes for greater stability than another we are as yet unable to say. But it is significant that the proportions of the various sugars found in both the glucose and galactose are, at least, of the same general character as those found in nature. It remains to be determined whether there exist certain enzymes which are capable of the same reactions as are produced by the alkalies. Recently Spoehr and Wilbur [43] have found that disodium phosphate and neutral mixtures of phosphates produce the same isomerizing effects.

Nef carried out a similar line of reasoning with the pentose sugars and concluded that only 12 pentoses can be formed in formose.

In considering this phase of the Baeyer theory which deals with the condensation of formaldehyde to sugars as was first discovered by Butlerow, there remains the question whether this method of synthesizing sugars has any application to the process taking place in the plant. The methods thus far discussed all postulate the action of more or less strong alkali on the formaldehyde. There is no evidence that the reaction of the cell sap or of protoplasm even approaches that of the solutions used in the methods of preparing formose, although protoplasm is probably slightly alkaline.

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[43] Spoehr and Wilbur, Carnegie Inst. Washington Yearbook 1925, p. 158.

d. The Condensation of Formaldehyde with Weak Alkalies and Under the Influence of Light.

Little consideration has been given to the question whether the condensation of formaldehyde actually takes place under conditions such as may exist in the chloroplast, at least as far as the hydrogen ion concentration of the chloroplast is concerned. The writer has made a series of tests to determine whether formose is formed from formaldehyde in *weak* alkaline or in neutral solution and at ordinary temperatures and, whether the reaction is influenced by sunlight. Pure 3 per cent solutions of formaldehyde (free from methyl alcohol and formic acid) were used, with the salts in tenth normal concentration, in glass flasks. These were exposed directly to the sunlight in Arizona for five months, and the amount of sugar formed determined every month. Not a trace of sugar was formed by $CaCO_3$, $3MgCO_3.Mg(OH)_2.3H_2O$, K_2CO_3, $KHCO_3$, colloidal $Fe(OH)_3$, in the dark or the sunlight. Potassium nitrate solutions which become slightly alkaline when exposed to sunlight with the formation of nitrite and traces of ammonia, produced very small quantities of sugar from formaldehyde in the light. Zinc carbonate also produced traces of sugar in the light. All of the solutions on evaporation at reduced pressure left a residue of polymerized formaldehyde (paraformaldehyde), but this is not a condensation product and disappears on repeated addition of water and evaporation at reduced pressure. Only such products which on evaporation at reduced pressure gave no test for formaldehyde in the water which had been distilled from them and which reduced Fehling solution were considered as condensation products. In almost every case some formic acid had been formed. With the stronger bases, lead hydroxide and calcium hydroxide, light increases the yield of the formose obtained. The writer has also endeavored to obtain condensation of formaldehyde with weak optically active bases, such as brucine and quinine, but the yields of sugar were always very small and never showed any optical rotation.

The condensation product obtained from formaldehyde is unquestionably a mixture of a great many different substances, probably more than Nef considered as being present. This becomes evident from the fact that the reducing power of the formose mixture as determined with a cuproalkaline solution is usually considerably lower than the amount calculated from the weight of condensation product taken as sugar. With stronger alkalies there are in all probability also some saccharinic acids formed.

Although the fact was not considered in the original Baeyer theory, it is possible that light is necessary, or at least plays a part in the condensation of formaldehyde. That the condensation is affected by light has been observed by various workers. Inghilleri[44] reported that by exposing sealed tubes containing formaldehyde to sunlight he obtained

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[44] Inghilleri, *Zeit. physiol. Chem.*, **71**, 105 (1911), **73**, 144 (1912).

"methylenitan" and from tubes containing formaldehyde and oxalic acid he claims to have obtained sorbose.

Pribram and Franke[45] obtained glycollic aldehyde by illuminating solutions of formaldehyde in quartz vessels with ultra-violet light.

Recently Baly, Heilbron and Barker[46] have also reported the formation of sugar from a solution of formaldehyde exposed to ultra-violet light. To the formaldehyde solution they added calcium carbonate and found that the optimum temperature during illumination was 37°. Baly states: "With an initial concentration of 40 per cent formaldehyde the maximum reducing power is 8 per cent calculated as glucose and with 20 liters of formaldehyde this can often be reached after 14 days of continuous illumination."

Irvine and Francis[47] have examined the mixture obtained by Baly from the action of ultra-violet light on formaldehyde solutions and found that the sirup behaved as though it were about 25 per cent sugar (calculated as glucose), but this value diminished when the sirup was heated with acid such as would hydrolyze a glucoside or polysaccharide. They conclude: "that part of the reducing power was therefore contributed by compounds other than sugars." From methylation experiments it is concluded that there was present 9.3 per cent of sugar in the original sirup and that "the bulk of the photosynthetical sirup, at least to the extent of 80 per cent, consists of nonsugar compounds which contain hydroxyl groups." Baly claims that glucuronic acid is present in the sirup, but he does not give sufficient evidence to support this claim.

Beyond the fact of demonstrating that it is chemically possible to obtain sugars which occur in the plant, all of the large amount of chemical work which has been done on the condensation of formaldehyde with strong and weak alkalies and with light are really of little significance in establishing the validity of Baeyer's theory. Condensing formaldehyde with strong alkalies or through the action of ultra-violet light and obtaining a great mixture of substances of which only a small per cent is in many cases hexose sugar, will, even to the most optimistic chemist, appear as a rather far cry to the method by means of which the plant forms glucose. Whether there are in the plant enzymes by means of which the condensation is effected, whether there exist in the chloroplast areas of high alkalinity or whether the great pressures which may exist in the surface layers of the cells play a rôle, are after all mere conjectures. Baeyer's theory is a good suggestion of a possible mechanism of photosynthesis; the chemical evidence which has been advanced in support of it may serve as a good clue for further investigations with very much more highly refined methods.

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[45] Pribram and Franke, *Monatsh.*, **33**, 415 (1912). Franke and Pollitzer, *ibid.*, **34**, 297 (1913).

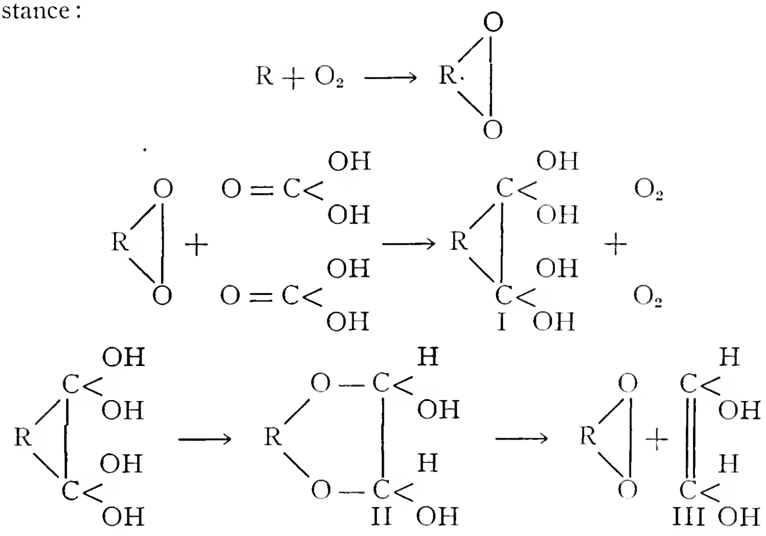
[46] Baly, Heilbron and Barker, *Jour. Chem. Soc.*, **119**, 1025 (1921). Baly, *Ind. Eng. Chem.*, **16**, 1016 (1924).

[47] Irvine and Francis, *ibid.*, **16**, 1019 (1924).

e. Glycollic Aldehyde.

The possibility that glycollic aldehyde may be an intermediate step in the formation of sugar has been suggested by various workers[48] and, as has been stated, it has been regarded as a possible intermediate step in the condensation of formaldehyde. Löb[49] observed that carbon monoxide and water under the influence of the silent electric discharge produce glycollic aldehyde and used this fact as the basis of a theory that the latter substance may be an intermediate product in photosynthesis.

Fincke[50] has developed a theory which is a departure from most others in that he considers glycollic aldehyde one of the first reduction products of carbonic acid. This viewpoint has certain distinct advantages over the formaldehyde theory. Glycollic aldehyde is very easily condensed to tetroses and hexoses *in vitro* and the condensation is known to take place in the animal organism, while formaldehyde is notoriously poisonous even in low concentrations and its condensation is not easily effected.[51] Fincke also considers that glyoxylic acid, which has been found in plants, may be related to photosynthesis. Of considerable interest in this theory is the point that it takes into account the fact that oxygen is necessary for photosynthesis. This is the action of a moloxide. Fincke calls it a superoxide. He regards that some component of the chlorophyll reacts with molecular oxygen: more properly this could be assumed to be a component of the chloroplast or related to the enzyme for the existence of which Willstätter and Stoll have brought considerable evidence. At any rate carbon dioxide is not reduced directly, but reacts first with another substance:



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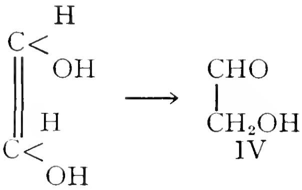
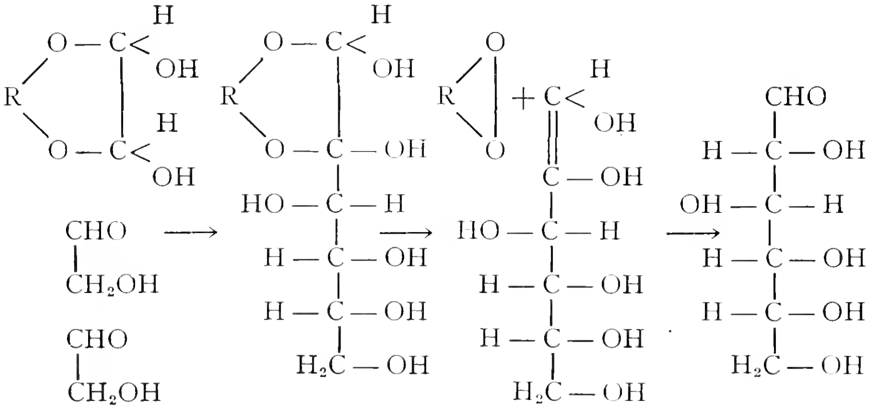
[48] Reinke, *Ber. chem. Ges.*, **14**, 2148 (1881). Lippmann, *ibid.*, **24**, 3306 (1891).
[49] Löb, *Landw. Jahrb.*, **35**, 541 (1906); *Biochem. Zeit.*, **63**, 93 (1914).
[50] Fincke, *Biochem. Zeit.*, **61**, 157 (1914).
[51] Barrenscheen, *Biochem. Zeit.*, **58**, 300 (1914).


IV

Fincke pictures that in the first stage the oxygen of the moloxide together with the carbonyl oxygen of the carbonic acid are split out by the action of light, thus an equal volume of oxygen is emitted for that of carbon dioxide absorbed. The second step is a rearrangement of compound I and splitting of compound II with the formation of the unsaturated alcohol III. The third stage is a rearrangement of this alcohol into glycollic aldehyde.

The intermediate substance II contains two asymmetric carbon atoms. If R is also asymmetric, and being a plant product this can be assumed, asymmetry can thus be induced in the further condensation products. Fincke thus pictures a stepwise condensation:



It will be observed that the third from the left of the above formulae represents the endiol of glucose, mannose and fructose so that all three of these sugars could be derived in the same manner.

Fincke's theory is perhaps somewhat fanciful in its present form, but it possesses some advantages over the formaldehyde theory. In another paper Fincke[52] does not include the moloxide; this does, however, not make for much greater simplicity.

f. The Theory of Willstätter and Stoll.

Willstätter and Stoll[53] on the basis of their extensive researches on chlorophyll and the mechanism of the photosynthetic process have formu-

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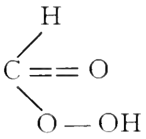
[52] Fincke, *Zeit. Nahrung. Genussm.*, 27, 8 (1914).

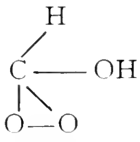
[53] Willstätter and Stoll, "Untersuchungen über die Assimilation der Kohlensäure," Berlin, 1918, pp. 236, 415.

lated a theory of the reduction of carbonic acid which, though not entirely novel, has several original features. The theory pertains more especially to the mode of "deoxidation" of carbonic acid. The fact is accepted that in photosynthesis the reduction of carbonic acid attains the level of formaldehyde, in fact, the formaldehyde theory so far as it postulates the intermediate formation of this substance is accepted. Willstätter and Stoll consider that carbonic acid is added to the magnesium complex of the chlorophyll, and the carbonic acid or a carbonic acid derivative thus becomes a component of the pigment. They believe the chlorophyll to be in a colloidal solution in the leaf and that the colloidal state and the optical properties of the chlorophyll are not altered by the addition of the carbonic acid. Chemical change is accomplished in the chlorophyll molecule itself by means of the absorbed light energy. The whole theory is thus based upon the supposed property of chlorophyll to form an addition compound with carbonic acid.

The result of light absorption by the chlorophyll-carbonic acid complex is a rearrangement of the molecule of carbonic acid: "eine Verschiebung der Valenzen im Kohlensäuremolekül, eine Umgruppierung der Atome, welche durch die zugeführte Energie bewirkt wird." This rearrangement of the carbonic acid molecule leads to the formation of a peroxide and two possible structures are proposed, namely:

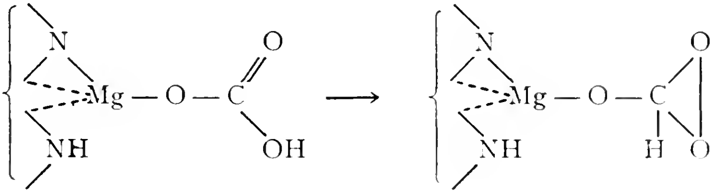


Performic acid



Formaldehyde peroxide

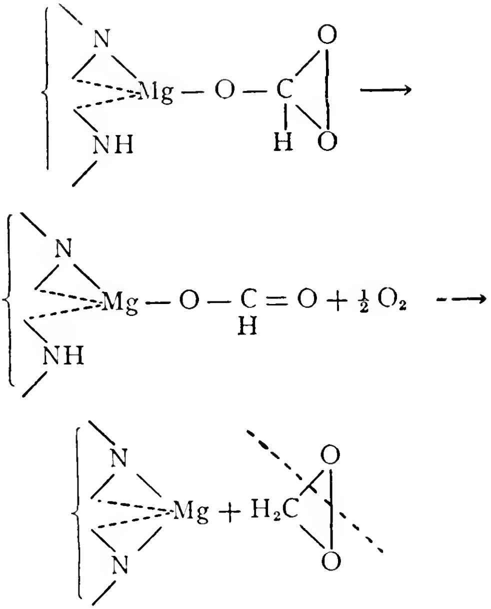
The performic acid is known and some evidence is presented for the possible existence of the formaldehyde peroxide. The possibility is also suggested that the addition to chlorophyll may not be of carbonic acid itself but of some carbonic acid derivative. The addition product may be written as follows:



Chlorophyll-carbonic acid compound

Chlorophyll-formaldehyde peroxide

The further rearrangement of the chlorophyll-formaldehyde-peroxide results in the splitting out of oxygen which may take place in two stages, with the intermediate formation of formic acid (or more properly a formic acid stage); this undergoes a similar rearrangement, with the absorption of energy, to a peroxide and a subsequent splitting off of oxygen:



Intermediate products are not liberated from the chlorophyll. A reduction product as, for instance, a strong acid such as formic acid, would not be freed from the magnesium, or only with destruction of the chlorophyll, which does not occur. All of the evidence of Willstätter and Stoll points to the conclusion that the reduction is carried straight through to formaldehyde or its equivalent in degree of reduction. This fact is used to support the conclusion that the reduction product remains combined with the chlorophyll until a molecule of oxygen is removed.

Formaldehyde does not remain combined with the chlorophyll. Willstätter and Stoll have shown that chlorophyll is not capable of forming an addition product with formaldehyde. The liberation of formaldehyde from the chlorophyll is brought about by the action of the carbonic acid or by water. For the condensation of formaldehyde to sugars energy is presumably not necessary.

The splitting out of oxygen from the peroxide also requires little energy. The radiant energy is used in the rearrangement of the chlorophyll-carbonic acid complex to the peroxide. It is possible that the splitting of the peroxide is accomplished by enzyme activity. As has been discussed in the section of this book dealing with the effect of temperature and chlorophyll-content on the rate of photosynthesis, Willstätter and Stoll have brought evidence to support the idea that there are two separate reactions involved in the photosynthetic process. One of these is a purely photochemical reaction, the other an enzymatic one. It is therefore assumed that the enzymatic reaction consists in the splitting off of oxygen from the chlorophyll-formaldehyde peroxide complex by means of a catalase-like enzyme.

Willstätter and Stoll endeavored to subject their theory to experimental test. They tried to detect the photoisomerization of the chlorophyll-carbonic acid complex in light by means of a reagent which would serve as a test for a peroxide. Their system consisted of chlorophyll, carbonic acid, peroxidase and pyrogallol. In the presence of a peroxide the pyrogallol is converted into purpurogallin by the peroxidase. After making special preparations of peroxidase and illuminating their system in an air stream containing 5 to 20 per cent of carbon dioxide, Willstätter and Stoll obtained large quantities of purpurogallin, but this was not the result of carbonic acid reduction, rather of the oxidation of chlorophyll itself, partially induced by light. While all of the experiments directed to establish the formation and subsequent splitting of a peroxide gave only negative results, the positive value of these experiments is to show the extreme complexity of the natural chlorophyll apparatus. The great protection which the chlorophyll in the chloroplast enjoys against photo-oxidation as well as against the splitting out of magnesium through the carbonic acid, indicates that there are conditions in the living chloroplast about which our analytical methods have given us very little information.

Interesting and suggestive as the theory of Willstätter and Stoll is, it must be realized that it rests upon certain assumptions. The first of these is that formaldehyde is the final reduction product of carbonic acid. Willstätter and Stoll base this assumption upon the fact that the photosynthetic quotient, CO_2 absorbed : O_2 emitted is unity. It has already been mentioned that the same quotient would be obtained for any compound of the formula $C_nH_{2n}O_n$, as glycollic aldehyde, for instance. Another assumption pertains to the absorption of carbonic acid by chlorophyll. While their experiments indicate that this may occur to a certain extent, it still seems somewhat questionable whether this can account for the total absorption of carbon dioxide by the leaf. The possibility of an absorption of carbonic acid by chlorophyll has been considered by a number of workers.[54] The investigations of Brown and Escombe, already discussed, have demonstrated that the leaf possesses a very high absorptive capacity for carbon dioxide during photosynthesis. This is of a very much greater magnitude than can be accounted for by the absorption of carbon dioxide by chlorophyll, or by any other means which we know of as existing in the leaf.

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[54] Hoppe-Seyler, "Physiologische Chemie," Berlin, 1881, p. 139. Hansen, *Arb. bot. Inst. Würzburg*, **3**, 426, 429 (1885). Luther and Hällström, *Ber. chem. Ges.*, **38**, 2288 (1891). Jörgensen and Kidd, *Proc. Roy. Soc.*, **89** B, 342 (1916).

g. The Blackman Reaction.

A conception of the mechanism of photosynthesis differing somewhat from that of Willstätter and Stoll was developed by Warburg.[55] He considers that photosynthesis is not a simple photolysis of carbonic acid. There are rather several steps, of which the first is the "photochemical primary action." In this action oxygen is not split out, but it consists rather in the action of light on the chlorophyll molecule with the formation of the "photochemical primary product." The rate of formation of this product is proportional to the amount of radiant energy absorbed per unit time. The "photochemical primary product" reacts in a secondary reaction with the "acceptor." The "acceptor" is not carbonic acid, but a carbonic acid derivative which is formed in the cell in a chain of chemical reactions. There are thus according to this view three reactions: 1. the photochemical primary reaction, 2. the secondary reaction and 3. acceptor formation. The acceptor formation takes place independently of light; it is a "preparatory" (vorbereitende) reaction, the product of which, the acceptor, is consumed in the secondary reaction.

Warburg considered that in the "primary photochemical action" 1. no oxygen is formed and 2. no substances are formed which automatically split out oxygen. This view is based partially upon the claim of Willstätter and Stoll that chlorophyll adds on carbon dioxide, and partially upon quantum theoretical reasoning which makes a primary splitting of carbon dioxide by the light which is active in photosynthesis (0.77 μ) highly improbable.

Warburg's theory of "acceptor" formation was based upon his investigations of the influence of carbon dioxide concentration and light intensity on the rate of photosynthesis. Above certain high concentrations of carbon dioxide and high illumination intensities the rate of photosynthesis does not increase when either of these factors are augmented. Also, under these conditions (high illumination intensity or high carbon dioxide concentration) a rise of 10° in temperature (15 to 25°) causes the rate of photosynthesis to double. This fact, discovered by Blackman, is taken by Warburg to indicate that in the mechanism of carbon dioxide reduction a slowly progressing chemical reaction plays a rôle and under these conditions determines the rate of photosynthesis. This reaction Warburg designated as the "Blackman reaction." When the light intensity is low a temperature coefficient close to unity is obtained; this is characteristic of photochemical reactions and indicates that under these conditions the light reaction is determining the rate of photosynthesis. When the light intensity is high, a high temperature coefficient is obtained; this is characteristic of an ordinary chemical reaction and indicates that under these conditions a thermal or "dark" reaction is determining the rate of photosynthesis.

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[55] Warburg, *Biochem. Zeit.*, **100**, 230 (1919); **103**, 188 (1920); *Naturwiss.*, **9**, 354 (1921). Warburg and Negelein, *Zeit. physik. Chem.*, **102**, 235 (1922); **106**, 191 (1923). Warburg and Uyesugé, *Biochem. Zeit.*, **146**, 486 (1924).

Another source of support for his theory of "acceptor" formation Warburg saw in the action of potassium cyanide on the rate of photosynthesis. In cells that have been treated with potassium cyanide, atmospheric carbon dioxide cannot be used in photosynthesis. When the illumination is very low, so that the rate of respiration exceeds that of photosynthesis, the rate of the latter is not depressed by concentrations of cyanide of 0.02 M. and less. Under these conditions atmospheric carbon dioxide is not used directly in photosynthesis, but in the light no carbon dioxide of respiration is liberated; it is concluded that this or possibly an intermediate product of respiration is used in photosynthesis under these circumstances. If a certain cyanide concentration inhibits the rate of photosynthesis by 50 per cent with high illumination intensity, the same concentration will have no effect with low illumination. This fact was taken to indicate that the secondary reaction, i.e. the reaction between the "photochemical primary product" and the "acceptor" is not affected by the cyanide. Nor is the "photochemical primary reaction" thus affected. Warburg considered that it was only the dark reaction, the Blackman reaction, which is affected by cyanide.

It was originally Warburg's idea that the Blackman reaction constituted the formation of the "photochemical acceptor." Willstätter and Stoll, on the other hand, made the assumption that the photochemical reduction of carbonic acid, their photoisomerization reaction, resulted in the formation of a peroxide and that the splitting out of oxygen from this compound was a "dark" reaction and constituted the Blackman reaction. If the theory of Willstätter and Stoll is correct it would be expected that if hydrogen peroxide were introduced into a living green cell it would be decomposed according to the known behavior of the Blackman reaction. From the effect of temperature, cyanide and various urethanes on the formation of oxygen from hydrogen peroxide by *Chlorella*, Warburg and Uyesugé concluded that the theory of Willstätter and Stoll is more nearly in accordance with the experimental facts than Warburg's original ideas. The agreement between the behavior of hydrogen peroxide in *Chlorella* and the Blackman reaction is not very close, though perhaps this is not to be expected in view of the fact that the peroxide of Willstätter is not hydrogen peroxide but supposedly formaldehyde peroxide. While much in these views may assist considerably in forming a conception of the mechanism of photosynthesis they must very clearly be considered as hypotheses.

h. Other Theories.

A number of other theories of the mechanism of photosynthesis have been advanced by various writers. Some of these are based upon observations regarding the behavior of the chlorophyllous cell, others are merely speculative. The latter will not be discussed here. Wurmser[56] has again

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[56] Wurmser, "Récherches sur l'assimilation chlorophyllienne," Paris (1921).

taken up the notion of Pringsheim[57] that the oxygen is not liberated in the chloroplasts. It will be recalled that Pringsheim on the basis of his studies of photosynthetic asphyxiation came to the conclusion that carbon dioxide reduction and oxygen liberation do not occur at the same place in the cell, that oxygen is not formed within the cell, but that during photosynthesis a substance is formed which migrates away from the chloroplasts, and oxygen is liberated from this substance only when it reaches the surface of the cells. Pringsheim's ideas were apparently refuted by the discovery of gas vacuoles and of oxygen bubbles in the cells. Wurmser now stresses the great susceptibility of chlorophyll to photo-oxidation and concludes that oxygen cannot possibly be liberated at the surface of the pigment; he returns to the conception of Pringsheim, though in a somewhat modified form.

Wurmser considers that the reduction of carbonic acid takes place in the protoplasm. This reduction is brought about by the absorption of energy from other reactions, apparently oxidation reactions. In photosynthesis a primary transformation takes place at the surface of the chlorophyll granules. He supposes that in the chloroplast there is a substance, the nature of which is yet unknown, which undergoes a chemical reaction with the absorption of energy:

$$\text{A} + \text{Light} \longrightarrow \text{A}' - \text{X cal.} \qquad \text{(I)}$$

The product A′ diffuses in the protoplasm and undergoes a spontaneous reaction with the regeneration of A and the liberation of energy:

$$\text{A}' \longrightarrow \text{A} + \text{X}' \text{ cal.} \qquad \text{(II)}$$

It is the energy liberated in the second reaction which makes possible the reduction of carbonic acid:

$$CO_2 \longrightarrow C + O_2 - 94{,}300 \text{ cal.} \qquad \text{(III)}$$
$$CO_2 + H_2O \longrightarrow CH_2O + O_2 - 127{,}200 \text{ cal.} \qquad \text{(IV)}$$

Reaction I is a photochemical reaction; the quantity of A′ formed in unity of time is proportional to the quantity of light absorbed. But the rate of photosynthesis is not dependent only upon the concentration of A′, but also on reaction II which depends upon the ability of the protoplasm to utilize the energy in A′. The substance A is not chlorophyll, for Wurmser has shown that the only known photochemical reaction of chlorophyll, namely that of photo-oxidation, shows maximum and minimum reaction in portions of the spectrum which are quite different from those in which photosynthesis exhibits its greatest activity. Wurmser considers that the substance A is therefore colorless. According to this view the reduction of carbonic acid is not a photochemical reaction and this reduction is not directly associated with chlorophyll.

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[57] Pringsheim, *Sitzber. Preussischen Akad. Wiss.*, **38**, 763 (1887).

This pigment acts as a photosensitizer of the substance A which probably has its maximum absorption in the ultra-violet. Wurmser's theory is as yet incompletely founded, but may prove of considerable value. In this theory the Blackman reaction would probably be reaction II, which is a dark reaction and one in which oxygen apparently plays a rôle.

Ostwald [58] has formulated a theory which has a certain similarity to that of Wurmser. Ostwald assumes that the primary step in photosynthesis consists in the formation by oxidation of a lipoid peroxide. However, the theory contains many assumptions which have little or no evidence for support.

Baudisch [59] has also made some interesting theoretical deductions relating to the mechanism of photosynthesis, more particularly in regard to the synthesis of amino acids.

The theories relative to the function of chlorophyll in the photosynthetic process are discussed in Chapter 7.

2. Attempts To Find in the Plant Substances Which Form the Intermediate Steps Demanded by the Theories

A very natural sequel to the construction of theories of the mechanism of photosynthesis has been the desire to obtain substantiation of these by finding in the plant the substances which, according to the theories, are the intermediate products between carbon dioxide and carbohydrates. A great many investigations have been carried out from this viewpoint. Most of these are concerned with the detection of formaldehyde as a means of testing the Baeyer hypothesis. We shall not discuss these investigations in detail; in many of them color reactions were employed as final tests for the presence or absence of formaldehyde, tests which are of very questionable value. The entire method is, in fact, of very limited significance for establishing the validity of any theory. Many of the older reports have been shown to be of no value on account of the fact that the chemical tests employed were later shown to be not specific for formaldehyde or had been improperly employed.

a. Formaldehyde.

The results of the earlier attempts to detect formaldehyde in illuminated leaves have been subjected to criticism by Curtius and Franzen,[60] Czapek [61] and Euler [62] and were found to be unreliable. The aldehyde reaction which had been obtained when the expressed juice or extracts of leaves were distilled could not be ascribed to formaldehyde, but un-

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[58] Ostwald, *Koll. Zeit.*, **33**, 356 (1923).

[59] Baudisch, *Science*, **58**, 451 (1923); *Ber. chem. Ges.*, **44**, 1009 (1911); **49**, 1159, 1176 (1916); **50**, 652 (1917).

[60] Curtius and Franzen, *Ber. chem. Ges.*, **45**, 1715 (1912).

[61] Czapek, *Bot. Zeitg.*, **58**, 153 (1900).

[62] Euler, *Ber. Chem. Ges.*, **37**, 3411 (1904). Bokorny, *Chem. Zeitg.*, **33**, 1141, 1150 (1909). Plancher and Ravenna, *Atti. Accad. Lincei* [5], **13**, 459 (1904).

questionably other substances are present in these preparations which also give an aldehyde reaction.

Curtius and Franzen proposed the detection of formaldehyde by conversion into formic acid which can be easily distinguished. Fincke demonstrated that this method was unreliable and that a macerated mass of fresh leaves fixes or destroys formaldehyde which has been added to the mass so that the aldehyde cannot be distilled off. This fixation of formaldehyde by plant material is not confined to portions containing chlorophyll. When formaldehyde is added to a macerated mass of plant material and the juice is expressed, the fixation is less than when the material is subjected to steam distillation. Under any circumstances this would indicate that it is extremely difficult to detect small quantities of formaldehyde in plants. If more drastic means are employed to liberate any formaldehyde which may be present, there is danger of decomposing material in the plant tissue which may result in the formation of formaldehyde.

Kimpflin[63] has endeavored to demonstrate the presence of formaldehyde in the immediate neighborhood of the chlorplasts by microchemical means. But these results as well as those of others who have announced the finding of formaldehyde are of very doubtful value in view of the findings of Curtius and Franzen that other aldehydes give the same or very similar reactions.

Another feature of the question of the presence of formaldehyde in plants is the necessity of deciding whether any formaldehyde which may be there is actually associated with the photosynthetic process. Spoehr[64] has shown that a number of substances which are components of plants yield formaldehyde when solutions of these substances are exposed to the light. Neuberg[65] has also shown that methyl alcohol and glycocoll do the same, and similar reactions have been observed by others.[66]

Fincke[67] employed the modified Schiff reagent which had been described by Denigés[68] and by Grosse-Bohle.[69] He showed that in illuminated leaves there was no formaldehyde in concentration greater than 1 : 200,000, the safe limit of his method.

Recently Sabalitschka and Riesenberg[70] have also reported that they

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[63] Kimpflin, "Essai sur l'assimilation photochlorophyllienne du carbon," Lyon (1908), p. 105. Gibson, *Ann. Bot.*, **22**, 117 (1908). Gentil, *Bull. Ass. Chim. Suc. Dist.*, **27**, 169 (1914). Kleinstück, *Ber. chem. Ges.*, **45**, 2902 (1912). Schryver, *Proc. Roy. Soc.*, **82** B, 226 (1910). Angelico and Catalano, *Gaz. chim. ital.*, **43**, 38 (1913). Priestley and Armstead. *New Phytologist*, **21**, 62 (1922).

[64] Spoehr, *Biochem. Zeit.*, **57**, 95 (1913).

[65] Neuberg, *ibid.*, **13**, 305 (1908); **29**, 279 (1910).

[66] Volmar, *Compt. rend.*, **176**, 742 (1923). Benrath, "Photochemie" (1912), p. 181. Rosenthaler, *Arch. Pharm.*, **251**, 587 (1914).

[67] Fincke, *Biochem. Zeit.*, **52**, 214 (1923); **51**, 253 (1913). Curtius and Franzen, *Ann. Chem.*, **404**, 93, 165 (1914).

[68] Denigés, *Compt. rend.*, **150**, 529 (1910); *Jour. Pharm. Chim.* (6), **4**, 193 (1896).

[69] Grosse-Bohle, *Zeit. Nahr.-Genussm.*, **14**, 88 (1907).

[70] Sabalitschka and Riesenberg, *Biochem. Zeit.*, **145**, 377 (1924).

were unable to detect any formaldehyde in the distillate from the juice of leaves by means of the phloroglucin reaction.

Curtius and Franzen substantiated the fact that the reagent used by Fincke gives only a temporary coloration with other aldehydes and that the aldehydes now known to be present in leaves give no reaction. The reagent is prepared by dissolving one gram of rosaniline hydrochloride or acetate in 500 cc. of water and adding to this solution 25 grams sodium sulfite and 15 cc. of hydrochloric acid (specific gravity 1.124); the whole is then diluted to one liter. The solution is slowly decolorized and can be used after a few hours. In pure solution formaldehyde can be detected in concentration of 1:500,000, but in the colored extracts of leaves 1:200,000 is the limit. The tests are carried out by adding 1 to 2 cc. of hydrochloric acid (specific gravity 1.124) to 10 cc. of the solution to be tested and then 1 cc. of the rosaniline reagent. Formaldehyde produces a blue to red violet coloration. With dilute solutions several hours are required for the development of the color, sometimes not reaching maximum intensity until after 20 hours. Willstätter and Stoll [71] have studied the conditions essential for the detection of small quantities of formaldehyde by means of the fuchsin test. They recommend a 1 per cent solution of parafuchsine or fuchsine which is treated with sulfur dioxide until 0.1 cc. of the solution when diluted to 10 cc. does not develop a color. Care must be exercised in using this reagent, for too great dilution, removal or neutralization of the sulfurous acid cause the formation of a colored solution which may erroneously be taken as a test for aldehyde. They employed the reagent by adding two drops of concentrated hydrochloric acid to 10 cc. of the solution to be tested and then 4 drops of the fuchsine reagent. The presence of the hydrochloric acid greatly retards the rate of reaction, requiring often a whole day, but the reagent is very sensitive.

The tests for formaldehyde of Leach, Rimini, Schryver and Bono have been subjected to a critical study by Salkowski.[72] Fosse and Hieulle [73] report that the color obtained by the Schryver test with formaldehyde is also produced by glyoxylic acid.

b. Other Intermediate Products.

Glycollic aldehyde has been suggested by several authors as a possible intermediate product in the reduction of carbon dioxide, but the evidence of its presence in leaves is very limited. Mazé [74] distilled various kinds of leaves under reduced pressure and reports obtaining glycollic aldehyde from the elder. He obtained no formaldehyde, and besides glycollic aldehyde, ethyl alcohol and acetaldehyde, he reports the presence of sub-

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[71] Willstätter and Stoll, "Unters. ü. Assim. Kohlensäure," p. 387.

[72] Salkowski, *Biochem. Zeit.*, **68**, 337 (1915).

[73] Fosse and Hieulle, *Compt. rend.*, **179**, 636 (1924). Larnal, *Bull. acad. roy. Méd. Belg.*, **27**, 701 (1913).

[74] Mazé, *Compt. rend.*, **171**, 1391 (1920).

stances to which he ascribed the formula $CH_3 . CHOH . CO . CH_3$ and $CH_3 . CHOH . CHO$. Rouge[75] has also endeavored to find support for the glycollic aldehyde theory. He also failed to find the slightest trace of formaldehyde, but by means of p-nitrophenylhydrazine obtained very small amounts of the p-nitrophenylhydrazone of glycollic aldehyde from different species of leaves. However, too much value cannot be placed upon any of this evidence as supporting the theory of glycollic aldehyde as an intermediate product in photosynthesis. In mixtures, color reactions are often deceiving and a few hundredths of a gram of a hydrazone also do not offer very conclusive evidence.

Glyoxylic acid, $COOH . CH : O$, has also been suggested as an intermediate product. Its presence in plants has been reported by several.[76] Whether this compound is actually associated with photosynthesis has, however, not been established. The same applies to the presence of glycollic acid which has been found in a wide variety of plants, both in the leaves and fruits.[77]

The presence of formic acid in leaves has also not been definitely established and there exist many contradictory reports. A question of importance in this regard is whether in those cases in which formic acid has been found, it was originally in the leaves or was formed from other substances through the process of distillation. In the older literature[78] there occur frequent references to the presence of formic acid, but its occurrence has since been questioned, especially in leaves, by Fincke.[79]

Of the presence of oxalic acid in leaves there exists very little doubt. Franzen[80] and his collaborators have made a very careful critical examination of all the publications in which malic, citric, tartaric and succinic acids are reported as constituents of plants. It is concluded that in a relatively small percentage of cases is the evidence which has been presented to show the presence of these acids, sufficient to warrant the conclusions.

An interesting substance has been isolated by Curtius and Franzen[81] by the distillation with steam of macerated leaves. After removal of the acids in the distillate, they were able to isolate α-β-hexylene aldehyde, $CH_3 . CH_2 . CH_2 . CH : CH . CHO$. This aldehyde gives many of the reactions of formaldehyde and in all probability in some cases it has been

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[75] Rouge, *C. A.*, **15**, 2294 (1921).

[76] Brunner and Chuard, *Ber. chem. Ges.*, **19**, 595 (1886). Lippmann, *ibid.*, **24**, 3299 (1891). Talladin, *Bull. acad. sci. Petrograd* (1916), 1021.

[77] Gorup-Besanez, *Ann. Chem.*, **161**, 229 (1872). Shorey, *Jour. Am. Chem. Soc.*, **21**, 45 (1899). Stolle, *Chem. Zent.* (1900), II, 343. Ordonneau, *Bull. soc. Chim.* (3), **6**, 261 (1891).

[78] Bergmann, *Bot. Zeitg.*, **40**, 731 (1882).

[79] Fincke, *Biochem. Zeit.*, **51**, 253 (1913); *Unters. Nahr. Genussm.*, **27**, 10 (1914).

[80] Franzen and Keyssner, *Biochem. Zeit.*, **135**, 183 (1923). Franzen and Helwert, *ibid.*, **135**, 384 (1923); **136**, 291 (1923). Franzen and Ostertag, *ibid.*, **136**, 327 (1923).

[81] Curtius and Franzen, *Ann. Chem.*, **404**, 93 (1914).

erroneously taken for formaldehyde. It is very doubtful, however, whether this compound is in any way associated with photosynthesis, though it is apparently widely distributed.

3. Feeding Experiments

Another line of reasoning which has been followed in endeavoring to determine the nature of the intermediate products of photosynthesis is the feeding to the plant of such substances which are supposed to constitute intermediate products, and to determine whether such feeding results in an increase in dry weight, of starch or of sugar by the plant. It is essentially a physiological method and experiments along this line have been carried out for a very long time. No decisive results have been obtained by the use of this method nor is it, in fact, to be expected that there would be. The difficulties associated with the use of this method are of a very fundamental nature. In the normal metabolism of an organism, and there is no reason why photosynthesis should form an exception, the products which constitute the intermediate steps in these activities bear a rather definite relation to the needs or capacity for the further transformation of such products. That is, the amount and concentration of the intermediate products in the normal course of the processes are adjusted to the capacity of the organism to utilize or dispose of them. An undue accumulation usually results in rather deep seated disturbances of all the processes of the organism. There is, moreover, good reason for believing that some reactions are carried out in localized areas within the cells; whether this is due to localization of enzymes, to differences in the pH or to other facts it is unnecessary to dwell on here. That such localizations play a rôle in photosynthesis the very nature of the chloroplasts would indicate. Finally, the *rate* at which the intermediate products are supplied to the centers where they are converted, undoubtedly plays a very important rôle in maintaining the normal course of the reaction. To these circumstances may be added that it is conceivable that in a complex series of reactions, such as photosynthesis, we are probably not dealing with a single intermediate product, but there exists the possibility that several such products are essential for the reaction, and increasing the concentration of only one may be of little influence on the total reaction or may, in fact, inhibit it.

In view of these considerations the feeding to a plant of a substance which according to theoretical views may constitute an intermediate product of photosynthesis has little chance of leading to very definite conclusions. The difference between the two conditions of the natural formation on the one hand and the artificial feeding on the other becomes evident in the case of formaldehyde. In the normal process, from all we know, at least one important step takes place in the chloroplasts in the cells, and let us assume that here formaldehyde is formed. From the fact that there have been no reliable tests obtained from illuminated leaves, or only the slightest traces, it can safely be assumed that this substance does not accumulate to any extent. Compare to this the method of bathing the entire plant (in case of an aquatic plant) in a formaldehyde solution or exposing a leaf, with its delicate stomatal mechanism, to gaseous formaldehyde, a notoriously poisonous substance, and the difference in the two sets of conditions must be evident. Consequently too much is not to be expected from the physiological experiments of this nature, for on the one hand, negative results with a certain substance may simply mean that it has not been supplied at the proper concentration, that it does not penetrate to the proper locality in the cell or that some other accessory factor has not been supplied, etc., while a positive result, on the other hand, may still mean very little toward establishing the substance fed as an intermediate product, for we know of a variety of substances from which the plant is capable of producing sugar and starch, but which for obvious chemical reasons we do not consider intermediate products of photosynthesis.

a. Formaldehyde.

Feeding experiments with formaldehyde have been carried out for a great many years and much has been written regarding the results obtained and the methods which must be followed.[82] The poisonous property of formaldehyde was recognized as a factor to be guarded against, wherefore compounds of formaldehyde were first used which in water solution produced only very small concentrations of this substance. Thus methylal, $CH_2(OCH_3)_2$, was at first used, though it was later realized that the methyl alcohol, which is simultaneously produced with formaldehyde from methylal, is capable of producing starch in *spirogyra*.

Similarly, the addition product which formaldehyde forms with sodium bisulfite was used.[83] The slight increases in weight and the decrease in reducing power of the solution in which the plants were placed are not satisfactory criteria from which a utilization of formaldehyde can be concluded.

Positive results under these conditions and by using pure formaldehyde solutions were claimed for algae when exposed to the light, but not when in the dark. The decrease in the reducing power of the formaldehyde-sodium sulfite solution may well have been due to oxidation of the formaldehyde. Schroeder[84] also considers that an increase in weight may be ascribed to the action of bacteria which may convert the formaldehyde into other organic compounds which the algae are capable of assimilating.

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[82] Bokorny, "Studien u. Experimente ü. den chemischen Vorgang der Assimilation," Erlangen, 1888. *Ber. bot. Ges.*, **6**, 116 (1888). Loew and Bokorny, *Jour. prakt. Chem.*, **36**, 285 (1887). Bouilhac, *Compt. rend.*, **133**, 751 (1901).

[83] Bokorny, *Ber. bot. Ges.*, **9**, 103 (1891); *Landw. Jahrb.*, **21**, 445 (1892); *Arch. f. Hyg.*, **14**, 202 (1892); *Pflüger's Arch.*, **125**, 474 (1908).

[84] Schroeder, "Die Hypothesen ü. die Chemische Vorgänge bei der Kohlensäure-Assimilation," Jena, 1917, p. 116

Later Bokorny [85] reported the formation of starch from a 0.001 per cent formaldehyde solution, but it is not absolutely certain from these experiments whether the starch was actually formed from the formaldehyde or from sugar already present in the plants.[86] Treboux [87] found that 0.005 per cent formaldehyde was poisonous to *Elodea,* while in a 0.0005 to 0.001 per cent solution the plants remained uninjured. However, Treboux could not detect any starch formation from formaldehyde in the dark nor in the light in absence of carbon dioxide. That these very dilute solutions of formaldehyde had not affected the photosynthetic apparatus is proven by the fact that when the plants were exposed to sunlight and carbon dioxide was added to the solution, starch appeared in the chloroplast within 1-2 hours.

An advance in the method of studying the problem was made by Grafe,[88] who used land plants, supplied the formaldehyde in gaseous form and protected the roots. Under these conditions formaldehyde could be tolerated to about 0.04 per cent in the air when the plant was illuminated, but toxic effects became noticeable in the dark and in the colorless portions of the plants. Definite conclusions as to whether formaldehyde is used by the plants cannot be drawn from these experiments. An increase in the size and development of the formaldehyde cultures in the light as compared with the controls free from carbon dioxide was probably the main result. Another interesting observation was that the formaldehyde cultures contained no starch but a great deal more of the reducing sugars than the plants grown normally.

Miss Baker [89] studied the effect of formaldehyde on the development of seedlings in the light, i.e. from the germination of the seed through a development of 22 to 28 days. She found that in the cultures in light there was a smaller decrease in the dry weight of the seedlings when formaldehyde was present than without this gas. She speaks of this as an "increase in dry weight"; it is questionable whether this is justifiable. In the formaldehyde cultures there was never a gain in dry weight over that of the original weight of the seed. This occurred only in the presence of carbon dioxide. This becomes clearer from the following example of one experiment. The seeds used were White Mustard, temperature 14 to 25°, "amounts of formaldehyde in air, about 0.52 per cent, or about fifteen times as much potential carbon as is present in normal air of 0.04 per cent of carbon dioxide." The duration of the experiment was 24 days:

Atmosphere	Orig. Dry Weight	Final Dry Weight	Final/Orig. Weight
Air — CO_2 + CH_2O	0.4015 gram	0.3585 gram	89.3 per cent
Air — CO_2	0.3985	0.2590	65.0
Air + CO_2	0.3965	0.4035	102.0

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[85] Bokorny, *Biochem. Zeit.*, **36**, 92 (1911).

[86] Bouilhac, *Compt. rend.*, **135**, 1369 (1902).

[87] Treboux, *Flora.*, **92**, 73 (1903).

[88] Grafe and Viser, *Ber. bot. Ges.*, **27**, 431 (1909); **29**, 19 (1911).

[89] Baker, *Ann. Bot.*, **27**, 411 (1913).

The only cultures which showed a final increase in dry weight in the light were those containing carbon dioxide; where formaldehyde was present the loss in final weight was less than without. Miss Baker showed that formaldehyde had no effect on the respiration of the seedlings in the dark, so that in all probability the smaller loss in dry weight in the presence of formaldehyde cannot be ascribed to a retarded rate of respiration. It is significant that in the dark formaldehyde does not show this effect of a smaller loss of dry weight. The question arises whether in the experiments of Baker, Grafe and others,[90] in which there appears to be a utilization of a small amount of formaldehyde in the light, these cannot be interpreted on the basis that the formaldehyde is oxidized in the light. Spoehr[91] found that formaldehyde vapor is oxidized to formic acid by air in sunlight. In the experiments just referred to, were the plants actually utilizing formaldehyde or was it formic acid, and is the fact that formaldehyde is less toxic in light to be explained on the basis that in the light it is partially oxidized?

More recently Jacoby has studied the effect of gaseous formaldehyde on nasturtium leaves in the dark. He took two sets of leaves, one exposed to an atmosphere of formaldehyde, the other in air; after 24.5 to 32 hours the dry weight was determined and in six experiments the formaldehyde cultures had a dry weight 1.7 to 5.4 per cent higher than the controls. In a later paper Jacoby[92] reports experiments in which oxygen was removed from the atmosphere containing the formaldehyde and here also he concludes that the formaldehyde was fixed by the leaves. Sabalitschka and Riesenberg[93] have also reported an increase in sugar and starch in leaves which were exposed to an atmosphere containing formaldehyde in the dark.

Contradictory and uncertain as much of the work on the feeding of formaldehyde is, it seems to indicate that small amounts can be utilized by the plant. The fact that it is so injurious to protoplasm naturally excludes the tolerance of high concentrations. Whether the formaldehyde, if formed as an intermediate product in photosynthesis, is combined with some other substance and thus is less toxic, or in this form undergoes condensation to sugars more easily, it is of course impossible to say. There is, however, no chemical evidence which would support such an opinion. The fact that formaldehyde may be used by the plant to form sugars may be regarded only as supplementary evidence for the formaldehyde theory, for there are many other substances from which the plant can manufacture sugar, and apparently far more easily than from formaldehyde.

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[90] Ewart, *Proc. Roy. Soc.*, **80** B, 30 (1908). Usher and Priestley, *ibid.*, **78** B, 321 (1906).

[91] Spoehr, *Plant World*, **19**, 15 (1916).

[92] Jacoby, *Biochem. Zeit.*, **101**, 1 (1919-1920); **128**, 119 (1922).

[93] Sabalitschka and Riesenberg, *ibid.*, **144**, 545 (1924). Boitreux, *Compt. rend. soc. biol.*, **83**, 737 (1920).

b. Other Substances From Which Sugar Can Be Produced by the Plant.

Reference has already been made to the fact that carbon monoxide cannot replace carbon dioxide in photosynthesis and is toxic to plants in higher concentrations. On the other hand, this gas has been found in quite high concentrations in the floaters of certain sea weeds, so that its toxicity is probably not as general as was at first concluded. In the earlier forms of the formaldehyde theory, carbon monoxide was assumed to be an intermediate product; for later modifications of the theory the behavior of carbon monoxide is, however, of little significance.

The results of studies of the nutritive value of carboxylic acids for plants in the light are of little value, because of the fact that most of these acids under the influence of light and mineral nutrients (notably iron) and through the action of micro-organisms are broken down into carbon dioxide and other organic compounds.[94] Thus it is not certain whether the plant is not forming carbohydrates from the carbon dioxide present, through the normal photosynthetic process rather than through the utilization of a carboxylic acid.

Treboux [95] has made an interesting study of the utilization of various organic acids by algae. He used 40 different species of algae and the gain in dry weight of the plants was determined after they had been in solutions of the ammonium and potassium salts of different organic acids in the dark for one to two months. About one-half of the plants used, developed with the organic acids as the only source of carbon. It was found that the organic acids with a long chain of carbon atoms were not as good nutrients as the relatively simple acetic acid. The optimal concentration of the potassium salts was about 0.25 per cent. The acids which could be used in this manner were acetic, lactic, citric and butyric acids; some of these also form starch. The amino acids glycocoll, alanine, leucin, asparagin and aspartic acid are apparently less favorable sources of carbon, while formic, oxalic, propionic, valeric, succinic, malic and tartaric acids are doubtful sources. Bokorny [96] has prepared a list of the acids that can be used by the plant, though some of those included are of questionable value. Different species of plants apparently vary greatly in their ability to utilize different organic compounds and there exists still a great lack of agreement on many of these questions.

Other substances which have been reported as capable of being used by plants for starch formation are methyl alcohol, glycol, phenol, glycerol and urea.[97] In considering intermediate products of photosynthesis these substances are not of very great interest beyond demonstrating the fact

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[94] Stutzer, *Ber. chem. Ges.*, **9**, 1395 (1876); *Landw. Versuchs.*, **21**, 104 (1878). Schmöger, *Ber. chem. Ges.*, **12**, 753 (1879). Spoehr, *Biochem. Zeit.*, **57**, 95 (1913).

[95] Treboux, *Ber. bot. Ges.*, **23**, 432 (1905).

[96] Bokorny, *Biol. Centralbl.*, **17**, 5 (1897). Loew and Bokorny, *Jour. Prakt. Chem.*, **144**, 272 (1887).

[97] Lippmann, "Chemie der Zuckerarten," II, 1763 (1904).

that the plant is capable of forming starch from a variety of substances. The formation of starch in itself is probably not a very good criterion to follow in endeavoring to judge as to possible intermediate products of photosynthesis. Starch formation occurs only when the sugar concentration has attained a sufficiently high value, and the enzymes which are active in starch synthesis are affected by a wide variety of substances and conditions which do not influence photosynthesis.

If, finally, we ask how much have these feeding experiments contributed to establishing or disproving the formaldehyde theory of photosynthesis, we are forced to the conclusion that it has been very little. There are other substances from which the plant can also produce starch and much more easily than from formaldehyde. Here it must be remembered also that the attempts to establish the value of formaldehyde as a substance which the plant can use have been more numerous and persistent than with any other substance. The first results with formaldehyde were also negative. Doubtless there are other substances, which if studied with the care used with formaldehyde, would also yield positive results. Probably as much as can safely be concluded is that these feeding experiments indicate that formaldehyde as a possible intermediate product need not be excluded. Accepting the positive results, these still do not prove that formaldehyde is the intermediate product in the reduction of carbon dioxide and water to carbohydrates. It should be mentioned here that the ability to form carbohydrates from formaldehyde is not confined to the chlorophyllous cells; the liver of some animals can convert formaldehyde to glycogen.[98] A definite proof of the formaldehyde theory is certainly not afforded by any of the feeding experiments.

The same may be said of the feeding experiments with organic acids, although their tolerance by the plant is notably higher than that of formaldehyde, and organic acids have long been recognized as components of plants, though their value as starch producing substances is largely confined to algae and very little is known of the manner in which this is accomplished. The feeding experiments with organic acids also give little support to the theory of Liebig.

4. The Reduction of Carbon Dioxide

a. The Direct Reduction of Carbon Dioxide.

Many different means have been employed to effect a reduction of carbon dioxide. While most of these are very different from any reaction that could take place in a living plant, and have therefore little direct bearing on the photosynthesis problem, they, nevertheless, are of interest in this connection and have thrown considerable light on the behavior of carbon dioxide. From the chemical viewpoint and especially

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[98] Grube, *Pflüger's Arch.*, **121**, 636 (1908).

from that of energetics the reduction of carbon dioxide is by far the most important and difficult step in photosynthesis. The reduction of carbon dioxide to the energy level of carbon or what is perhaps more accurate, the reduction of carbonic acid to the energy level of carbohydrates, requires a great deal of energy. As to the means by which this is accomplished in the plant we have but a few clews. We know that it takes place in the visible portion of the spectrum, that chlorophyll, water and oxygen are essential and that the action is dependent upon conditions necessary for the maintenance of the life activities of the living organism, such as a moderate temperature and the presence of certain inorganic salts. In what manner the light acts and just what the relations are to the other activities of the living cell are largely matters of conjecture. All information regarding analogous reactions are therefore not without interest to the problem of photosynthesis and may contribute directly or indirectly to a clearer understanding of the phenomenon.

The reduction of carbonic acid to formic acid has been accomplished by various means. Lieben [99] and Ballo [100] were able to do this with sodium and other amalgams and Moisson [101] by the action of potassium hydride on carbon dioxide obtained potassium formate.

Wieland [102] showed that in the presence of palladium, carbon monoxide can be oxidized in the absence of oxygen according to $CO + H_2O = CO_2 + H_2$. Bredig and Carter [103] were able to reverse this reaction, so that under certain conditions they obtained 100 per cent formic acid. This required high pressures of hydrogen (50 atm.) and of carbon dioxide (20 atm.) and the presence of some inorganic carbonate, besides the palladium. They used $KHCO_3$, Na_2CO_3, $CaCO_3$, and $Na_4B_4O_7$ and found that the yield of formic acid with sodium carbonate was much lower than with potassium bicarbonate, the velocity of formic acid formation depending apparently not only on the partial pressure of the hydrogen, but also on that of the carbon dioxide and on the undissociated molecule of the base. The catalyst, palladium, is also essential to the reaction. The determination of the equilibrium, $CO_2 + H_2 \rightleftarrows CO + H_2O \rightleftarrows HCO_2H$ gave no indication of the formation of formaldehyde. Lieben also concluded that bicarbonates are most easily reduced while carbonates are reduced with difficulty, and free carbon dioxide is reduced by sodium amalgam only in traces. It is apparently the HCO_3^- ion which is most easily attacked. The amount of hydrate which is formed when carbon dioxide is dissolved in water is small. The proportion of dissolved CO_2 which is in the form of H_2CO_3 varies with dilution. Carbonic acid, which may be considered as hydroxy formic acid, is accord-

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[99] Lieben, *Monatsh. Chem.*, **16**, 211 (1895); **18**, 582 (1897); **19**, 333 (1898).

[100] Ballo, *Ber. chem. Ges.*, **17**, 7 (1884).

[101] Moisson, *Compt. rend.*, **134**, 18, 261 (1902); **136**, 723 (1903).

[102] Wieland, *Ber. chem. Ges.*, **45**, 679 (1912); **46**, 3327 (1913).

[103] Bredig and Carter, *ibid.*, **47**, 541 (1914); *Zeit. elektrochem.*, **20**, 489 (1914); *Chem. Ztg.*, **39**, 72 (1915).

ing to Thiel and Strohecker[104] a strong acid. In what form the carbon dioxide is used in photosynthesis, whether it is dissolved CO_2, HCO_3^-, or CO_3^- has received little attention. Considering the energetics of the reaction, this is a question of considerable importance. There is some evidence (Wilke)[105] that in water solution carbon dioxide also forms a higher hydrate, $C(OH)_4$, which is a very weak acid. Schaum[106] in a theoretical consideration of the mechanism of photosynthesis assumes the formation of $C(OH)_4$, which then undergoes a rearrangement with the formation of a peroxide, the latter, through the action of catalase, analogous to the conception of Willstätter and Stoll, breaking down to formaldehyde, water and oxygen:

$$C(OH)_4 \leftrightarrows H_2C(OOH)_2 \rightarrow H_2CO + H_2O + O_2.$$

Schaper[107] studied the action of carbon dioxide on ferro oxalate. While at ordinary pressures carbon dioxide does not oxidize the complex $K_2Fe(C_2O_4)_2$, at pressures of 40-60 atmospheres about 10 per cent oxidation was observed. At ordinary pressures and saturation of potassium oxalate Schaper calculates that equilibrium would be attained when 0.2 per cent of the ferro complex had been oxidized.

A direct reduction of carbon dioxide in water solution to formaldehyde was accomplished by Fenton[108] by the use of metallic magnesium. The presence of weak alkalies greatly favors the amount of reduction, due probably to bicarbonate formation. Formic acid is also reduced in this manner to formaldehyde. That a similar rôle should be ascribed to the magnesium which constitutes part of the chlorophyll molecule in the photosynthetic process there is no reason for believing.

Many attempts have also been made to reduce carbon dioxide under conditions which more nearly simulated the process of photosynthesis than is the case in the reactions with metallic magnesium or atomic hydrogen. Thus Losanitsch and Jovitschitsch,[109] Berthelot[110] and especially W. Löb[111] have studied the effect of the silent electric discharge on mixtures of carbon dioxide and water. By this means a variety of products was obtained; among these were formic acid and formaldehyde. The following reactions are involved: $2CO_2 = 2CO + O_2$; $CO + H_2O = HCOOH$; $CO + H_2O = CO_2 + H_2$; $3O_2 = 2O_3$; $2H_2 + 3O_2 = 2H_2O_2 + O_2$; $H_2 +$

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[104] Thiel, *Ber. chem. Ges.*, **46**, 241 (1913). Thiel and Strohecker, *ibid.*, **47**, 945 (1914). Vörlander and Straube, *ibid.*, **46**, 172 (1913).

[105] Wilke, *Chem. Ztg.*, **39**, 309 (1915). Walker, *Jour. Chem. Soc.*, **77**, 1 (1900). Schipley and McHaffie, *Jour. Soc. Chem. Ind.*, **42**, 319, 321 (1923). Kolthoff, *Zeit. Nahr. Genussm.*, **43**, 184 (1922).

[106] Schaum, *Ber. chem. Ges.*, **51**, 1372 (1918). Pusch, *Zeit. Elektrochem.*, **22**, 206 (1916).

[107] Schaper, *Zeit. physik. Chem.*, **72**, 320 (1910).

[108] Fenton, *Jour. Chem. Soc.*, **91**, 687 (1907).

[109] Losanitsch and Jovitschitsch, *Ber. chem. Ges.*, **30**, 135 (1897).

[110] Berthelot, *Compt. rend.*, **126**, 610 (1898); **131**, 772 (1900).

[111] Löb, *Landw. Jahrb.*, **35**, 541 (1906); *Zeit. Elektrochem.*, **11**, 745 (1906); **12**, 282 (1906). Holt, *Jour. chem. Soc.*, **95**, 30 (1909). Gibson, *Ann. Bot.*, **22**, 118 (1908).

$CO = H_2CO$. The final products obtained depend very much upon the amount of water vapor present. Löb was also able to obtain glycollic aldehyde from carbon monoxide and hydrogen.

An electrolytic reduction of carbon dioxide under pressures of 10 to 15 atmospheres was also accomplished by Fischer and Priziza;[112] under these conditions formic acid with traces of methyl alcohol were obtained. Though it has also been claimed that carbon dioxide is reduced to formaldehyde by alpha and beta particles, recent investigations of Lind and Bardwell[113] show that carbon dioxide is not decomposed chemically by alpha-radiation.

Lunt[114] was not able to detect any formaldehyde or formic acid in mixtures of carbon dioxide and hydrogen in the corona due to alternating currents of high frequency.

With the development of the quartz mercury vapor lamp a convenient source of light of short wave length became available.[115] These lamps have been made in many different designs for laboratory work so that ultra-violet light of high intensity could be used for a variety of experimental work. As carbon dioxide has no absorption bands in the visible portions of the normal spectrum but does show absorption in the ultra-violet, and as it is one of the fundamental principles of photochemistry that there can be no photochemical action without absorption of light, it seemed but natural to try the effect of ultra-violet light on carbon dioxide.

That water is decomposed by ultra-violet light was reported by Kernbaum[116] who obtained hydrogen and hydrogen peroxide on illuminating water for ten hours. The formation of hydrogen peroxide from water and oxygen in ultra-violet light was also observed by Thiele.[117] Coehn[118] found that hydrogen and oxygen unite to form water in ultra-violet light and that the latter is also decomposed into detonating gas by the light. There is thus attained an apparent equilibrium which is not altered by a change of temperature of 150° to 800°. Under the conditions of Coehn's experiment the decomposition of water vapor is 0.00087 to 0.0010 per cent; this corresponds to the thermal equilibrium at about 1250°. Weigert[119] has made the following calculation in order to give an idea of the magnitude of the factors involved in these reactions. For the photochemical decomposition of liquid water into gaseous hydrogen and oxygen, which have an equilibrium pressure at 17° of 3.4 and 1.7×10^{-28}

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[112] Fischer and Priziza, *Ber. chem. Ges.*, **47**, 256 (1914).

[113] Stoklasa, Sebor, Zdobnicky, *Compt. rend.*, **156**, 646 (1913). Usher and Priestley, *Proc. Roy. Soc.*, **84** B, 102 (1912). Lind, "The Chemical Effects of Alpha Particles and Electrons," Chemical Catalog Co., 1921, pp. 85, 93, 121. Lind and Bardwell, *Jour. Amer. Chem. Soc.*, **47**, 2675 (1925).

[114] Lunt, *Proc. Roy. Soc.*, **108** A, 172 (1925). Moset and Isgarischew, *Zeit. Elektrochem.*, **16**, 613 (1910).

[115] Ellis and Wells, "The Chemical Action of Ultraviolet Rays," The Chemical Catalog Co., 1925, p. 30.

[116] Kernbaum, *Compt. rend.*, **149**, 273 (1909).

[117] Thiele, *Zeit. angew. Chem.*, **22**, 2472 (1909).

[118] Coehn, *Ber. chem. Ges.*, **43**, 880 (1910).

[119] Weigert, "Die Chemische Wirkung des Lichts," Stuttgart, 1911, p. 19.

atmospheres, 1.62 cal. of the radiant energy would have to be fixed as chemical energy in order that the first cubic millimeter of gas were formed. With the electric arcs not more than 0.1 per cent of the electric energy can be converted into chemical energy in this manner. With an illumination surface of one square millimeter and an arc lamp of 50 volts and 10 amperes, at a distance of 5 cm., the first gas bubble from a cubic millimeter of water would appear in about two minutes.

That dry carbon dioxide is decomposed by ultra-violet light was shown by Herchefinkel[120] who illuminated this gas over mercury and obtained carbon monoxide and the yellow oxide of mercury.

In 1910 D. Berthelot and Gaudechon[121] endeavored to test the old ideas of M. Berthelot, published almost half a century before, that photosynthesis consisted in a decomposition of carbon dioxide into carbon monoxide and oxygen, and that the former was reduced by hydrogen formed by the decomposition of water into hydrogen and oxygen, formaldehyde being formed. They found that in ultra-violet light carbon dioxide is decomposed into carbon monoxide and oxygen, especially if there is a substance present to react with the oxygen formed, as phosphorus or hydrogen. Carbon monoxide also reacts with hydrogen to form formaldehyde. As water is decomposed in ultra-violet light into hydrogen and oxygen, they were able to obtain formaldehyde from carbon monoxide and water. The formaldehyde polymerized rapidly in ultra-violet light. They were not able to obtain formaldehyde directly from carbon dioxide and water, only from carbon dioxide in the presence of hydrogen. Usher and Priestley[122] then reported that they were able to obtain tests for formaldehyde by exposing quartz tubes containing water and carbon dioxide to ultra-violet light. It is not without interest that when they used open dishes containing water and placed these immediately beneath the lamp, they also obtained tests for formaldehyde even when no carbon dioxide was bubbled through the water. This formaldehyde must have originated from the action of the ultra-violet light on the dust or other impurities which got into the water or from atmospheric carbon dioxide. The latter must have been very small, while it is well known that there are a great many substances which form formaldehyde in ultra-violet light.

Immediately following these publications appeared those of Stoklasa[123] and his collaborators. The results of these authors are again at variance with those of Berthelot and Gaudechon and of Usher and Priestley. Stoklasa concluded: that by the action of ultra-violet light on water and carbon dioxide in the absence of potassium hydroxide neither formaldehyde nor carbohydrates are formed. Formaldehyde is formed from carbon dioxide by the action of ultra-violet light only when there is present hydro-

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[120] Herchefinkel, *Compt. rend.*, **149**, 395 (1909).

[121] Berthelot and Gaudechon, *Compt. rend.*, **150**, 1690 (1910).

[122] Usher and Priestley, *Proc. Roy. Soc.*, **84** B, 101 (1911).

[123] Stoklasa and Zdobnicky, *Biochem. Zeit.*, **30**, 432 (1911). Stoklasa, Sebor and Zdobnicky, *ibid.*, **41**, 333 (1912); **54**, 330 (1913). Löb, *ibid.*, **48**, 257 (1913).

gen in the "nascent" condition and potassium hydroxide. The presence of potassium hydroxide is necessary, according to these authors, for the formation of potassium bicarbonate which is reduced, rather than the carbon dioxide. They seem to regard the condition of the potassium bicarbonate ("in Entstehung begriffen") as well as the "nascent" state of the hydrogen as essential to the formation of formaldehyde. The applications which are made of these conclusions to the photosynthetic process are somewhat fanciful especially because of the "nascent" hydrogen required. In a later publication Stoklasa alters his scheme to describe the reactions of photosynthesis. He points to the fact that an alkaline solution of formaldehyde is oxidized to formic acid and the latter in ultraviolet light is oxidized to carbon dioxide. This he regards as the reversal of the photosynthetic reactions. The latter he consider progresses as follows:

$$K_2CO_3 + CO_2 + H_2O = 2KHCO_3$$

$$2KHCO_3 + \text{Light} = K_2CO_3 + HCOOH + O$$

$$HCOOH + \text{Light} = HCOH + O$$

$$n(HCOH) = (HCOH)n.$$

Stoklasa thus considers that formic acid is an intermediate step in the reduction of carbonic acid (i.e., potassium bicarbonate) to formaldehyde. It is important to note, however, that Stoklasa gives no experiments to show that formic acid on illumination with ultra-violet light yields formaldehyde. Stoklasa also reports that by illuminating potassium bicarbonate he obtained a hexose. Into 300 cc. of a 7 per cent of potassium hydroxide solution was run a stream of carbon dioxide (the source of the carbon dioxide is not given) and to the solution was added 80 cc. of a 15 per cent solution of ferrous sulfate, while the whole was illuminated for 8 hours. After precipitating the inorganic salts with potassium phosphate and alcohol he obtained an alcoholic extract containing 0.219 gram of reducing substance giving a phenylosazone melting at 204-205° and analyzing for a hexosazone.

Recently Baly, Heilbron and Barker [124] claim to have obtained a direct synthesis of formaldehyde from carbon dioxide and water by the use of ultra-violet light. Their results are more analogous to those of Usher and Priestley than to those of Berthelot and Gaudechon or of Stoklasa in that they do not use hydrogen, which is considered essential for the formation of formaldehyde by the three last mentioned. In the opinion of Baly and his collaborators the formation of formaldehyde takes place in light of very short wave length ($\lambda = 220\ \mu\mu$) and the polymerization of formaldehyde in light of longer wave length ($\lambda = 290\ \mu\mu$). The quantity of formaldehyde formed from carbon dioxide is, according to

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[124] Baly, Heilbron and Barker, *Jour. Chem. Soc.*, **119**, 1025 (1921). *Nature*, **112**, 323 (1923); *Jour. Soc. Chem. Ind.*, **40**, 377 (1921); *Ind. and Eng. Chem.*, **16**, 1018 (1924).

Baly, very small, two to eight parts per million, though this can be increased if calcium or potassium bicarbonate is present. It is asserted that when the solution of carbon dioxide is not agitated the formaldehyde produced is so rapidly polymerized to carbohydrates that no tests for formaldehyde can be obtained. If the solution is agitated some of the formaldehyde is carried to the back of the vessel where it escapes polymerization. Baly reports having obtained tests with Benedict solution which indicate the presence of about 0.005 per cent reducing sugar. In some experiments Baly used various organic compounds to protect the formaldehyde "from those specific rays which cause polymerization." For this purpose he used paraldehyde and sodium phenoxide as well as colored compounds as malachite green and methyl orange. It is extremely doubtful whether these substances when illuminated with ultra-violet light do not themselves form formaldehyde or compounds which give reactions which may be mistaken for formaldehyde. The same applies to the experiments in which a solution containing sodium carbonate, sodium citrate and copper sulfate (Benedict solution) was illuminated and reduction of the copper observed.[125] That cupro-alkaline solutions such as Fehling's solution are decomposed by light has been known for a long time and there is little reason to believe that the same does not apply to Benedict's solution.

There is unfortunately very little agreement in the results of various experimenters on the reduction of carbon dioxide in ultra-violet light. Berthelot and Gaudechon obtained formaldehyde only in the presence of hydrogen, Stoklasa only when the hydrogen was in "statu mascendi," Usher and Priestley obtained formaldehyde "in a polymerized form" and Baly and his collaborators claim formaldehyde and sugar. With these must be included the experiments in which only negative results were obtained.

Spoehr[126] was unable to obtain any definite evidence of the formation of formaldehyde from carbon dioxide in several different series of experiments. Quartz mercury vapor lamps of several makes were used, the temperature, pressure of carbon dioxide, distance from the source of ultra-violet light and duration of illumination were all varied. Stoklasa's experiments were repeated using hydrogen prepared electrolytically. No definite test for formaldehyde was ever obtained. Small amounts of formic acid were formed and when formic acid was illuminated with ultra-violet light a small amount of a non-volatile sirup was obtained. This reduced Fehling's and Tollen's solutions and algae developed in solutions thereof in the dark; it was also decomposed in ultra-violet light so that only small amounts were obtained. Thiele[127] has observed a similar action of ultra-violet light on formic acid.

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[125] Plotnikow, "Lehrbuch der Photochemie," 1920, p. 330.

[126] Spoehr, *Biochem. Zeit.*, **57**, 110 (1913); *Plant World*, **19**, 1 (1916); *Jour. Amer. Chem. Soc.*, **45**, 1184 (1923).

[127] Thiele, *Zeit. angew. Chem.*, **22**, 2472 (1909).

Coehn and Sieper[128] also studied the effect of ultra-violet light on carbon dioxide under different conditions. While many photochemical reactions are apparently sensitized by the presence of small amounts of water this is not the case with carbon dioxide. The decomposition of carbon dioxide is very greatly reduced by the merest trace of water vapor. Mixtures of carbon dioxide and water yielded no formaldehyde. Bauer and Rebmann[129] and Baur and Büchi[130] repeated the experiments of Baly, Heilbron and Barker, but were unable to obtain positive results of the formation of formaldehyde from carbon dioxide and water. They showed that formaldehyde is formed when various dyestuffs such as malachite green, eosine, rhodamine, and phosphine are exposed to ultra-violet light. They were also unable to obtain formaldehyde from carbon dioxide in the presence of colloidal ferric hydroxide or uranium oxide.

Porter and Ramsperger[131] have made a very careful reinvestigation of the action of ultra-violet light on carbon dioxide and water. They found that "in every run where the illuminated gas came in contact with rubber tubing, sealing wax, de Kootinsky cement or stop-cock grease, small quantities of formaldehyde were obtained, but the results were never quantitatively reproducible." When the entire apparatus was constructed of quartz-to-glass seals, and conductivity water and carbon dioxide made from pure sodium bicarbonate were used, after 36 to 60 hours' illumination, not a trace of formaldehyde or reducing sugars was obtained. The failure to obtain positive results cannot be attributed to the fact that the transparency of the quartz for light of short wave length had been impaired by prolonged exposure to this light, for the same negative results were obtained with new quartz apparatus, and spectrographs showed transmission down to 210 $\mu\mu$.

It is difficult to understand to what the discrepancy in the results of the action of ultra-violet light on carbon dioxide and water can be attributed. The tests for very small quantities of formaldehyde must be carried out with the greatest care in order to avoid spurious results. It is possible that the positive results are to be attributed to dust particles or other impurities as the results of Porter and Ramsperger suggest. Or in those cases in which it is claimed that formaldehyde is formed there existed conditions favorable to the formation of formaldehyde which were unknown to the experimenters. Porter and Ramsperger conclude: "These experiments compel us to believe that if formaldehyde is formed from carbon dioxide and water alone, under the influence of ultra-violet radiation, the reaction is reversible and the ordinary mercury arc provides the energy required to decompose formaldehyde as fast as it is formed. The question must be settled by determining whether or not there is a definite region in the ultra-violet spectrum that will

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[128] Coehn and Sieper, *Zeit. physik-chem.*, **91**, 347 (1916). Chapman, Chatwick and Ramsbottom, *Jour. Chem. Soc.*, **91**, 942 (1907); **89**, 22 (1908).

[129] Bauer and Rebmann, *Helv. chim. Acta*, **5**, 828 (1922).

[130] Bauer and Büchi, *ibid.*, **6**, 959 (1923).

[131] Porter and Ramsperger, *Jour. Amer. Chem. Soc.*, **47**, 79 (1925).

invariably supply the energy required for the reduction of carbon dioxide and which lacks the power to bring about polymerization or decomposition of formaldehyde. The next experiment in this field must be made with monochromatic ultra-violet light."

The question may well be asked of what significance for the natural process of photosynthesis are experiments on the action of ultra-violet light on carbon dioxide and water? That the ultra-violet portion of the solar spectrum plays only a very insignificant rôle or none at all in photosynthesis is almost certain, for the intensity of wave lengths shorter than 300 μμ in the solar spectrum is very small and, moreover, photosynthesis is carried on quite normally when the plants are behind glass which is opaque to ultra-violet light. This is also substantiated by measurements of the rate of photosynthesis with different wave lengths of light. On the other hand the problem of photosynthesis is in a stage of development in which any information relative to the action of light on the components of the reaction may be of value. It is impossible to say from which direction a solution will come. The danger lies in jumping at conclusions, in hasty attempts to explain the whole phenomenon on the basis of partial knowledge or of limited experimental observations.

Photochemistry is still an undeveloped science. Chemists have not been able to describe satisfactorily the mechanism of thermal reactions, and photochemical reactions are apparently even more difficult to interpret. The nature of fluorescence and the rôle which fluorescent substances may play in photochemical reactions is a field which will probably have to be worked over before we can understand the chemistry of photosynthesis. This is suggested by the striking fluorescence of chlorophyll and some of the pigments which are associated with it in the cell. It is true that at present there is little information which could lead us to believe that ultra-violet light is of any significance in relation to the function of chlorophyll. In blue light chlorophyll fluoresces in the red, about at 700 μμ. Carbon dioxide and carbonic acid have no absorption bands in the visible spectrum. Is it possible that in the photosynthetic apparatus there can take place a "stepping up" of energy in the sense that the absorption of a number of quanta in red light, for example, may result in the reradiation of one quanta of ultra-violet? Have we in photosynthesis a photochemical reaction taking place in a nonabsorbing acceptor by reacting with a primary photo-acceptor? It is doubtful whether we are as yet in possession of sufficient experimental data to expect profitable speculation.

b. The Reduction of Carbon Dioxide in the Presence of "Catalysts" and Chlorophyll Preparations.

There are some photochemical reactions proceeding easily in ultra-violet light which require a catalyst in order that they may take place in sunlight. Uranium and iron salts have been extensively used to bring about these reactions and the term photocatalysts has been applied to them rather freely and with little discrimination as to whether the reactions in question are really cases of catalysis or not. Some attempts have also been made to apply various salts of iron and uranium in the photochemical reduction of carbon dioxide. Here again the results are quite contradictory. Moore and Webster [132] claimed to have obtained formaldehyde by exposing to sunlight solutions of colloidal uranic and ferric hydroxides, through which carbon dioxide was passed. In this case it is not a question of using ultra-violet light, for glass vessels were used and in some cases a "Uviol" mercury arc. On the other hand, entirely negative results with these conditions were obtained by Baur and Rebmann and by Spoehr in the work previously cited.

Other attempts in this direction were made by using chlorophyll preparations in various forms. Several claims of having attained photosynthesis in preparations of this nature have been made, the basis of these being the formation of formaldehyde, of hydrogen peroxide or of oxygen in the illuminated preparations. Thus Usher and Priestley [133] obtained tests for formaldehyde by using chlorophyll in films of gelatine in the presence of catalase. These, as well as the claims of Schryver [134] and of Chodat and Schweizer,[135] have been subjected to criticism by Ewart,[136] Mameli and Pollacci,[137] Euler,[138] and by Curtius and Franzen.[139]

Of direct bearing on these claims are also the results of Warner [140] who reported that formaldehyde was produced by the photo-oxidation of the chlorophyll films in air. No formaldehyde was formed when such films were illuminated in a moist atmosphere of nitrogen or of carbon dioxide. The bleaching of the films was found to be effected by hydrogen peroxide "in the formation of which carbon dioxide can have no share, since there is no decolorisation in moist carbon dioxide free from oxygen." Extracts of carotin also produce formaldehyde and are bleached under similar conditions. At the same time Wager [141] published a paper giving his results of a study of the action of light on chlorophyll. He comes to conclusions very similar to those of Warner. The decomposition of chlorophyll and formation of aldehyde in the light take place as readily in the absence of carbon dioxide as when this is present. These results clearly show that from the formation of formaldehyde in arti-

[132] Moore and Webster, *Proc. Roy. Soc.*, **87** *B*, 163 (1913); **91** B, 196 (1920).

[133] Usher and Priestley, *Proc. Roy. Soc.*, **77** B, 369 (1906); **78** B, 318 (1906); **84** B, 101 (1911).

[134] Schryver, *ibid.*, **82** B, 226 (1909-1910).

[135] Chodat and Schweizer, *Arch. Sci. Phy. et Nat.* (4), **39**, 334 (1915).

[136] Ewart, *Proc. Roy. Soc.*, **80** B, 30 (1908).

[137] Mameli and Pollacci, *Atti. accad. Lincei* (5), **17**, 739 (1908).

[138] Euler, *Zeit. physiol. Chem.*, **59**, 122 (1909).

[139] Curtius and Franzen, *Sitzber. akad. Wiss. Heidelberg, Math.-nat. Kl.*, 1912, 7, 17.

[140] Warner, *Proc. Roy. Soc.*, **87** B, 378 (1914).

[141] Wager, *ibid.*, **87** B, 386 (1914).

ficial preparations of chlorophyll no conclusions can be drawn regarding the photosynthetic process.

Willstätter and Stoll[142] have given this problem critical examination and find that it is not the chlorophyll from which the aldehyde is formed, but from some substance which accompanies the leaf pigment. The attempts of these investigators to obtain evidence of an extracellular photosynthesis with preparations of chlorophyll contaming catalase or peroxidase were entirely negative or gave such slight tests for formaldehyde that they did not consider them sufficiently decisive. Regarding the formation of formaldehyde from chlorophyll Willstätter and Stoll point out that a chlorophyll preparation for investigations of this nature may possess two sources of error: it may be contaminated with some foreign substance or it may have undergone decomposition during preparation. Although the physiological experimentation in the work of Usher and Priestley and of Chodat and Schweizer were carried out with great care, these workers were, according to Willstätter and Stoll, quite disregardful of the principles of making chlorophyll preparations and of chemical technique. The latter find that chlorophyll which is illuminated in an atmosphere of oxygen is quite stable, and that in the first stages of the oxidation of chlorophyll in light no formaldehyde or any other aldehyde is formed. Nor are there any lower peroxides formed in this process. They conclude that in those cases in which formaldehyde was found as a product of photo-oxidation of chlorophyll, the substances which contaminated the chlorophyll probably were subjected to photo-oxidation and that this resulted in the formation of formaldehyde directly, or indirectly by the action of these oxidation products (peroxides) on the chlorophyll. Pure chlorophyll is apparently quite resistant to oxidation; even after illumination of 4 to 8 hours in the presence of oxygen it is not bleached. Jörgensen and Kidd[143] have also shown that formaldehyde is not formed from carbon dioxide when chlorophyll preparations are illuminated.

Recently Eisler and Portheim[144] have described experiments in which oxygen was liberated from illuminated chlorophyll preparations. The latter were prepared by extracting leaves with 95 per cent alcohol and adding diluted horse serum to the alcoholic chlorophyll solution. A flocculent precipitate is formed, from which the alcohol is removed, and the precipitate is dissolved in 0.85 per cent sodium chloride solution or in M. .01 sodium carbonate-sodium bicarbonate mixture. Similar chlorophyll-albumin preparations have been prepared from a number of plants; they possess the color, spectrum and fluorescence of a living leaf and the chlorophyll can again be extracted from them with alcohol.

When solutions of such chlorophyll preparation (from grass) are

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[142] Willstätter and Stoll, "Untersuchungen ü. die Assimilation der Kohlensäure," p. 395.

[143] Jörgensen and Kidd, *Proc. Roy. Soc.*, **89** B, 342 (1916).

[144] Eisler and Portheim, *Biochem. Zeit.*, **135**, 293 (1923); **130**, 497 (1922).

illuminated in the presence of carbon dioxide, it is claimed the gas in the flasks containing the preparations, when compared to the unilluminated preparations, is higher in oxygen content. The difference in oxygen content between the illuminated preparations and those kept in the dark is 0.9 to 4.5 per cent. Should these findings be verified the method may prove a valuable one for further investigation.

5. The Role of Water in Photosynthesis

The assumption is almost universally made that photosynthetic reaction may be represented by the equation:

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

This conclusion is based largely upon the relation of the amount of carbon dioxide absorbed to that of oxygen emitted. That water enters into the reaction was already assumed by Senebier, although with the exception of two experiments carried out by de Saussure in 1803 no experimental work has been carried out to establish this assumption. It is, of course, largely based upon the fact that carbohydrates are the first products which accumulate as a result of photosynthesis and that the dry material of the plant is largely of carbohydrate composition.

The experiments of de Saussure[145] on the utilization of water during photosynthesis gave the following results. Seven plants of *Vinca Minor L.*, grown in atmospheric air, absorbed 431 cc. carbon dioxide; this according to the constants available to de Saussure, he calculated as equivalent to 217 mg. of carbon. The plants increased their dry weight by 531 mg. De Saussure concluded that during photosynthesis the plants had fixed 315 mg. of water. It is evident that there are in de Saussure's calculations some minor errors due largely to the fact that the density of carbon dioxide had not been determined accurately. But there is a fair agreement between his results and the values which can be calculated on the basis of the usual equation. With 431 cc. of carbon dioxide absorbed the increase in dry weight calculates to 514 mg. as compared to the 531 mg. found by de Saussure.

A second experiment with *Mentha aquatica* showed that 309 cc. of carbon dioxide were absorbed, which de Saussure makes equivalent to 159 mg. of carbon. The plants gained 318 mg.; therefore it is concluded that 159 mg. of water had been fixed. Here the agreement with the theoretical values is not very good, for from 309 cc. of carbon dioxide there should be formed 360 mg. of dry material on the basis that this is carbohydrate of the molecular weight 180.

Experiments of this nature are rather difficult to carry out and there are a number of factors which must be considered which were omitted in de Saussure's experiments. It is essential that the plants be not injured in the slightest degree which is very difficult to avoid. The rela-

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[145] de Saussure, *Ostwald's Klassiker*, **15**, 26; **16**, 36 (1890).

tion of $H_2O:CO_2$ is 108:264 on the basis of the hypothetical equation; moreover, from what we know of the products of photosynthesis probably only a small amount is present in the form of hexoses with a molecular weight of 180 so that corrections would have to be applied for disaccharides and starch if these are present. Undoubtedly, however, carefully executed experiments on this problem would be a contribution of considerable value to the whole problem of photosynthesis.

6. The Absorption of Carbon Dioxide by the Unilluminated Leaf

Through the researches of Brown and Escombe there was made a very considerable advance in our knowledge of the manner in which carbon dioxide gains access to the centers of photosynthetic activity in the leaf. These investigations demonstrate that the absorption of atmospheric carbon dioxide by an illuminated leaf proceeds at about one half the rate which the same surface of the leaf would possess if it were covered by a constantly renewed film of a solution of caustic alkali. Or if only the area of the stomatal openings is considered, the absorption by the leaf per unit area would be 43 to 64 times as fast as the absorption of a unit area of a freely exposed solution of caustic alkali. The explanation for this remarkable phenomenon as given by Brown and Escombe has been discussed in Chapter 2. They dealt especially with the purely physical process of the diffusion of carbon dioxide through stomata. They did not consider the agent or leaf constituents which effectuate the absorption of carbon dioxide.

In considering the absorption of carbon dioxide by the leaf it must be realized that the leaf surface cannot strictly speaking be compared to an equal area of a caustic alkali solution. The stomata open into intercellular spaces in the parenchyma which possess a very much larger surface than that of the leaf. This large surface of cell membranes, impregnated with water, must greatly aid in the absorption of carbon dioxide. On the other hand, the leaf is normally exposed to partial pressures of carbon dioxide which are very small and the diffusion of dissolved carbon dioxide is so very slow as compared with its gaseous diffusion, that it is exceedingly difficult to explain the relatively rapid rate of the photosynthetic activity. At 0.03 volume per cent, 1,000 cc. of water dissolves at 25° 0.033 cc. carbon dioxide. 1,000 grams of *Helianthus* leaves, with an area of 3.3 square meters, contain 850 cc. water and would dissolve only 0.027 cc. carbon dioxide under normal conditions. During photosynthesis there is absorbed about 1,500 cc. carbon dioxide by these leaves per hour. There is much to favor the opinions, variously expressed by Willstätter and Stoll, Warburg and others that the first step in photosynthesis is not a simple splitting of carbon dioxide under the influence of light, but that the carbon dioxide undergoes a primary change through absorption by some constituent of the leaf.

It has been known for some time that vegetable tissue is capable of absorbing carbon dioxide in quantities considerably above that accounted for by the solubility of this gas in the water of the tissues.[146] Willstätter and Stoll observed a decided absorption of carbon dioxide by leaves that had been dried and powdered and had subsequently been allowed to absorb water again. Their method of determining the amount of carbon dioxide absorbed by the leaf material consisted in passing a stream of air containing 5 to 10 per cent of carbon dioxide over the leaf material at 5°; on raising the temperature to 30° more carbon dioxide was given off than could be accounted for by the difference in solubility between the two temperatures and after making proper allowance for the post mortal respiration. Two species of leaves were used, *Helianthus annuus* and the nettle, *Urtica dioica*. The absorption was determined at different partial pressures of carbon dioxide, 1 to 19 volume per cent. The amount of absorption of carbon dioxide which is attributed to some constituent in the leaf is obtained by subtracting the quantity dissolved in the amount of water present (at the particular temperature and pressure) from the total absorption observed by the moist leaf material. They thus find that the leaf absorbs considerably more carbon dioxide than can be accounted for by the solubility of this gas in the water present. The difference between the amounts absorbed by water and the leaf substance becomes greater with smaller partial pressure of carbon dioxide. Thus, with the sunflower, in 5 per cent carbon dioxide, the absorption of the leaf material is four times that of the solubility in water, while with 1 per cent carbon dioxide it is six times the solubility, and it is calculated that with 0.1 per cent carbon dioxide the absorption of the leaf substance would be twelve times that of the solubility of carbon dioxide in water. In view of the low partial pressure of carbon dioxide in the atmosphere, there can be little doubt but that this high absorption capacity of the leaf material for carbon dioxide would result in making more of it available for photosynthesis.

Willstätter and Stoll did not determine the nature of the substance or substances which are responsible for the absorption of carbon dioxide, although they show that this is not related to the pigments of the leaf. They did not regard it as improbable that the absorption of carbon dioxide by the leaf material was due to inorganic carbonates, but favored the idea that the absorption was due to organic compounds. They thus came in accord with the conceptions which had previously been developed by Siegfried. The latter considered that it was not carbon dioxide which was reduced in the photosynthetic process, but that this gas united with components of the protoplasm on the basis of the carbamino reaction. Siegfried[147] showed that carbon dioxide combines with amino acids, sugars, alcohols and hydroxyacids. Thus the carbamino reaction with neutral amino acids or proteins and bicarbonates may be represented as follows:

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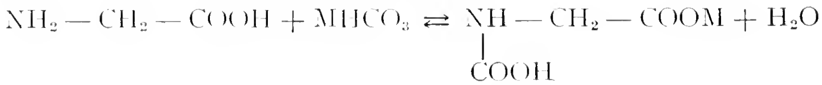
[146] Böhm, *Ann. Chem.*, **185**, 248 (1876). Carey, *Physiological Researches*, **2**, 407 (1923). Willstätter and Stoll, "Unters. ü. Kohlensäureass.," 174 (1918).

[147] Siegfried, *Zeit. physiol. Chem.*, **44**, 85 (1905); **46**, 401 (1905); **54**, 423, 437 (1908); **59**, 376 (1909).



$$NH_2-CH_2-COOH + MHCO_3 \rightleftarrows \underset{\displaystyle COOH}{\underset{|}{NH}}-CH_2-COOM + H_2O$$

The resulting products are carbamino acids and the reaction is usually carried out in alkaline solution so that the secondary salts are formed. Willstätter and Stoll suggested that the absorption of carbon dioxide by the leaf material was also based upon this type of reaction.

On the basis of preliminary experiments Spoehr and McGee came to conclusions which supported those of Willstätter and Stoll, that the leaf absorbs carbon dioxide from the atmosphere by a mechanism similar to that by which the blood serves in freeing the tissues of this gas. Willstätter and Stoll found that 100 grams of dry leaf material absorb from an atmosphere containing about 3.8 per cent carbon dioxide 160 mg. of this gas. In the experiments of Spoehr and McGee [148] the absorption of carbon dioxide was determined directly; that is, the leaf material was first freed from carbon dioxide by passing an air-stream, free of carbon dioxide, over the leaf material for 24 hours and then the amount of the gas which the leaf material took up was determined directly by exposing it to air containing a known quantity of carbon dioxide and analyzing the air after several hours. Thus, they obtained an absorption by 100 grams leaf material with 1.3 per cent carbon dioxide, at 25°, of 495 mg. or 272 cc. of carbon dioxide.

On further examination of the phenomenon of the absorption of carbon dioxide by dried leaf material by Spoehr and Newton it was found that the theory which ascribes the absorption to amino acids or proteins is untenable. The leaves were dried at about 45° in a stream of dry air. This material was powdered and extracted with water saturated with ether. The water extract from sunflower and nettle leaves, on the addition of alcohol, formed a precipitate which showed decided absorption of carbon dioxide, about 25 mg. per gram of the sunflower material. A number of other species of leaves was tested for absorption and the alcoholic precipitates from these absorbed either only slightly or not at all. The absorption of the dried leaf material itself could be estimated only in the case of the sunflower and nettle; in the case of the other plants studied the evolution of carbon dioxide due to post mortal respiration overbalanced any absorption.

That the amino acids and proteins in the alcoholic precipitates mentioned were not responsible for the absorption of carbon dioxide was indicated by the following facts: the amount of total nitrogen and of amino nitrogen in the precipitates bore no relation to the amount of carbon dioxide absorbed; the amount of nitrogen was also too small to account for the amount of carbon dioxide absorbed on the basis of the carbamino reaction. The alcoholic precipitates were dializable; the material which passed through the membrane, on reprecipitation with alcohol,

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[148] Spoehr and McGee, *Science*, **59**, 513 (1924).

absorbed carbon dioxide. All of these alcoholic precipitates had a high ash content, varying from 20 to 50 per cent according to the species of leaves used.

By employing the apparatus which Van Slyke[149] designed for determining the carbon dioxide in blood, it was possible to demonstrate that the larger part of the absorption of carbon dioxide by the dried leaf material and the alcoholic precipitates obtained therefrom could be ascribed to bicarbonate formation. By the use of this apparatus the amount of carbon dioxide liberated from a solution of the alcoholic precipitates or from suspensions of the finely powdered leaf material, in a Torricellian vacuum, and after the addition of sulphuric acid could be determined. It is a noteworthy fact that the two species of leaves with which Willstätter and Stoll studied the absorption of carbon dioxide were the sunflower and nettle. With these leaves Spoehr and Newton also observed highest absorption. Moreover, with the exception of these two leaf materials, others, including spinach, *hydrangea*, turnip, lettuce, alfalfa, rhubarb, grass and *Ramilina reticulata* showed no absorption by the leaf material and only slight absorption by the alcoholic precipitates in some cases, and in most cases none at all. Also, with the exception of sunflower and nettle, after saturation of the leaf material with carbon dioxide, the evolution of this gas, on addition of sulfuric acid, was exceedingly small. When this leaf material (excepting sunflower and nettle) was saturated with carbon dioxide the amount of this gas which was liberated in vacuo was equivalent to the amount which could be dissolved in the quantity of water present. Hence if there is an absorption factor in this leaf material, it is too small to be detected by this method.

The sunflower and nettle material is noteworthy because both the dried leaf material and the alcoholic precipitates absorb more carbon dioxide than can be accounted for by the solubility of this gas in the water added to the dried or precipitated material. When preparations of this sort (e.g. dried and powdered leaves to which a quantity of water equivalent to that originally present in the fresh leaves was added) were exposed to an atmosphere free of carbon dioxide for several hours, and then acidified, considerable carbon dioxide was liberated. When similar material was saturated with carbon dioxide and then acidified, the amount of this gas which was liberated was approximately double the quantity evolved in the case just described. These facts would indicate that the absorption of carbon dioxide is due to a carbonate : bicarbonate reaction. The values for the material that was saturated with carbon dioxide were obtained by acidifying the suspension after removing the carbon dioxide which came off under a Torricellian vacuum. The alcoholic precipitates behaved in a similar manner. For instance, a precipitate obtained from sunflower after being in an atmosphere free of carbon dioxide for 4 hours evolved 8.6 mg. carbon dioxide on acidification, and after having

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[149] Van Slyke and Stadie, *Jour. Bio. Chem.*, **49**, 1 (1921). Van Slyke and Cullen, *ibid.*, **30**, 289 (1917).

been saturated with carbon dioxide, the amount of this gas evolved on acidification was 17.3 mg. Similarly, when the precipitate was dialyzed and again precipitated this, after being in a carbon dioxide-free atmosphere, evolved 28.6 mg. on acidification, and after saturation with carbon dioxide, evolved 56.6 mg. In Table 32 are given some of the results obtained with different leaf material.

TABLE 32

CARBON DIOXIDE EVOLVED (MG.) FROM 1 GRAM DRIED AND POWDERED LEAVES AND FROM ALCOHOLIC PRECIPITATES OBTAINED BY EXTRACTING THE DRIED LEAVES WITH ETHER-WATER AND PRECIPITATION WITH 95 PER CENT ALCOHOL. IN EACH CASE A QUANTITY OF WATER WAS ADDED TO THE DRY PREPARATIONS EQUIVALENT TO THAT ORIGINALLY PRESENT IN THE FRESH LEAVES. DETERMINATIONS MADE WITH THE VAN SLYKE BLOOD GAS APPARATUS.

Material	Original Condition	After Being in CO_2-Free Atmosphere for 4 Hours	After Saturation with CO_2
Sunflower	13.9 mg.	13.7 mg.	27.7 mg.
Nettle	6.2	5.9	12.7
Spinach	0.2	0.0	0.7
Hydrangea	0.1	0.1	0.2
Turnip	0.5	0.3	0.5
Alfalfa	0.4	0.2	0.3
Rhubarb (leaves)	0.02	0.0	0.02
Grass	0.1	0.1	0.6
Ramilina recticulata	0.0	0.0	0.2
Sunflower alc. ppt.	—	24.4	49.5

An examination of Table 32 will show that the sunflower and nettle leaves occupy a peculiar position in regard to their capacity to absorb carbon dioxide. Moreover, a comparison of the values obtained for the evolution of carbon dioxide, on acidification, after the preparation had been in a carbon dioxide-free atmosphere and after saturation with carbon dioxide, show that the former is very close to one half the latter. This is what would be expected if the absorption were due to an inorganic carbonate. The same relation holds for the alcoholic precipitates obtained from water-ether extracts of the dried leaves. When the latter are dialyzed and the dialysate is again precipitated with alcohol, the absorbing capacity and the ash content of the latter are considerably higher than the original precipitate, though the carbonate: bicarbonate ratio still maintains. There is, however, no direct relation between the absorption capacity and the amount of ash in the leaves and alcoholic precipitates. It is, of course, possible that all leaves have an absorption capacity for carbon dioxide, but from these results it would appear that with many this is very small. Certainly it is not justifiable to draw conclusions from the behavior of the sunflower and nettle as to the behavior of all plants. Whether, finally, this absorption capacity for carbon dioxide has any direct relation to the photosynthetic process will require special experiments. It is suggestive, at least, that both the sunflower and nettle are known as possessing a high photosynthetic activity.

Chapter 6

The Energy Relations in Photosynthesis

1. The Energy Used in Photosynthesis

When viewed in the broadest prospect, the most important feature of the phenomenon of photosynthesis centers about the fact that the radiant energy of the sun is transformed into potential energy of the products synthesized. These are largely of the composition of carbohydrates, although the plant elaborates, in a more or less indirect manner, considerable amounts of fats and proteins as well. The significance of this storing of the sun's energy for life on the earth has already been touched upon, in general terms, in the first chapter. The need of more specific knowledge regarding the manner of transformation of energy in the photosynthetic process has been recognized for a long time. There have been numerous speculations regarding the manner in which the absorbed radiant energy is utilized in bringing about the chemical reactions constituting the reduction of carbon dioxide and water with the synthesis of carbohydrates. There have, however, been few experimental attacks on the problem. As a consequence our knowledge of the energy relations of photosynthesis is exceedingly meagre.

Undoubtedly the reason for this lack of more precise knowledge of the energy relations in photosynthesis lies in the difficulties which are encountered in endeavoring to subject the photosynthetic reaction to experimental conditions in which all the factors which influence the reaction are carefully controlled and measured. The complex nature of photosynthesis has been repeatedly emphasized; this arises from the fact that there are so many factors which exert an influence on the process. It is but necessary to recall that the factors of light intensity, temperature, carbon dioxide-concentration, chlorophyll-content, water-content and certain yet vaguely recognized enzymatic factors control the rate of photosynthesis; these are not independent of each other, but the intensity of each must always be considered. Probably the most serious difficulty of all arises from the fact that a photochemical reaction is involved, and the laws governing the kinetics of photochemical reactions, even in the simplest systems, are as yet but imperfectly understood.

Our information regarding the energy used in photosynthesis by the leaves of land plants is largely confined to the results obtained from the investigations of Brown and Escombe and of Puriewitsch. The energy relations of the leaves of land plants are more complicated than those of aquatic plants. In the former the situation becomes complicated through the transpiratory activity, the opening and closing of the stomata and the exchange of heat between the leaf and its surroundings due to a difference in temperature between the two. In aquatic plants where there is no factor of transpiration and consequent cooling of the plant, the energy relations are somewhat simpler. Warburg and Negelein have taken advantage of this fact in determining the photosynthetic efficiency of a unicellular alga. Determinations of the energy used in photosynthesis show a considerable variation. As these results have been arrived at by widely different methods it will be necessary to describe the experiments in some detail in order to be able to compare their relative worth.

The experiments of Brown and Escombe[1] constitute the first thorough attempt to construct a balance sheet for the energy relations of an illuminated leaf. Not the least of the contributions of these authors has been an analysis of the factors which must be considered in attempting such a calculation. It is doubtful whether they are justified in drawing conclusions as to some of the fundamental physical properties of leaves from the relatively limited data, especially as to the constancy of such properties as thermal emission under different experimental conditions. Nevertheless, their experiments are of considerable value from the viewpoint of a general approach to the problem and their results still constitute practically the only data which have been obtained by a method which endeavors to account for all the energy taken in and expended by the leaf.

The absorption of radiant energy by a leaf may result in 1, the vaporization of water and 2, in photosynthesis; both of these are endothermic in character. Also in the leaf, 3, the process of respiration is continually going on; the end result of the chemical reactions comprising respiration is exothermic. The values of these three processes, vaporization of water, photosynthesis and respiration, as measured in units of heat absorbed or evolved per unit of time, are very dissimilar and vary with different external conditions to which the leaf is exposed. The loss or gain of heat, due to each of these three processes, can be determined and referred to the total amount of energy received by the leaf, either by direct radiation or through the convective and conductive action of the air. In order to accomplish this, it is, however, essential to establish the absorptive power of the leaf for the different forms of radiant energy to which it is exposed, as well as the gain or loss of heat due to radiation and air convection and conduction due to differences in temperature between the leaf and its surroundings.

The coefficient of absorption of a leaf was taken by Brown and Escombe as "the difference between the solar radiant energy falling on the leaf in full sunshine and the amount transmitted" through the leaf. The measurements were made by means of a Callendar radiometer; a measurement

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[1] Brown and Escombe, *Proc. Roy. Soc.*, **76** B, 69 (1905).

was made with direct solar radiation, then one with the interposition of the leaf and finally a second observation of the direct radiation. The difference between the mean of the first and third observations and the second gave the coefficient of absorption when the direct radiation was taken as unity. A considerable variation was found in the values for the coefficient of absorption in leaves of different species, although fairly constant values were obtained for mature leaves of the same species. There is, however, a dearth of accurate experimental determinations of the coefficient of absorption of leaves especially using different frequencies and intensities of illumination. In Table 33 are given the values of the absorption coefficient of various leaves determined by Brown and Escombe.

TABLE 33

COEFFICIENT OF ABSORPTION OF THE RADIANT ENERGY OF SUNLIGHT FOR LEAVES OF DIFFERENT PLANTS. (Brown and Escombe.)

Plant	Coefficient of Absorption
Helianthus annuus	0.686
Polygonum Weyrichii	0.647
" Sachalinense	0.691
Petasites officinalis	0.728
Silphium terebrinthaceum	0.699
Arctium majus	0.728
Verbascum olympicum	0.758
Senecio grandifolius	0.774

The green leaf is very selective in its absorbing power. This fact is brought out by the results of the absorption of light by a number of similar leaves superimposed. If there were no selective absorption by the leaf the quantity of light transmitted would be decreased in geometric proportion as the superimposed leaves were increased in arithmetic proportion. Thus, if one leaf has a coefficient of transmission of 0.3, two superimposed leaves should have a coefficient of transmission of 0.09 and three leaves 0.027, etc. That this is not the case is shown by the experiments of Brown and Escombe who found the following quantities transmitted by *Polygonum Weyrichii:* one leaf 35.3 of the total radiation; two leaves 19.2 per cent and three leaves 15.5 per cent; similarly for *Helianthus annuus:* one leaf 31.3 per cent, two leaves 17.4, three leaves 11.6.

Similar results were obtained by Timiriazeff who found that the chlorophyll which had been dissolved from a unit area of one maple leaf absorbed 27 per cent of the direct radiation while the absorption of a solution three times this concentration was only 31 per cent.

That a single leaf is capable of absorbing most of the rays which induce photosynthesis, although the total radiation may be many times that which the leaf is capable of utilizing, has also been demonstrated by Willstätter and Stoll.[2] They found that when the light incident on

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[2] Willstätter and Stoll, "Untersuchungen ü. Kohlensäureassim.," p. 125.

a leaf is filtered through another similar leaf, the rate of photosynthesis of the first leaf is reduced to $\frac{1}{20}$ of the original rate and when two leaves are interposed, photosynthesis ceases.

Brown and Escombe found but a very small increase in the values of the coefficient of absorption with increasing age of the leaves. Their values are considerably higher than those obtained by Puriewitsch.[3] The latter by the use of a bolometer found that leaves of different plants absorbed 18 to 27 per cent of the incident solar radiation, while Brown and Escombe's results show an absorption of about 64 to 77 per cent. The bolometer of Puriewitsch was probably more accurate than the method of measuring the solar radiation used by Brown and Escombe. It is difficult to determine the cause for the discrepancy between values obtained by these workers.

In this connection the observations of Timiriazeff[4] are of some interest. This worker endeavored to determine the absorption caused by the chlorophyll of a single leaf. Thus, he found that the absorption of the direct sunlight by the green pigment dissolved in a volume of alcohol just sufficient to fill a glass cell of the same cross-section as the area of the leaf, ranged from 20 to 29 per cent for different leaves. These values are obviously nearer to those of Puriewitsch than to those of Brown and Escombe. At the same time it should be noted that the latter workers also compared the absorption of green and albino leaves. They found, for example, that the coefficient of absorption of the white portion of leaves of *Negundo aceroides* was 0.745 while that of the green portion was 0.787. This would indicate that in the method employed by Brown and Escombe only a relatively small portion of the radiant energy absorbed by the leaf can be ascribed to the absorption by the chlorophyll. However, there are other differences between the green and colorless portions of a leaf besides the chlorophyll-content which may influence the difference in absorption.

Brown and Escombe consider that the error due to reflection from the leaf surface which would tend to increase the values of the coefficient of absorption, was, under the conditions of their experiments, very small. Jörgensen and Stiles,[5] on the other hand, state that a black cloth may reflect as much as 1 per cent of the radiant energy falling on it.

In order to make an estimation of the energy exchanges in an illuminated leaf it is necessary to know, besides the coefficient of absorption of the leaf, also something of the thermal relations of the leaf to its surroundings. The radiant energy which is absorbed by a leaf tends to raise its temperature. If, for example, the solar radiation is taken as 0.8 calories per square centimeter per minute (a low value), the coefficient of absorption as 0.78, the weight of a square centimeter of leaf as 0.020 gram and its specific heat as 0.879, there would result a rise

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[3] Puriewitsch, *Jahrb. wiss. Bot.*, **53**, 209 (1914).
[4] Timirazeff, *Proc. Roy. Soc.*, **72** B, 449 (1903).
[5] Jörgensen and Stiles, "Carbon Assimilation," p. 141.

in temperature of the leaf expressed by $\frac{0.8 \times 0.78}{0.02 \times 0.879} = 35.^\circ4$ per minute. Such an increase in temperature of a leaf on illumination by direct sunlight would soon be fatal. A means of dissipating the absorbed energy is offered by: 1. the vaporization of water and 2. photosynthesis, both endothermic processes. Under circumstances the dissipation of the absorbed energy by vaporization of water and by photosynthesis is less than the amount of solar energy absorbed. As a consequence the temperature of the leaf will rise above that of its surroundings and a steady thermal state will be attained only when, with a sufficiently high temperature gradient, the excess of energy received by the leaf is dissipated by re-radiation and convective cooling. There is also the other possible condition, that through photosynthesis and vaporization of water the leaf is cooled more rapidly than energy is supplied by the incident radiation. Under these circumstances the temperature of the leaf will fall below that of its surroundings. In order to make determinations of the energy relations of the leaf during photosynthesis it therefore becomes essential to have some knowledge of the interchange of heat between the leaf and its surroundings, that is, what Brown and Escombe termed the thermal emissivity of the leaf surface.

The relation of thermal emission to the other modes of energy change in the photosynthetically active leaf was deduced by Brown and Escombe about as follows:

$R =$ Incident solar radiation in calories per square centimeter per minute.
$a =$ Coefficient of absorption of the leaf.
$\underset{\sim}{Q} =$ Heat loss per square centimeter of leaf lamina per minute.
$c =$ Photosynthesis, i.e., cc. of carbon dioxide absorbed by 1 square centimeter of leaf lamina per minute.
$W =$ Generalized expression of work effective in vaporization of water.
$w =$ That due to the photosynthetic process.
$r =$ Loss or gain of heat by the leaf through radiation, convective cooling or absorption of heat from the air surrounding the leaf.

It is important to note that R, W, w and r are calculated in terms of the "area of leaf lamina," while the thermal emissivity is calculated in terms of the "surface area of the leaf."

The means of obtaining the value of the total radiant energy falling on the leaf and the amount absorbed by the leaf have already been mentioned. A measure of the heat dissipated by the vaporization of water was obtained by a determination of the water lost through transpiration per unit area and time. The latent heat of vaporization of 1 gram of water at 20° was taken as 592.6 calories, so the heat dissipated by vaporization of water per unit area and time will be 592.6 $\underset{\sim}{Q}$.

The energy used in photosynthesis was determined from the volume of carbon dioxide absorbed in unit time and area on the assumption that a hexose is the product formed and that the heat of combustion, with a reversed sign, equals the heat of formation. The heat of combustion of glucose was taken as 3760 calories per gram, and as 1 cc. of carbon dioxide corresponds to 0.001336 gram of a hexose, this quantity of carbon dioxide absorbed in photosynthesis would correspond to an absorption of $0.001336 \times 3760 = 5.02$ calories. The energy used in photosynthesis expressed in calories per square centimeter per minute is $5.02\ c$. The total internal work to be ascribed to the vaporization of water and to photosynthesis may be expressed by

$$592.6\ Q + 5.02\ c = W + w.$$

The term "work" is perhaps not altogether fortunate and is to be taken as a generalized expression.

When the energy used in vaporization of water and in photosynthesis $(W + w)$ equals the total solar radiation absorbed by the leaf, R*a*, there is a steady thermal state. This is rarely the case; however, R*a* is usually either greater or less than $(W + w)$. When R*a* is greater than $(W + w)$ the excess of radiant energy absorbed will raise the temperature of the leaf above that of its surroundings. The thermal steady state can then be represented by

$$Ra = (W + w) + r$$

and when R*a* is less than $(W + w)$ heat must be absorbed from the surroundings and the steady state becomes

$$Ra + r = W + w$$

While R*a* and $(W + w)$ are values determined experimentally, *r* is a difference value. The actual difference of temperature between the leaf and its surroundings can be determined from *r* if the thermal emissivity of the leaf is known. Brown and Wilson [6] determined the thermal emissivity of the leaves of different plants. In Table 34 are given some of these values.

TABLE 34

Thermal Emissivity of Leaves of Various Species of Plants under Still Air Conditions. (Brown and Wilson.)

Plant	Thermal Emissivity in Calories per Minute per sq. cm. of Surface for 1° C. Temperature Excess
Liriodendron tulipifera	0.01194
" "	0.01274
Helianthus multiflorus	0.01499
Tropeolum majus	0.01427
Tilia europea	0.01598

Brown and Wilson also determined the thermal emissivity of leaves in moving air. Under these conditions the rate of cooling is consider-

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[6] Brown and Wilson, *Proc. Roy. Soc.*, **76** B, 122 (1905).

ably higher. Table 35 taken from the publication of Brown and Wilson illustrates the influence of air currents.

TABLE 35

THERMAL EMISSIVITY OF LEAVES OF *Liriodendron tulipifera* IN MOVING AIR. (Brown and Wilson.)

Rate of Air Current, Meters per Minute	Thermal Emissivity in Calories per Minute per sq. cm. for 1° C. Temperature Excess
Still air	0.0119
36.2	0.0173
71.4	0.0238
108.0	0.0304
139.0	0.0361

In order to illustrate the manner in which Brown and Escombe calculated their results, their own description of an experiment is here given:

"In (1) the leaf was enclosed in a glazed case through which ordinary air was passed, and the amount of carbon dioxide assimilated was estimated from its determination in the entering and emergent air, the results being corrected to the partial pressure of the carbon dioxide in the outer atmosphere, which in this instance corresponded to 2.71 parts per 10,000 of air.

"Leaf (2) for determining the rate of transpiration was freely exposed to the air, which had an average temperature of 16.°9 C., and an average velocity of 25.7 kilometers per hour. The partial pressure of the water vapor in the air was 9.21 mm. of mercury as determined from the average readings of the wet and dry bulb thermometers. The area of each leaf was, of course, accurately determined.

"The results were as follows:

(R) Average total solar radiation falling on the insolated leaves per square centimeter per minute	0.2596 calories
(a) Coefficient of absorption of the leaves	0.686

The actual amount of solar energy intercepted was therefore $Ra = 0.2596 \times 0.686 = 0.1762$ calory per square centimeter per minute.

"The amount of carbon dioxide assimilated, reduced to free air conditions, was 2.134 cc. per square decimeter per hour or 0.000355 cc. $\times 5.02 = 0.0017$ calory per square centimeter per minute (w).

"The water transpired from the leaf under free air conditions amounted to 1.259 grams per square decimeter per hour, or 0.000209 gram per square centimeter per minute. The amount of internal work of vaporization, W, is therefore $0.000209 \times 592.6 = 0.1243$ calory per square centimeter per minute.

"The total amount of internal work $W + w$ performed by the leaf is therefore equivalent to $0.1243 + 0.0017 = 0.1260$ calory per square centimeter of leaf lamina per minute.

"In this instance Ra, the solar radiation absorbed by the leaf, exceeds

$W + w$, the sum of the internal work of the leaf, by $0.1762 - 0.1260 = 0.0502$, which represents the value of r in calories in the equation

$$Ra = (W + w) + r.$$

"This value for r of 0.0502 calory per square centimeter per minute represents the *only part of the solar radiation which can have had any heating effect on the leaf.* From the value of r we can determine the mean temperature-difference between the exposed leaf and its surroundings $\Theta_n - \Theta$, if we know the thermal emissivity of the leaf, for $\Theta_n - \Theta = \frac{r}{2e}$.

"The thermal emissivity of a leaf of this nature, for 'still air' conditions, is approximately 0.015 calorie per square centimeter of leaf *surface* per minute for a temperature excess of 1°, and the emissivity increases by 0.00017 calorie per square centimeter per minute for an increased air speed of 1 meter per minute. Hence, since the average velocity of the wind in this case was 25.7 kilometers per hour, or 428 meters per minute, the corrected 'emissivity' becomes $0.0150 + 0.00017 \times 428 = 0.0577$ calorie per square centimeter of *surface* per minute for a temperature excess of 1°. Hence the temperature excess of the leaf-lamina above its surroundings will be

$$\Theta_n - \Theta = \frac{r}{2e} = \frac{0.0502}{0.1154} = 0.^\circ 43 \text{ C.}$$

"Since the average temperature of the air during the experiment was 16.°9 C., that of the leaf was about 17.°3 C.

"We are now in a position to state with a fair approach to accuracy the manner in which the leaf has disposed of the energy incident upon it, and to obtain some idea of the 'economic coefficient' of the leaf under these average conditions. If we denote R, the total energy incident on unit-area of the leaf in unit time, by 100, then the disposal of this radiation will be accounted for in the following manner:

(w) Energy used in photosynthesis	0.66
(W) " " " transpiration	48.39
($W + w$) Total energy expended in internal work	49.05
$R - Ra$. Solar radiant energy transmitted by leaf	31.40
(r) Energy lost by thermal transmission	19.55
	100.00"

From the results of Brown and Escombe it is apparent that a very considerable portion of the energy absorbed by the leaf is used in transpiration. That the rate of transpiration varies greatly with changes in the external conditions is, of course, a familiar fact. The changes brought about by variations of the transpiration rate in the distribution of the radiant energy absorbed by the leaf and probably also in the photosynthetic rate are too complicated to predict.

Relatively, the amount of energy liberated through the respiratory activity is small. Brown and Escombe calculate for a leaf of *Helianthus annuus,* emitting 0.70 cc. carbon dioxide per square decimeter of leaf-lamina per hour, 0.000582 calorie per sq. cm. per minute would be evolved, and since the weight of a sq. cm. of this leaf is 0.020 gram, with a specific heat of 0.879, the heat of respiration would raise the temperature of the leaf at the rate of 0.°033 per minute, assuming that the leaf was not losing heat in any way.

From the results of Brown and Escombe it is apparent that the energy used in photosynthesis varies considerably. In Table 36 are given the results of some of their experiments. Simultaneous experiments were carried out in which all conditions were kept the same except the insolation, R. In one case, A, the leaf was exposed to full insolation; in the other case, B, only a portion of that used in A was allowed to fall on the leaf; revolving sectors were used to reduce the insolation. It was attempted thus to alter but one factor, that of light intensity.

TABLE 36

Disposal of Energy Incident on a Leaf. The Total Energy Received by the Leaf Is Taken to Equal 100. (From data of Browne and Escombe.) Nos. 1 to 8 Are for *Helianthus annus,* with a Coefficient of Absorption, *a*, of 0.686, and 9 to 11 for *Senecio grandifolius* with *a* of 0.744. R Is Given in Calories per Sq. Cm. of Leaf Lamina per Minute.

No.	*w* Energy Used in Photosynthesis	W Energy Used in Transpiration	R-R*a* Radiant Energy Lost by Transmission	*r* Energy Lost by Reradiation	R Actual Incident Solar Radiation	Portion of Full Insolation Falling on Leaf
1	0.65	69.22	30.13	—	0.4547	A. Full
	0.94	77.35	21.71	—	0.2273	B. ½
2	0.66	69.02	31.32	—	0.4798	A. Full
	0.94	77.44	21.62	—	0.2399	B. ½
3	0.56	70.12	29.32	—	0.5492	A. Full
	0.72	80.38	18.90	—	0.2746	B. ½
4	0.67	74.04	25.29	—	0.3931	A. Full
	0.78	77.10	22.12	—	0.2948	B. ¾
5	0.57	72.18	27.25	—	0.4993	A. Full
	0.63	76.64	22.73	—	0.3744	B. ¾
6	0.44	76.40	23.16	—	0.5504	A. Full
	0.47	77.70	21.83	—	0.4806	B. ⅞
7	0.55	71.17	28.28	—	0.5337	A. Full
	0.60	72.66	26.74	—	0.4666	B. ⅞
8	2.79	34.26	31.40	31.55	0.1179	A. Full
	4.48	45.24	31.38	18.90	0.0736	B. ⅝
9	0.76	50.16	22.59	26.49	0.4288	A. Full
	1.46	77.01	21.53	—	0.2144	B. ½
10	0.80	42.10	22.59	34.51	0.4080	A. Full
	1.59	76.13	22.28	—	0.2040	B. ½
11	1.33	59.29	22.60	16.78	0.2468	A. Full
	2.23	78.90	18.84	—	0.1234	B. ½

The values of *w* (energy used in photosynthesis) may be taken as a measure of the efficiency of the photosynthetic process in the leaf. In the results of Brown and Escombe given in Table 36 a number of points should be noted. The quantity of energy incident on the leaf, R, when full insolation was used is not high, about 0.5 calorie per sq. cm. per hour. This is no doubt due to the condition of the London air. Unfortunately, in the experiments, the results of which are given in Table 36, the actual rates of photosynthesis were not determined. But from other experiments in the same publications, it is apparent that an increase in the light intensity from about 0.2 calorie per sq. cm. per mi. to 0.5 calorie does not produce a corresponding increase in the rate of photosynthesis. Brown and Escombe cite one case in which the solar radiation (0.5 calorie per sq. cm. per minute) was reduced to one-twelfth its original value (0.04 calorie) before any effect on the rate of photosynthesis was observed. The rate at this intensity of illumination was 0.00034 cc. per sq. cm. per minute and they consider that under these conditions "practically the whole of the rays capable of producing photosynthesis were utilized for that purpose." They estimate the energy used in photosynthesis under these conditions at 0.0017 calorie per sq. cm. per minute, so that with an incident intensity of 0.041 calorie per sq. cm. per minute 4.1 per cent "also represents the percentage of photosynthesizing energy in the original unscreened solar radiation." It is doubtful whether the results of Brown and Escombe can be accepted in the strict quantitative sense. The experiments do show that the incident radiation can be reduced appreciably without affecting the rate of photosynthesis. Weigert,[7] on the basis of Brown and Escombe's results, has calculated the efficiency of the photosynthetic reaction as being 98 per cent of the energy absorbed by the green parts of the leaf converted into chemical work.

In other words, in these experiments light was not the limiting factor controlling the rate of the reactions. It is apparent that with a decrease in R, the per cent of incident energy used in photosynthesis increases. The leaf was thus receiving a great deal more energy than it could utilize in photosynthesis. The greater portion of the absorbed energy was dissipated in the vaporization of water. In all probability the concentration of carbon dioxide was the limiting factor. Nevertheless, the experiments at least approximate the conditions existing in nature and go to show that the photosynthetic process, taken as a whole, with solar radiation, is of a low order of efficiency and that the proportion of the absorbed energy used in photosynthesis is a variable quantity. The results also indicate that the efficiency will be at a maximum when the illumination is just of sufficient intensity to produce maximal photosynthetic rate under the given conditions of temperature, carbon dioxide-concentration and chlorophyll-content. The experiments, furthermore, show what an important rôle, in the utilization of the absorbed energy,

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[7] Weigert, *Zeit. wiss. Phot.*, 11, 381 (1911).

is played by transpiration and that, under circumstances, the cooling effect of the transpiration is such that the leaf absorbs heat from the surrounding air.

It should perhaps also be pointed out that these experiments of Brown and Escombe constitute the first steps in determining the energy relations of illuminated leaves, that they have served to indicate the various factors which must be considered and the experimental difficulties which are encountered in such an undertaking. The accuracy of the results depends upon a number of determinations, foremost of which are the determinations of R, the total incident radiation, the absorption coefficient of the leaf, the accurate estimation of very small quantities of carbon dioxide in an air-stream, and the amount of water lost by transpiration. Due consideration must, of course, also be given to the effect of illumination on the opening and closing of the stomata and the effect of this on the gaseous exchange and on transpiration. Finally, it should be mentioned that the experiments of Brown and Escombe were carried out with detached leaves, and that these show a rate of photosynthesis considerably higher, as measured by the carbon dioxide taken in (about 45 per cent) than leaves attached to the plant.

Puriewitsch[8] endeavored to determine the energy used in photosynthesis from the increase in the heat of combustion of leaves after insolation and by calculating the ratio of this increase to the total amount of radiant energy falling on the leaf. For measuring the intensity of the solar radiation a bolometer was used; this was kept at right angles to the incident light. Photosynthesis was determined with excised leaves; these were kept in the dark for 24 hours prior to their use. The leaves were cut in half along the mid-rib; one half was immediately dried and its heat of combustion determined, the other half, with the petiole in water was exposed at right angles to the sunlight for several hours. The area of both halves of the leaves was determined by making a photographic print and measuring the area with a planimeter. The half leaves were then dried and their heats of combustion determined or in some cases the heat of combustion was calculated, though it is not clear how this was done. The results are tabulated in Table 37. In all these experiments (for convenience the numbering is not that followed by Puriewitsch) with the exception of 4 and 8 the incident solar radiation fell on the bolometer and the leaves at right angles; in 4 and 8 they were kept in one position at an angle of 50° with the horizon. In experiments 4, 7 and 10 the heats of combustion were determined, in the others they were calculated.

In the experiments of Puriewitsch there is a great variation (0.6 to 7.7 per cent) in the amount of solar energy utilized. The source of illumination was the sun; the bolographs also indicate great fluctuations in the intensity of illumination during the course of the experiments. Nevertheless the light was undoubtedly greatly in excess of the amount the leaves were capable of utilizing. This is also indicated by the fact that roughly

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[8] Puriewitsch, *Jahrb. wiss. Bot.*, **53**, 229 (1914).

TABLE 37

Utilization of Solar Energy by Various Leaves. (From Puriewitsch.)

No.	Species	Duration of Insolation Hours	Total Incident Energy, g. Cal. per sq. cm.	Increase in Heat of Combustion, g. Cal. per sq. cm.	Per Cent of Solar Energy Used in Photosynthesis
1	Acer plantanoides	6.0	361.03	2.208	0.6
2	" "	5.0	162.59	1.332	0.8
3	" "	6.0	240.33	6.508	2.7
4	" "	5.0	202.20	2.630	1.3
5	Polygonum sacchalinense	1.33	70.85	5.509	7.7
6	"	3.0	122.33	5.076	4.1
7	"	1.82	97.62	2.585	2.6
8	"	2.33	123.18	4.656	3.7
9	"	5.0	136.81	1.540	1.1
10	"	5.0	177.00	4.514	2.5
11	Helianthus annuus	4.5	132.48	5.977	4.5
12	Saxifraga cordifolia	2.33	68.16	3.450	5.0

there is an inverse ratio between the amount of energy used and the total energy falling on the leaves. The experiments were carried out in the open air during the summer in Kiew, so that the concentration of carbon dioxide was probably limiting the rate of photosynthesis, although the temperatures are not given. The temperature, which is an exceedingly important factor, would vary with the rate of transpiration of the leaf which in turn is dependent upon the "evaporating power of the air," a function of temperature, air movement and relative humidity. These experiments serve to demonstrate the extreme difficulty of determining the photosynthetic efficiency of a plant in the field and the necessity of taking into consideration many metereological factors in order to interpret the results obtained.

The wide variation in the results obtained by Puriewitsch are also noticeable in experiments with the same species. Thus *Polygonum sacchalinense* showed a utilization in 1.33 hours of 7.7 per cent, in 2.33 hours of 3.7 per cent and in 5 hours 1.1 and 2.5 per cent. This Puriewitsch considers as indicating a decreasing utilization with longer periods of time due to an accumulation of the products of photosynthesis. It has, in fact, frequently been observed that the rate of photosynthesis decreases as the products of photosynthesis accumulate; this phenomenon has been described under the discussion of the Time Factor.

The experiments carried out by Puriewitsch, it must be recognized, are not sufficiently extensive to permit drawing very satisfactory conclusions. They constitute an advance over the methods of Krascheninnikoff[9] who also determined the increase in the heat of combustion of leaves that had undergone photosynthesis, in relation to the amount of carbon dioxide absorbed. Krascheninnikoff's results indicate that carbohydrates

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[9] Krascheninnikoff, "Accumulation of Solar Energy in Plants" (Russian), Moscow, 1901.

are not the only products of photosynthesis. Apparently substances are formed in photosynthesis with a higher heat of combustion than that of glucose.

Puriewitsch also determined the coefficient of absorption of leaves, that is, the per cent of the total incident radiation which is absorbed by leaves. By means of a bolometer he determined the total amount of radiant energy falling on the leaf, and with another bolometer the energy which passes through the leaf when this is in an atmosphere containing carbon dioxide and when it is in a carbon dioxide-free atmosphere. He found that a leaf which is photosynthetically active (in an atmosphere containing carbon dioxide) absorbs more of the energy falling on it than a leaf in which no photosynthesis is taking place (in an atmosphere free of carbon dioxide). The amount of energy absorbed varied considerably with different species of leaves, from about 18 to 27 per cent. The leaves which were in an atmosphere containing carbon dioxide absorbed from 1 to 2.6 per cent more energy than those in an atmosphere without carbon dioxide. This excess of absorption by leaves in which photosynthesis is taking place is greater when the incident radiation is filtered through solutions which absorb a portion of the solar rays. When filtered through an alum solution leaves of *Acer plantanoides,* in an atmosphere containing CO_2, showed an excess of 4.93 per cent over the leaves in a CO_2-free atmosphere; when the light was filtered through a solution of gentian violet which was transparent for rays of 720-597 μμ and from 490 μμ to the end of the visible spectrum, there was an excess absorption of 4.25 per cent by the leaves in an atmosphere containing carbon dioxide. When the light was filtered through a ruby glass, transparent to rays of 590-690 μμ the excess absorption was 11.7 per cent. There is no adequate explanation for this excess absorption by leaves in which photosynthesis is taking place over those which were in an atmosphere free of carbon dioxide. It is possible that the products formed in photosynthesis may increase the absorption of the leaves in a measure. Results similar to those of Puriewitsch had previously been obtained by Detlefsen,[10] though by less accurate means. Ursprung[11] reinvestigated the problem, using a filament electric lamp as a source of light with a maximum intensity of 70,000 Lux. and air containing as much as 8 per cent carbon dioxide. He could not detect the effect reported by Detlefsen and by Puriewitsch so that we have here two sets of directly contradictory results.

This phenomenon, of an excess absorption of light due to photosynthesis, if it actually exists, is not without some theoretical interest. In photosynthesis the radiant energy of the sun is converted into potential chemical energy. Draper's fundamental law states that only the light which is absorbed is chemically active. Also it may be assumed that the light which is transformed into chemical energy must disappear as such. In two leaves, one of which is photosynthetically active and the other not,

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[10] Detlefsen, *Arb. bot. Inst. Würzburg,* 3, 534 (1888).

[11] Ursprung, *Ber. bot. Ges.,* 36, 124 (1918).

only in the former will there be a transformation of radiant into chemical energy, and in this leaf it might be expected that a greater absorption of radiant energy takes place. In the leaf which is not photosynthetically active there are *a priori* two possibilities of the disposition of the energy. It is possible that the amount of energy used in photosynthesis, in the case of the first leaf, is not absorbed by this leaf, but passed through the leaf which thus shows lower absorption. This would correspond to the findings of Detlefsen and of Puriewitsch. Or it is possible that in both leaves the same quantity of radiant energy is absorbed. In the leaf which is photosynthetically active a portion of the absorbed energy is converted into potential chemical energy; the rest is used in transpiration or is reradiated. In the leaf which is not photosynthetically active all of the absorbed energy is dissipated in transpiration or by reradiation. It might be expected that in the latter case there would occur a higher heating effect made evident by higher transpiration or thermal emission than in the photosynthetically active leaf. Photosynthesis would thus decrease the rate of transpiration. That this is the case has, in fact, been claimed by Déhérain[12] and Jumelle.[13] The point is, however, one of dispute,[14] though it ought not to be very difficult to establish which of the two possibilities of the disposition of energy, just referred to, occurs in the plant. In either case the possibility suggests itself, of using either the difference in absorption or in heating effect between a leaf which is photosynthetically active and one which is not, as a means of measuring photosynthesis. It must be borne in mind, however, that as the results of Brown and Escombe have shown, a great deal more of the radiant energy absorbed by the leaf is used in transpiration than in photosynthesis, under most conditions, so that the lower rate of transpiration, should such be caused by photosynthesis, in most cases would be very small. Also the indirect effects due to the absence of carbon dioxide in the one case may be such as to obliterate the slight increase in heating effect produced when photosynthesis is not taking place.

The results of Detlefsen and of Puriewitsch have a further theoretical implication, that is in connection with the chemistry of the photosynthetic process. They recall the early observations of Bunsen and Roscoe who found that the absorption of light by chlorine and hydrogen separately was less than when these two gases reacted in light to form hydrogen chloride. Bunsen and Roscoe concluded that the absorption of the reacting mixture was greater because energy is used to bring about the chemical reaction. The absorption of light by the separate components is termed optical or photo-thermal absorption and that absorbed to bring about the chemical reaction is "photochemical extinction."[15] The question of photochemical

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[12] Déhérain, *Ann. Sci. Nat.* (6), **4**, 177 (1876).

[13] Jumelle, *Rev. gén. Bot.*, **1**, 37 (1890); **2**, 417 (1891); **3**, 241 (1892).

[14] Burgerstein, "Die Transpiration der Pflanzen.," Jena, 1904.

[15] Sheppard, "Photochemistry," 1914, p. 187. Weigert, "Die Chemischen Wirkungen des Lichtes.," 1911, p. 49.

extinction has become a much debated subject, and, owing to the complex nature of photochemical reactions, has become quite involved. There is some evidence that Bunsen and Roscoe overestimated the magnitude of light absorption which can be ascribed to chemical extinction, and Pringsheim would ascribe this absorption to intermediate products in the photochemical reaction. Some distinction must, however, probably be made between the types of photochemical reactions, i.e., 1, those in which light performs work against chemical forces and 2, those in which the action of light is catalytic. In the former, light corresponding to the chemical work is used, there is a diminution of the entropy and the light must vanish; photochemical extinction is in such a case at least highly probable. As far as we know, photosynthesis is this type of reaction. To the second type of reaction can be counted those in which light accelerates a reaction proceeding slowly in the dark. There are as a matter of fact few endothermal photochemical reactions known and none in which the increase in potential approaches that of the photosynthetic reaction. It is obvious that a discussion of photochemical extinction in relation to photosynthesis raises many more questions than are capable of being answered, considering the meagre state of the experimental data available. The rôle of the chlorophyll pigments in photosynthesis, the fluorescence of these, the influence of surface layers are problems which have hardly been approached and the importance of which cannot be determined until a great deal more experimental work has been carried through.

Warburg and Negelein [16] approached the problem of the efficiency of the photosynthetic activity in a different manner. They studied the problem by using an aquatic plant, the unicellular alga *Chlorella vulgaris*, and thus avoided the difficulties attendant on determining transpiration and the indirect effect of changes in the size of stomatal openings.

If the radiant energy absorbed by the plant is designated by E and the energy utilized by U, the quotient U/E can be regarded as an expression of the efficiency. The ratio U/E changes with alterations in the intensity of the absorbed radiation. The ratio is less with high intensity; with decreasing intensity the value of the ratio increases and approaches a limit. It is this limit or point of maximum efficiency which Warburg and Negelein endeavored to determine by using low light intensities. The manner of measuring the rate of photosynthesis was in principle the same as that employed in their other investigations on photosynthesis, in which they made use of the Barcroft mannometer, which has been described in the chapter on Methods of Measuring Photosynthesis. Light of a limited spectral region was used, 570-645 μμ and the intensity was measured with a bolometer. The vessel in which the photosynthesis was carried out was filled with the alga so that all the light was absorbed and the absorbed radiation was thus equal to the incident radiation. In order to obtain a definite value for the ratio U/E, values for two different incident radiations were determined and then by extrapolation from these two values

[16] Warburg and Negelein, *Zeit. physik. Chem.*, 102, 235 (1922)

the value for the limiting case, where E is equal to zero, was calculated. In view of the fact that the course of the ratio U/E with changing light intensity was known for only two points, the values obtained by extrapolation must be subject to some error. This method of calculation was later altered in a subsequent publication. Some typical results are given in Table 38.

TABLE 38

Values of U/E Obtained by Warburg and Negelein.

Incident Radiation, Cal. per sq. cm. per mi. × 10^{-4}	E	U	$\frac{U}{E} \cdot 100$	$\lim_{E=0} \frac{dU}{dE} \cdot 100$
0.162	0.136	0.078	57	72
0.327	0.275	0.116	42	
0.203	0.171	0.096	56	67
0.406	0.341	0.155	45	
0.215	0.181	0.099	55	69
0.430	0.362	0.147	41	

As an average, it was found that 70 per cent of the absorbed radiant energy was utilized in photosynthesis. A later recalculation gave a mean value of 59 per cent and a maximum value of 63.5 per cent in red light. These results are of quite a different order from those obtained by Brown and Escombe and by Puriewitsch. They are, however, hardly comparable, for while the authors just mentioned used solar radiation, Warburg and Negelein worked with a limited portion of the spectrum, 570-645 μμ, also the plants in the two series of investigations were very different. Warburg and Negelein used very low light intensities and this factor was probably in relative minimum; their results also show an increasing percentage of energy utilized in photosynthesis with decreasing incident radiation. The results given in the table may be taken as the highest values obtained, for the amount of energy utilized in photosynthesis depends to a large extent upon the condition of the plants and the conditions under which these were cultivated. This applies especially to the chlorophyll-content of the algae used. Plants were kept under culture conditions of low light intensity and thus developed the capacity for utilizing the high percentage of absorbed radiant energy given in the table.

Warburg and Negelein[17] also determined the per cent of absorbed radiant energy utilized in photosynthesis with incident radiation of different frequencies. A quartz mercury vapor lamp was used as a source of light and the following lines were isolated: the yellow line $\lambda = 578$ μμ, the green line $\lambda = 546$ μμ, and the blue line $\lambda = 436$ μμ. These lines were obtained by the use of filters prepared according to the instructions of Hübl.[18] A red portion of the spectrum $\lambda = 610\text{-}690$ μμ with maximum intensity at 660 μμ was also used.

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[17] Warburg and Negelein, *Zeit. physik. Chem.*, **106**, 191 (1923).
[18] Hübl, "Die Lichtfilter," Halle, 1921.

The efficiency was determined in a manner quite similar to the method used in the experiments just described; E designates the absorbed radiant energy and W the energy transformed in photosynthesis. The latter was determined in the same manner, with the assumption that the following equation represents the reactions: $6\ (CO_2) + 6\ (H_2O) = (C_6H_{12}O_6) + 6\ (O_2) - 674{,}000$ calories.

The ratio W/E, or the quantity of chemical work done by one calorie of absorbed radiant energy is designated by Φ and is dependent upon the intensity of incident radiation. With increasing intensity Φ becomes smaller, and with decreasing intensity approaches a limiting value of Φ_0. In the previous determinations as already noted, Φ_0 was calculated by extrapolation from two values of Φ measured at two different intensities. As the course of curve of Φ with different intensities of light is not definitely known, Φ was determined at very low light intensities. Φ_0 was taken as the value obtained with the lowest light intensity. It has already been mentioned that the rate of photosynthesis varies greatly with differences of cultural conditions of the alga, and in these experiments cultures which showed a high photosynthetic efficiency were used. Warburg and Negelein conclude that the efficiency decreases with decreasing wave length. This is shown in Table 39. The value in the green light is somewhat questionable on account of the uncertainty of complete absorption. It was also found that there is apparently no relation between Φ_0 and the coefficient of absorption of the different wave lengths by the methyl alcoholic solution of the pigments of the alga.

TABLE 39

PHOTOSYNTHETIC EFFICIENCY OF CHORELLA WITH LIGHT OF DIFFERENT WAVE LENGTHS. (From Warburg and Negelein.)

	Red $\lambda = 660\ \mu\mu$ 610-690 $\mu\mu$	Yellow $\lambda = 578\ \mu\mu$	Green $\lambda = 546\ \mu\mu$	Blue $\lambda = 436\ \mu\mu$
Φ_0 per cent............	59	53.5	44.4	33.8

Warburg and Negelein endeavored to apply Einstein's law of photochemical equivalence to these results. This law is based upon the quantum theory of Plank, according to which energy is absorbed or radiated only in integral units equal to hv in which h is the Plank constant (6.547×10^{-27} ergs per sec.) and v is the frequency. Einstein formulated his theory of photochemical action on the assumption that the occurrence of a photochemical reaction is due to the absorption of quanta of radiation, each molecule requiring one quantum, hv, of a frequency v, characteristic of the absorbing molecule. Thus Einstein proposed the following formula:

$$n = \frac{Q}{hv}$$

in which Q is the absorbed heat required to produce the chemical reaction, n is the number of molecules dissociated by light of the frequency v. Each wave length thus has its own energy and the shorter the wave lengths of light, the larger are the units of energy as becomes evident from Table 40.

TABLE 40

ENERGY CORRESPONDING TO VARIOUS WAVE LENGTHS OF LIGHT.

Color	Wave Lengths in μμ	hv ergs	$Q = N hv$ cal.
Red	750–650	$2.62–3.02 \times 10^{-12}$	37,800–43,630
Orange	650–590	$3.02–3.33 \times 10^{-12}$	43.630–48,060
Yellow	590–575	$3.33–3.42 \times 10^{-12}$	48,060–49,320
Green	575–490	$3.42–4.01 \times 10^{-12}$	49,320–57,880
Blue	490–455	$4.01–4.32 \times 10^{-12}$	57,880–62,330
Violet	455–395	$4.32–4.97 \times 10^{-12}$	62,330–71,800

Einstein's principle was deduced for an ideal, reversible, unimolecular reaction of a gas or a dilute solution exposed to monochromatic radiation. It has been found that in some cases the photochemical equivalent is less than that demanded by theory and in other reactions it is many times greater.

The equation $n = \frac{Q}{hv}$ is apparently not directly applicable to the photosynthetic reaction, for one quantum of the wave-length used in photosynthesis is not sufficient to decompose one mole of carbon dioxide. Warburg and Negelein therefore proposed the formula (k a proportionality factor):

$$n = k \frac{Q}{hv} \qquad (1)$$

The equation would indicate that the number of molecules of carbon dioxide decomposed is proportional to the number of absorbed quanta, and assumes that each quantum absorbed produces the same chemical reaction. The proportional number of molecules of carbon dioxide decomposed by light of different wave lengths was thus calculated and k was taken to indicate the number of molecules of carbon dioxide decomposed by one quantum or $\frac{1}{k}$ the number of quanta necessary for the decomposition of one molecule of carbon dioxide. Thus the following number of quanta of different wave lengths have been calculated as required to decompose equivalent quantities of carbon dioxide. This has been obtained by dividing equation (1) by N_0, the Avagadro constant:

$$\frac{n}{N_0} = k \frac{Q}{hv\,N_0}$$

or $k = \Phi_0 \cdot hv \cdot N_0$, where Φ_0 is expressed in $\frac{\text{moles}}{\text{cal.}}$ (mean values).

It is apparent from these results of Warburg and Negelein for the decomposition of one molecule of carbon dioxide that there are required about 4 quanta of red and yellow light and about 5 quanta of blue light.

TABLE 41

Wave Length	$\Phi_0 \left[\frac{\text{Moles}}{\text{cal.}}\right]$	$N_0 h\nu$ (cal.)	k	$\frac{1}{k}$
660	5.25×10^{-6}	43,000	0.226	4.4
578	4.75×10^{-6}	49,200	0.234	4.3
4.36	3.01×10^{-6}	65,100	0.196	5.1

Adams[19] has recalculated some of the results of Warburg and Negelein. The latter based their calculations on the assumption that the photosynthetic reaction consisted in the immediate formation of glucose and that the heat of combustion of glucose with reversed sign equals the heat of formation. Instead of this, Adams used the heat absorbed in the following reaction:

$$CO_2 + 3\,H_2O = 2\,H_2O_2 + HCHO$$

The heat of this reaction is calculated as — 171.0 calorie-mole and the energy absorbed by the chlorophyll as 174.4 calorie-mole. On the assumption of the formation of glucose from six molecules of formaldehyde the maximum efficiency to be expected would be 64.4 per cent and on this basis Adams concludes that Warburg and Negelein's results, on a formaldehyde plus hydrogen peroxide basis, become 91.6 per cent for the mean observed efficiency and 98.6 per cent for the maximum observed efficiency in red light.

Weigert[20] has also developed a conception of the energetics of the photosynthetic reaction with the assumption of hydrogen peroxide as an intermediate product. That the primary step in photosynthesis consists in a splitting off of oxygen from carbon dioxide is considered as most unlikely by Weigert. Rather, he regards the formation of hydrogen peroxide of primary importance in the first photochemical step. According to his view chlorophyll is to be regarded as an optical sensitizer. The primary effect of the absorption of light by quanta results in an internal photoelectric effect, i.e., a shifting of an electron from one atom to another. There is thus formed, not a highly unstable structure of brief life, as a Bohr atom in a higher quantum state, but a new chemical system. The degree of probability of this new system passing to the final reaction product is of the same order as that of its passing back to its original condition. This conception of photochemical reactions has been developed by Weigert[21] for a number of other reactions. According to this view of the first step in the primary photochemical reaction, the value of $h\nu$ of the absorbed energy quantum (and accordingly the active frequency) is related to the kinetic energy with which the electron leaves its place (or orbit) in the atom or ion. Whatever reactions follow this primary reaction have no relation to the primary photoelectric process. Whether a number

[19] Adams, *Jour. Am. Chem. Soc.*, **48**, 292 (1926).

[20] Weigert, *Zeit. physik. Chem.*, **106**, 313 (1923).

[21] Weigert, *Zeit. physik. Chem.*, **101**, 414; **102**, 416 (1922); *Zeit. f. physik.*, **14**, 383 (1923).

of such primary photoelectric processes lead to an accumulation of a photochemical reaction product will depend thermodynamically upon the energy balance.

The necessity of oxygen for the photosynthetic process has been repeatedly demonstrated. The formation of hydrogen peroxide on illumination of light sensitive substances in the presence of water is well known. It is assumed that the primary reaction in photosynthesis is the loss of an electron from the chlorophyll molecule.

$$\begin{array}{c}
2\ \text{chlorophyll} + 2\ h\nu + 2\ O_2 = 2\ \text{chl}^+ + 2\ O_2^- \\
2\ O_2^- + 2\ H_2O = 2\ H + 2\ OH^- + 2\ O_2 \\
2\ H + 2\ OH^- + 2\ \text{chl}^+ = H_2 + H_2O_2 + 2\ \text{chl} \\
\hline
2\ H_2O + 2\ h\nu = H_2 + H_2O_2 \\
CO_2 + H_2 + H_2O_2 = HCOH + H_2O + O_2 \\
\hline
CO_2 + H_2O + 2\ h\nu = HCOH + O_2
\end{array}$$

It is undetermined whether in this scheme the free hydrogen atoms and hydroxyl groups or the H^-_2 and H_2O_2 react with carbonic acid.

According to these equations 2 energy quanta suffice to reduce 1 mole of carbon dioxide to formaldehyde, i.e., it is necessary that only one electron be carried by the molecular oxygen to each of the two water molecules. This will occur only with low light intensities; with increased intensity there will be a reduction in efficiency.

The thermochemical reactions are as follows:

$$\begin{array}{c}
2\ H_2O = H_2 + H_2O_2 - 92{,}000\ \text{calories} \\
CO_2 + H_2 + H_2O_2 = HCOH + H_2O + O_2 - 18{,}000\ \text{calories} \\
\hline
CO_2 + H_2O = HCOH + O_2 - 110{,}000\ \text{calories}
\end{array}$$

It is evident that this is an endothermic reaction, and the required energy is supplied by 2 N $h\nu$ in which N is the Avagadro constant. The magnitude of N $h\nu$ increases with decreasing wave length, i.e., for 700 $\mu\mu$ it is 40,500 calories and for 600 $\mu\mu$, 47,500 calories. These values must be doubled for the equation given above in which case there is still a deficit, *viz.:*

$$\begin{array}{r}
CO_2 + H_2O + 2\ N\ h\nu = HCOH + O_2 - 15{,}000\ \ (600\ \mu\mu) \\
- 29{,}000\ \ (700\ \mu\mu)
\end{array}$$

A complete balancing of the energy in the equation is attained only at 517 $\mu\mu$ when

$$2\ N\ h\nu = 110{,}000\ \text{calories}$$

This lessens the validity of the equations upon which the theory is based. Direct evidence hereto can be supplied only through experimentation in which the efficiency of the leaf is determined, i.e., the ratio of the radiant energy absorbed to that converted into potential chemical energy. The experiments of Warburg and Negelein are the most recent and probably the most exact bearing on this subject. Suffice it to recall that from these experiments it is concluded that for the reduction of one molecule of carbon dioxide there are required four quanta in red and yellow light and five quanta in the blue.

Nevertheless, Weigert considers that his scheme represents the actual state of affairs. He bases this largely upon the high temperature coefficient of the photosynthetic process. The temperature coefficient has been extensively investigated and it is evident that for purely photochemical reactions the photosynthetic process exhibits some decided abnormalities. Thus under conditions of high light intensity and high carbon dioxide-concentration, the temperature coefficient varies as follows for each 10° :

	5.4-10°	10-20°	20-30°
Q_{10}	4.3	2.1	1.6

With low light intensities the temperature coefficient decreases, and between 25 and 32° it is unity, indicating that under these circumstances the photosynthetic process is independent of temperature. Such is the case for most photochemical reactions. In these the energy requirements of the chemical reactions following the primary reaction is less than the energy of the absorbed quanta; i.e., the number of quanta absorbed, per unit time, regulates the rate of formation of the photochemical end products. Thus the rate of reaction is independent of the temperature of the system.

In the photosynthetic process the outstanding facts are that this is an endothermic reaction and that the absorption of energy quanta takes place in a long wave length region of the spectrum. Thus the two quanta calculated as necessary for the reaction do not suffice to cover the total energy requirements. In order that the process may take place at all the deficit of energy must be supplied by the system. This according to Weigert's theory would result in a cooling of the system and an automatic inhibition of the whole process. Thus, at higher temperatures, the supplying of the deficit is more easily accomplished than at lower temperatures and on this basis Weigert accounts for the abnormally high temperature coefficient at low temperatures. Moreover, the fact that with low light intensity the temperature coefficient approaches unity is explained by the fact that under these conditions, the rate of the primary process, as the slowest one in the system of step-reactions, determines the rate of the total reaction.

The theory of Weigert is of interest in connection with that proposed by Spoehr and McGee[22] on the relation between photosynthesis and respiration. They have considered the probability that a portion of the energy required for the reduction of carbonic acid is derived from the oxidation of carbohydrates in the chlorophyllous cells. If the energy liberated by the oxidative processes first appears in the form of light

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[22] Spoehr and McGee, Carnegie Inst. of Wash., Pub. No. 325, 76 (1923).

of low frequency[23] it is more conceivable how this would be of value to the photochemical process than the heat *per se*. Although these theoretical considerations are still to be tested by much experimental work, they are not without interest in the light of the results claimed by Detlefsen and by Puriewitsch which have already been discussed.

Results of the efficiency of photosynthesis somewhat different from those of Warburg and Negelein have been obtained by Wurmser.[24] He concludes that *Ulva lactuca* shows a photosynthetic efficiency in red light, 590-700 μμ, of 59 per cent and in green light 490-590 μμ, of 83 per cent. Wurmser had previously found a high value of photosynthetic efficiency in the green portion of the spectrum and considers that this maximum efficiency in the green is due to the rôle which the colorless portion of the protoplasm plays in photosynthesis. He is of the opinion that the liberation of oxygen cannot take place in the presence of chlorophyll, for this is very sensitive to photooxidation. Wurmser considers that the method of Warburg and Negelein gave values which are too low for the portions of the spectrum which are feebly absorbed, as the green. It should be stated that Warburg and Negelein did not put particular value on their determinations in this portion of the spectrum. Wurmser also considers that Warburg and Negelein's results in the blue-violet region are too high because of the absorption of pigments other than chlorophyll. According to Wurmser, neither his results nor those of Warburg and Negelein represent the maximum efficiency obtainable in the red. He is of the opinion that the efficiency is practically the same for all wave lengths and probably in the neighborhood of 70 to 80 per cent.

2. The Storing of Energy by Crop Plants

In the previous section the question has been discussed what proportion of the radiant energy absorbed by the chlorophyllous portions of a plant are utilized in photosynthesis. In the earlier experiments, this was based upon total solar radiation incident on a leaf, while in the later, more refined experiments, the source of light was confined to a portion of the spectrum. As a practical matter, it would be desirable to know what portion of the total energy of solar radiation, falling on an area of land under cultivation, is transformed into the potential chemical energy of the material constituting the body of the plant.

That this is not an easy matter to determine hardly needs emphasis. Nor can such a determination be taken as a measure of the photosynthetic efficiency of the plants. The growth and development of a plant depend upon a number of factors, of these, the formation of substances formed in the photosynthetic process, is but one. Pütter[25] has endeavored to

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[23] Becking, L. B., "Radiation and Vital Phenomena," Dissertation, Utrecht, 1921, p. 62.

[24] Wurmser, *Ann. de Physiol. Physiochim. Bio.*, **1**, 47 (1925); *Compt. rend. Soc. Biol.*, **83**, 437 (1920); *Bull. Soc. Chim. biol.*, **6**, 487 (1923).

[25] Pütter, *Die Naturwissenschaften*, **2**, 169 (1914). Mayer, *Landw. Versuchsstat.*, **40**, 205 (1892).

calculate what portion of the energy of solar radiation incident on a unit area of open field is utilized in the synthesis of organic material. As a basis of his calculations he used the yields of various field crops. He included in these calculations the original weight of the seed, and the loss of weight of the seedling through respiration. The total production of the crop was increased by 15 per cent to allow for the material consumed in respiration. Other material losses of the plant, such as pollen, were disregarded. The analyses of different crops grown in Germany were used and the energy determined on the basis that 1 gram crude protein was equivalent to 4.8 calories, 1 gram crude fat to 9.2 calories, 1 gram nitrogen-free extract to 4.0 calories and 1 gram crude fiber to 4.0 calories. The organic dry materials (ash free) remaining in the soil were taken as: wheat 2669 kilograms, rye 4044 kilograms, barley 1802 kilograms, and oats 2111 kilograms, and the heats of combustion of these were calculated on the basis of 3.6 calories per gram. The heats of combustion of the grain calculated to 3.59 calories per gram and that of the straw to 3.45 calories per gram. The incident solar radiation was calculated for a growing season of 18-21 weeks.

The values in Table 42 illustrate the results of Pütter's calculations of the per cent utilization of solar energy. These show an average utilization of about 3 per cent.

TABLE 42

Utilization of Solar Energy of Crop Plants in the Field. (Pütter.)

Plant	Mean Incident Solar Radiation, cal. per sq. m.	Unusually High Crop Yields, cal. per sq. m.	Per Cent Utilization of Solar Energy	Per Cent Utilization of Solar Energy, Including Material Used in Respiration
Summer wheat	221,500	6270	2.83	3.26
Summer rye	209,000	4670	2.25	2.60
Summer barley—				
2 rowed	195,000	4375	2.25	2.60
4 rowed	154,000	3602	2.34	2.68
Hooded	123,000	3930	3.20	3.68
Oats	218,000	6270	2.87	3.31
Potatoes	250,000	6530	2.62	3.02
Beets	300,000	5500	1.84	2.12

Chapter 7

Chlorophyll and the Chloroplasts

1. Chlorophyll

The green pigment of leaves has been the subject of scientific investigation for a great many years. Even prior to the discovery of photosynthesis and before the importance of the green color for the development of the plant was recognized, much study was devoted to the chemistry of the pigments of leaves, and several names which are famous in the annals of chemistry, such as Berthollet, Proust and Berzelius are associated with the early attempts to determine the nature of the leaf pigment. With the realization of the significance of the green pigment to virtually all life on the planet, the interest in this substance also grew rapidly and it was soon regarded as one of the most important substances in nature. That the large number of investigations of the nature of the leaf pigments yielded relatively little valuable information must be ascribed to the great instability of these compounds and to the fact that chemical technique had not been sufficiently developed to enable workers to handle these compounds.

The suggestion of the term "chlorophyll" is usually ascribed to Pelletier and Caventou;[1] these authors also finally recognized the fact that this was not a single substance but a mixture of a number of compounds. We cannot here go into a discussion of the development of the chemistry of the leaf pigments; the subject has been adequately treated by others.[2] Most of the efforts have been directed toward establishing the chemical composition and the constitution of the very complex molecules of the chlorophyll pigments and to determine the physical properties of these, especially in relation to the absorption of light. Relatively little work has been done on the rôle which the chlorophyll pigments play in the photosynthetic process.

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[1] Pelletier and Caventou, *Ann. Chim. Phys.* (2), **9**, 194 (1818); (2), **51**, 182 (1832).

[2] Czapek, "Biochemie der Pflanzen.," 2 Ed., Jena, 1913, Vol. I, p. 555. Willstätter and Stoll, "Untersuchungen über Chlorophyll," Berlin, 1913. Marchlewski, "Chemie der Chlorophylle," Braunschweig, 1909. Tswett, "Chlorophylls in the Plant and Animal Worlds" (Russian), Warsaw, 1910. Palmer, "Carotinoids and Related Pigments," Chemical Catalog Co., N. Y., 1922. Tschirch, "Untersuchungen über das Chlorophyll," Berlin, 1884.

a. The Extraction and Separation of the Leaf Pigments.

A method which has aided considerably in the separation of the different leaf pigments and has been of much value in demonstrating the presence of the different pigments is that devised by Tswett.[3] The method is based upon the difference in adsorption affinities of the various pigments by dry, powdered calcium carbonate or other adsorbents. A dry benzol, petroleum ether or carbon disulfide extract of the leaf pigments is filtered through a column of powdered calcium carbonate. The latter thus becomes differentiated into zones of different color, the various pigments being adsorbed in different portions of the column. There is thus formed a stratified column called a chromatogram, each zone of which contains a different pigment. The various zones can be sharply separated and the pigments contained therein can be extracted by means of different solvents.

Tswett prepared the solutions of the leaf pigments in the following manner. The leaves were ground with emery and a small quantity of magnesium oxide or calcium carbonate and extracted with alcoholic petroleum ether (1 : 10). The alcohol was removed by washing and the solution was dried. Less successful were the methods of extracting with petroleum ether leaves which had previously been treated with boiling water or of extracting the neutralized leaf material with alcohols, acetone, ether or chloroform, distilling off these solvents and redissolving the pigment in petroleum ether or carbon bisulfide. Tswett's chromatograms had the following adsorption-zones: I. (from top) colorless; II. yellow, containing xanthophyll β; III. dark olive green designated by Tswett as chlorophyllin β; IV. dark blue green, designated as chlorophyllin α; V. yellow, containing two xanthophyll pigments; VI. colorless; VII. orange yellow, xanthophyll α. The yellow carotin was not adsorbed by the calcium carbonate. In this manner Tswett attained a separation of the leaf pigments and by extracting the various zones he was able to differentiate the pigments spectroscopically. Sufficiently large quantities for exhaustive chemical analysis could, however, not be obtained by this method. It remained for Willstätter and his co-workers to devise methods for the isolation of the different leaf pigments.

Although the pigments in almost all leaves are the same, they cannot be obtained from different species with equal ease and with the same degree of purity. For obtaining preparations of the different pigments it is very essential that proper consideration be given the choice of the plant material. Leaves which have relatively little chlorophyllase, an enzyme which partially breaks down chlorophyll, are to be preferred. The nettle has proved to be very satisfactory material for extraction and was used extensively by Willstätter and some earlier workers.

Most of the early workers extracted the leaf pigments from fresh leaves usually by means of boiling alcohol. Willstätter recommends previously drying the leaves. This procedure has several advantages; a much

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[3] Tswett, *Ber. bot. Ges.*, **24**, 316, 384 (1906).

smaller volume and weight of leaf material can be used, there is an economy in solvents, because these do not become diluted by the water in the leaves (this is 70-80 per cent of the fresh weight), the extraction is not dependent upon the season of the year or the location of the plants, and very considerable quantities of the pigments can be prepared. It should also be stated, however, that in the drying of the plant material there are some disadvantages. The leaves must be dried very carefully in order to avoid destruction of the chlorophyll; some leaves, as, for example, elder and the needles of conifers, lose considerable chlorophyll during drying. Some plant material, also, cannot be stored without loss of chlorophyll unless the powdered material is kept in vacuum desiccators over sulfuric acid.

Precautions must also be taken in the extraction of these pigments from the dry material. Chlorophyll is easily soluble in benzol and dry acetone, but the pigment cannot be extracted by these solvents. The ease of extraction is dependent not only upon the solubility in a given solvent. The dry leaf material is extracted only very slowly by absolute alcohol, ether, chloroform and acetone and not at all by benzol, petroleum ether and carbon bisulfide. On the other hand, extraction can be accomplished rapidly with methyl alcohol. Most of the solvents just mentioned extract chlorophyll quite readily if there is a certain amount of water present; the exception is methyl alcohol which extracts more easily in the anhydrous condition. The cause for this seems to lie in the colloidal state of the chlorophyll in the leaf. From colloidal aqueous solutions of chlorophyll no pigment can be extracted by shaking with pure ether or benzol, according to Willstätter and Stoll. If a small quantity of salt, e.g., calcium chloride, is added to the colloidal chlorophyll solution, practically all the chlorophyll passes rapidly into the organic solvents. Willstätter and Stoll have made determinations of the amount of chlorophyll extracted by different organic solvents containing varying quantities of water.

For most purposes 80 per cent acetone by volume is most useful. Willstätter and Stoll's method was to place the finely powdered leaf material in a large Nutsch filter and the acetone is poured into this. By alternate suction and percolation and adding the solvent in several portions, the leaf material is extracted to a straw yellow color. The leaf material must not form too thick a mat on the filter. In small quantities, e.g., 500 grams of material the chlorophyll can be extracted with 1500 to 1600 cc. of solvent and in half an hour yield 900 cc. of extract containing 4.5 grams of chlorophyll.

For extracting the pigment from fresh material, this is first ground very thoroughly, which forms a thick olive brown paste. Thus, for example, starting with 2.5 kilos of fresh nettle leaves, the paste is shaken with 1500 cc. of acetone and filtered with suction. The filtrate contains no chlorophyll. The residue is subjected to a pressure of 200 atmospheres in a Buchner press, and ground again. It is then shaken for five minutes with 1500 cc. of acetone which, with the water remaining in the plant material, becomes diluted to about 80 per cent by volume, and to this another liter of 80 per cent acetone is added. It is filtered and washed three times with 80 per cent acetone. The extract contains the chlorophyll. This method is very serviceable for the extraction of plant material which is dried with difficulty, e.g., mucilaginous plants.

For the separation of the 80 per cent acetone extract into the different pigments Willstätter and Stoll recommend the following procedure. The acetone extract (from 2 kilograms of leaves) is poured into a separatory funnel containing 4000 cc. of petroleum ether (0.64-0.66) and 500 cc. of water are added, shaking carefully. The water is drawn off, and the petroleum ether layer is washed twice, each time with a liter of 80 per cent acetone; this removes colorless impurities. The acetone which has been dissolved in the petroleum ether layer is removed by washing four times with 500 cc. each of water. The petroleum ether is now shaken with three lots of 2000 cc. each of 80 per cent methyl alcohol. If the last extract is still appreciably colored, the petroleum ether is shaken once or twice more with methyl alcohol. The methyl alcohol extracts contain xanthophyll and a small quantity of chlorophyll *b*. The xanthophyll may be purified according to the method described below.

The isolation of chlorophyll from the petroleum ether solution is accomplished by first washing this with water, four times, each with 2000 cc., in order to remove the methyl alcohol and acetone. Thereby the petroleum ether solution loses its fluorescence, becomes turbid and the chlorophyll begins to precipitate. The suspension in petroleum ether is shaken with anhydrous sodium sulfate and talc and is filtered through a thin layer of talc with suction. The filtrate contains besides a little chlorophyll, considerable *carotin*. The talc holding the chlorophyll is washed with petroleum ether until the filtrate is colored but slightly yellow and then with petroleum ether of boiling point 30-50°. This is sucked off completely and the chlorophyll is dissolved immediately from the talc in ether. The ether solution is filtered through anhydrous sodium sulfate and concentrated to 100 cc., filtered again and concentrated to 25 cc. The chlorophyll is precipitated by the addition of 800 cc. low-boiling petroleum ether. A blue-black powder is formed or sometimes a suspension of fine particles which can be filtered only on talc. This is again dissolved in 20 cc. of ether and permitted to evaporate in a desiccator. There are thus obtained 6.5 grams of chlorophyll from a kilo of leaf material.

Certain tests can be applied to the chlorophyll preparation, obtained in the manner described, in order to determine its purity. The ash should amount to 4.5 per cent and consist of pure magnesium oxide. The ash of pure chlorophyll contains no phosphorus nor iron; the presence of both of these elements has been a matter of dispute for some time. The chlorophyll preparation should contain no yellow pigments. The presence of the yellow pigments becomes evident in the following test. The ether solution is shaken with methyl alcoholic potassium hydroxide, thereby the color becomes brown, and in a few minutes the original chlorophyll color appears in the alkaline medium. During this operation the ether should remain colorless; if carotin or xanthophyll are present, these pass into the ether on the addition of water. A quantitative determination of the phytol-content of the preparation can also be used as a test of its purity.

Willstätter and Stoll have concluded that the chlorophyll from different plants does not differ in composition. This conclusion is based upon the analyses of chlorophyll from a large number of different species, including marine algae, fresh water plants and land plants from the temperate zone and the tropics, representing many different families. They concluded that most plants contain four pigments, viz., two yellow pigments, carotin and xanthophyll and two green pigments, chlorophyll *a* chlorophyll *b*. Thus far in this chapter the two latter pigments have been treated together as "chlorophyll." That the latter is actually composed of two pigments was already considered probable by Stokes [4] and, as has already been mentioned, by Tswett who by means of his chromatograms was able to demonstrate the existence of two chlorophyll pigments. These he designated as α- and β-chlorophyllin which Willstätter and Stoll called chlorophyll *a* and chlorophyll *b*. Marchlewski [5] used the terms neo- and allochlorophyll for the two components.

The separation of chlorophyll a and chlorophyll b is a rather tedious process; Willstätter and Stoll accomplished this by taking advantage of the fact that cholorophyll *a* is more soluble in petroleum ether while chlorophyll *b* is more soluble in methyl alcohol. Eight grams of chlorophyll are dissolved in 150-200 cc. of ether, and the dark solution is filtered into 4000 cc. of petroleum ether (0.64-0.66). In order to prevent the precipitation of the chlorophyll 50-100 cc. of methyl alcohol are added. The ether is removed by shaking with 2000 cc. of 80 per cent methyl alcohol.

The petroleum ether solution is now extracted with methyl alcohol in order to remove the chlorophyll *b* component. For this purpose 85 per cent methyl alcohol is saturated with petroleum ether and 0.01 gram oxalic acid is added to each liter. About 14 extractions with 2000 cc. each of 85 per cent methyl alcohol are required to extract the chlorophyll *b;* the chlorophyll *a* remains in the petroleum ether. The first methyl alcohol extract, after separation, is brought to a concentration of 90 per cent by the addition of 1000 cc. of methyl alcohol, washed with 1000 cc. of petroleum ether, added to 2000 cc. of ether and then considerable water is added to this.

To the second methyl alcohol extraction a liter of methyl alcohol is also added, washed with the petroleum ether used for washing in the first extraction to which another 500 cc. has been added. This methyl alco-

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[4] Stokes, *Proc. Roy. Soc.*, **13**, 144 (1864). Sorby, *ibid.*, **21**, 442 (1873).

[5] Jacobson and Marchlewski, *Biochem. Zeit.*, **39**, 174 (1912); **40**, 296 (1912).

hol extraction is then added to the ether containing the first extract and another liter of ether is added. The petroleum ether, used for washing, is freed from methyl alcohol by a stream of water, whereby some dissolved pigments are precipitated.

The third and fourth methyl alcohol extracts are treated in the same manner, but the amount of the *b* component becomes less in each extraction. To the sixth methyl alcohol extract, before washing with petroleum ether, only 900 cc. of methyl alcohol are added, to the seventh 800 cc., the eighth 700 cc. and to the fourteenth only 100 cc. They are all washed with the same petroleum ether used in the first and second extractions and are added to the same ether solution, always with the addition of more ether, at first 1000 cc. and after the tenth extraction 500 cc.

When there is a great deal of chlorophyll *b* in the original chlorophyll, the sixth and seventh extraction are also brought to 90 per cent by the addition of methyl alcohol; to the following extractions less of the methyl alcohol is added.

The sixteenth and seventeenth extractions have the purpose of freeing the chlorophyll *a* from the last traces of chlorophyll *b*. These methyl alcohol extractions are rich in chlorophyll *a* and are therefore well washed with petroleum ether.

The petroleum ether solution, thus freed from chlorophyll *b*, contains the chlorophyll *a* component. The solution is washed with water until the chlorophyll has been precipitated quantitatively. This precipitate is adsorbed with 30-100 grams of talc and the whole is filtered carefully with suction through a layer of talc, and washed with low-boiling petroleum ether. The suction is continued until there is no odor of petroleum ether. The chlorophyll *a* is then extracted from the talc with pure ether and filtered several times. Most of the ether is evaporated and the last portion of the ether is permitted to evaporate in a vacuum desiccator. The chlorophyll *a* remains as a beautifully shining, blue-black mass.

The chlorophyll *b* component, which has been extracted by means of methyl alcohol, is now in an ether-petroleum ether solution. This is washed with water to free it from methyl alcohol and, after drying with sodium sulfate, is concentrated to about 500 cc. and finally at reduced pressure, to 30 to 40 cc. From this solution the main portion of the chlorophyll *b* is precipitated by the addition of 300 cc. petroleum ether (30-50°). This precipitate is filtered immediately onto talc. The filtrate contains considerable quantities of chlorophyll *a*. The precipitate also contains some chlorophyll *a*, and has to be dissolved again in ether and reprecipitated with low-boiling petroleum ether. This dissolving and reprecipitation process has to be repeated two to three times if there are relatively large quantities of chlorophyll *a* present. Chlorophyll *b* is more easily filtered than the *a* component; the former can therefore be collected on a hard filter and finally dried in a desiccator.

The yellow pigments carotin and xanthophyll are widely distributed in nature, in both plants and animals, and as they apparently occur in different isomorphous and isomeric modifications these have, at the suggestion of Tswett, been grouped under the term carotinoids. We shall here confine the discussion to the yellow pigments in the chloroplasts of green leaves and not include the pigments of "autumn leaves" nor of yellow, orange and red flowers and fruits.

Arnaud[6] succeeded in isolating carotin from green leaves by extraction with petroleum ether. Leaves of spinach, vacuum dried and powdered, were extracted with petroleum ether. The petroleum ether was distilled and from the concentrated solution the solvent was evaporated at ordinary temperature. The waxy substances were removed by washing the residue with a little cold ether and the pigment was recrystallized from petroleum ether. When the leaf material is perfectly dry rapid extraction with petroleum ether removes no chlorophyll nor xanthophyll.

Willstätter obtained the yellow pigments as a side product in the preparation of chlorophyll. The plant material was extracted with 80 per cent acetone and the dissolved pigments were transferred to petroleum ether, as has already been described. The petroleum ether solution is freed from xanthophyll by washing three times with 80 per cent methyl alcohol. These extracts are bright green. The pigment is extracted from the methyl alcohol by the addition of ether (4 to 5 liters) and dilution with water. The ether solution contains xanthophyll and some chlorophyll *b*. This latter can be removed by shaking the ether solution with 30-50 cc. of concentrated methyl alcoholic potassium hydroxide and subsequent extraction with water. Direct crystallization of the xanthophyll, without removal of the chlorophyll *b*, can be accomplished by drying the ether with sodium sulfate, concentrating to about 30 cc. and adding 200-300 cc. methyl alcohol. This solution is again heated gently to evaporate the ether and is filtered. On cooling the xanthophyll crystallizes in shining plates. In order to have the xanthophyll crystallize out more completely water may be added to the methyl alcohol solution; under these conditions the xanthophyll separates in crystal aggregates which gradually change into plates. The yield of xanthophyll is about 0.8 gram for 2 kg. of dried nettle leaves.

The petroleum ether chlorophyll solution from which the xanthophyll has been removed with 80 per cent methyl alcohol, as just described, still contains *carotin*. The petroleum ether solution is washed four times, each with 2000 cc. of water. Thus the methyl alcohol is removed and the chlorophyll is precipitated. The petroleum ether, after filtration, is concentrated in vacuum at 40° and the oily residue is treated with 300 cc. 95 per cent alcohol. The carotin rapidly separates out in steel-blue crystals. In order to free the carotin from impurities it is dissolved in

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[6] Arnaud, *Compt. rend.*, **100**, 751 (1885). Van Wisselingh, *Flora*, **107**, 371 (1915). Coward, *Biochem. Jour.*, **18**, 1114, 1123 (1924).

200-300 cc. of petroleum ether, it is filtered rapidly, and washed with a mixture of 2 volumes of petroleum ether and 1 volume of alcohol. The yield is about 0.25 gram. Carotin can, of course, be obtained very much more easily and in larger yields from carrots than from green leaves. Willstätter obtained 125 grams of pure carotin from 5000 kilograms fresh or 472 kilograms dry carrots.

Recently Schertz[7] has published detailed methods for the isolation and purification of xanthophyll and carotin by methods based upon those of Willstätter and Stoll. Schertz states that to obtain the purest preparations of xanthophyll, crystallization must first be made from methyl alcohol and that the pigment must then be precipitated from chloroform by the addition of petroleum ether.

The pigments of the algae differ in certain respects from those of most land plants, although there is little reason for believing that the course of the photosynthetic reactions in the two types of plants is not the same. The difference lies rather in the fact that while in most flowering plants only four pigments are to be found, the algae present a variety of color, red, brown and blue-green. These plants usually contain the same four pigments, i.e., chlorophyll *a* and *b*, carotin and xanthophyll, although the proportion of these is different, and in addition they contain other pigments which give them their characteristic color and more or less mask the color of the chlorophyll. A tremendous amount of work has been done on the development of these pigments in the plants under different circumstances, on the physiological rôle of the pigments as well as on their chemical composition.[8] From the brown algae there has been isolated a pigment which is typical of these plants, fucoxanthin, of the empirical formula $C_{40}H_{54}O_6$, and due to the efforts of Willstätter[9] and his collaborators, the methods of obtaining this pigment, its properties and chemical composition have been quite well established. The red pigment, phycoerythrin and the blue pigment phycocyanin, though extensively investigated, have as yet not been subjected to exhaustive chemical study. They are complex nitrogenous substances, related to protein but of a lower nitrogen content. There has been much speculation regarding the possible rôle which these pigments play in the photosynthetic process, though no satisfactory conclusions have been arrived at. Fucoxanthin is a carotinoid and its absorption spectrum is similar to that of carotin and of xanthophyll. Phycoerythrin and phycocyanin are strongly fluorescent, a property which has been the subject of much study.[10]

[7] Schertz, *Jour. Agri. Res.*, **30**, 469, 575 (1925).

[8] Czapek, "Biochemie der Pflanzen," 2nd Ed., I, 594.

[9] Willstätter and Stoll, "Untersuchungen ü. Chlorophyll," 141, 247 (1913).

[10] Lloyd, *Science*, **58**, 91, 229 (1923); **59**, 241 (1924); *Trans. Roy. Soc. Canada* (3), **17**, 129 (1923). Boresch, *Biochem. Zeit.*, **119**, 167 (1921); *Ber. bot. Ges.*, **39**, 93 (1921). Hanson, *New Phytologist*, **8**, 337 (1909). Kylin, *Zeit. physiol. Chem.*, **69**, 169 (1910); **74**, 105 (1911); **76**, 396 (1912); **82**, 221 (1912); **94**, 337 (1915). Schütt, *Ber. bot. Ges.*, **6**, 36, 305 (1888). Wille, *Ber. bot. Ges.*, **40**, 188 (1922). Teodoresco, *Rev. Gén. Bot.*, **32**, 145 (1920).

b. The Physical Properties of the Leaf Pigments.

Chlorophyll *a* and *b* precipitated from ether by means of petroleum ether are microcrystalline. On slow evaporation of solution in an ether-petroleum ether mixture the *a* component crystallizes in bundles of narrow, thin plates. Dry chlorophyll *a*, as usually obtained, is a blue-black, soft powder, chlorophyll *b* is a dark green, almost black powder.

Chlorophyll *a*, according to Willstätter and Stoll, softens and runs to a tough mass when heated to 117-120°; the *b* component softens between 86-92°, becomes molten at 120-130° and swells.

Mention has already been made of the difference between the solubility and the ease of extraction of the pigments from the leaf. Chlorophyll *a* is very soluble in absolute alcohol, ether, acetone, chloroform, carbon bisulfide and benzol. In methyl alcohol, in the cold, it is not very soluble, though easily when the reagent is warm. In 95 per cent ethyl alcohol it dissolves readily, in 80 per cent with difficulty; in 90 per cent methyl alcohol, even when warm, it is only slightly soluble; in 80 per cent methyl alcohol it is almost insoluble. In petroleum ether it is only slightly soluble, in ligroin it is more soluble, easily when warm. A very small amount of alcohols in the petroleum ether greatly increases the solubility in the latter solvent.

The solubility of chlorophyll *b* is in general somewhat less than that of the *a* component; this is especially noticeable in petroleum ether in which chlorophyll *b* is insoluble in the cold and the solution becomes but only slightly colored on boiling. Chlorophyll *b* is easily soluble in absolute alcohol, ether and the reagents which dissolve the *a* component.

Chlorophyll *a*, in ethyl alcohol solution, is blue-green, with a deep red fluorescence. In concentrated ether solution the color is almost blue which takes on a green hue on dilution. A colloidal solution of chlorophyll *a*, which can be kept for weeks, is produced by diluting rapidly a concentrated solution in alcohol or acetone with much water. This solution is green and does not fluoresce, but has a blue-green opalescence. This solution can be extracted with ether only by prolonged shaking with the solvent; however, on addition of a little calcium chloride it goes into the ether immediately.

The color of solutions of chlorophyll *b* does not vary as that of the *a* component in different solvents. The ether solution is bright green. In comparison to solutions of chlorophyll *a*, those of chlorophyll *b* have a yellow hue; in carbon bisulfide the latter are yellow-green. The fluorescence of chlorophyll *b* solutions is brown-red.

The absorption spectra of solutions of chlorophyll have been examined very extensively, and these have been used to some extent to determine the purity of the chlorophyll preparations. Tswett[11] was able to isolate individual pigments from his chromatograms and study the

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[11] Tswett, *Ber. bot. Ges.*, **25**, 388 (1907). Englemann, *Zeit. wiss. Mikrosk.*, **5**, 289 (1888). Brdlik, *Compt. rend.*, **147**, 990 (1908).

absorption spectra of the various components. The absorption of chlorophyll in the infra-red and ultra-violet has been studied by Van Gulik[12] and in the latter region also by Marchlewski and Moroz.[13]

In the publication of Willstätter and Stoll are reproduced the spectrographs of chlorophyll solutions of various concentrations. Chlorophyll *a* has seven sharply defined absorption bands in the visible portion of the spectrum and the end absorption in the violet. The strongest absorption is evidenced by one band in the red, one in the indigo-blue and one in the violet portion; the weakest absorption bands are in the yellow and green. The following absorptions are given by Willstätter and Stoll for 0.0431 gram of chlorophyll *a* in 1000 cc. of ether (0.001 mole in 20 liters).

	Thickness of Layer in mm.			
	2.5	10	40	80
Band I	669— —655	675—648	680—637.625	684—596.587
" II	619 | 605	623..603	625— —600	
" III	—	585.570	586...564	587—561
" IV	—	—	539..523	541...521
" V	—	—	504 | 489	504.488
" VI	462 | 455	465.453	471. 468—	473—
" VII	439—427	444—		
End absorption VIII.	...415—			

The absorption spectrum of chlorophyll *b* has nine bands between $\lambda = 700$ and 410 μμ and the end absorption beginning at 400 μμ. The intensity of these bands diminishes in the following order: VIII, II, IX, X, I, IV, III, V, VII.

The following absorption bands are given by Willstätter and Stoll for 0.0431 gram of chlorophyll *b* in 1000 cc. of ether:

	Thickness of Layer in mm.			
	2.5	10	40	80
Band I	666 . 659	667...659.651	673—625	677—582..
" II	648...638	651—635		
" III	—	615 | 611	615.609	574...559
" IV	—	599.585	600...583	
" V	—	—	571.559	
" VI	—	—	547..530	549...530
" VII	—	—	506 | 500	
" VIII	467—446	474—	483—	508.489
" IX	433..424			
End absorption X....	407—			

Warburg and Negelein[14] also determined the coefficient of absorption of a solution of an extract of all the leaf pigments obtained from *Chlorella* according to the method of Willstätter. A methyl alcohol solution thus showed lowest absorption in the green (546 μμ), 1.8 times this

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[12] Van Gulik, *Ann. Physik.* (4), **23**, 277 (1907); **46**, 147 (1915). Eckerson, *Bot. Gaz.*, **40**, 302 (1905).

[13] Marchlewski and Moroz, *Bull. soc. chim.*, **35**, 705 (1924). Ursprung, *Ber. bot. Ges.*, **36**, 73 (1918).

[14] Warburg and Negelein, *Zeit. physik. Chem.*, **106**, 195 (1923).

in the yellow (578 μμ), 9 times the absorption in the red (660 μμ) and 23 times in the blue (436 μμ). The yellow pigments, which were included in the solutions examined, it was found, do not absorb any red, yellow or green light; in the blue-green region of the spectrum the yellow pigments are responsible for about 46 per cent and in the blue for about 30 per cent of the absorption of the mixture of leaf pigments.

In Table 43 are given some results of Tswett[15] who made spectrophotometric measurements of the two chlorophyll components in alcohol solution. An Englemann microspectrophotometer with a grating and a Nernst lamp, as a source of light, were used.

TABLE 43

PER CENT TRANSMISSION OF LIGHT OF DIFFERENT WAVE LENGTH BY CHLOROPHYLL *a* AND *b* IN ALCOHOLIC SOLUTION. (From Tswett.)

Chlorophyll *a*		Chlorophyll *b*			
		I		II	
λ	J/J_0	λ	J/J_0	λ	J/J_0
680–690	53.5	—	—	—	—
675–680	15.0	—	—	—	—
670–675	4.8	—	—	—	—
660–670	1.9	660–670	71.5	665–675	74.5
657–660	7.9	650–660	54.2	655–665	61.0
650–655	18.0	640–650	45.8	645–655	48.0
640–650	32.1	630–640	65.8	635–645	53.0
620–640	51.2	—	—	—	—
500–510	74.2	—	—	—	—
490–500	72.4	—	—	490–500	71.8
480–490	66.5	—	—	485–490	54.0
470–480	63.0	—	—	480–485	41.7
460–470	53.8	475–480	34.5	475–480	40.2
555–460	42.2	470–475	27.3	470–475	26.3
450–455	33.3	460–470	15.7	460–470	18.8
445–450	20.0	450–460	20.1	450–460	21.7
440–445	10.6	440–450	33.0	440–450	40.2
430–440	0.9	430–440		430–440	40.3
420–430	2.4	—	—	420–430	62.0

As can be seen in Table 43, Tswett found maximum absorption in the blue end of the spectrum. Ursprung summarizes the results of various observers of the location of the maximum absorption of chlorophyll solutions thus: Reinke, 676-663 μμ; van Gulik, 673-666 μμ; Englemann, 665-660 μμ, and Donath, 638 μμ. Ursprung reports maximum absorption with a narrow slit at 661 μμ, and with a wide slit at 637 μμ. Iwanowski[16] reports maximum absorption of chlorophyll *a* at 665 μμ; this is also the location of the maximum absorption of mixtures of the two chlorophyll components. He also finds a second maximum absorption in the blue at 440 μμ.

The bands of the absorption spectrum of a colloidal solution of chloro-

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[15] Tswett, *Ber. bot. Ges.*, **25**, 388 (1907). Ursprung, *ibid.*, **36**, 74 (1918). Englemann, *Zeit. wiss. Mikro.*, **5**, 289 (1888).

[16] Iwanowski, *Ber. bot. Ges.*, **32**, 433 (1914).

phyll are shifted toward the red end as compared to those of a true solution of chlorophyll. This becomes evident in the following table of Willstätter and Stoll for the absorption spectrum of 0.044 gram chlorophyll *a* in 1000 cc. of water containing 1 per cent of acetone (0.001 mole in 20 liters) when compared to the table giving the absorption spectrum of a true solution of chlorophyll *a*.

	Thickness of Layer in mm.		
	10	20	40
Band I	692—664	712—658	732—650..
" II	—	637 | 615	640...609
" III	—	595 | 581	598. .575
" IV	—	—	554 . 534
" V	—	—	510 . 490...
" VI	466...455—	471—	477—
End absorption			

It is interesting that the absorption spectrum of green leaves also shows the shifting of the bands toward the red end of the spectrum as compared with those of a solution of chlorophyll in ether or acetone. In order to obtain the absorption spectra of leaves it is necessary to remove the gases from the intercellular spaces. This can be accomplished by displacing the air with water by drawing out the gas under a vacuum. The leaves in this condition are more transparent and the light-absorption of a number of superimposed leaves can be determined. The difference in the position of the absorption bands between leaves and chlorophyll solutions can thus be determined.

The condition of chlorophyll in the living plant. The location of the absorption bands of chlorophyll in true and colloidal solution and in the leaf has been used as evidence to establish the condition of the chlorophyll in the leaf. For many years this has been a question of much dispute,[17] it being variously claimed that the chlorophyll was present in the chloroplast in the solid condition, that the chlorophyll was in the colorless stroma, that it was adsorbed on the surface of the stroma, that it was dissolved in an oil and that it was combined with lipoidal substances of the chloroplast. Lubimenko [18] was of the opinion that the chlorophyll is chemically combined with the protein in the chloroplast. It is now realized that some of these assumptions are improbable. Iwanowski [19] compared the absorption spectrum of leaves with that of colloidal solutions and found that the two are similar but not identical. The absorption spectrum of a colloidal solution lies between that of the leaf and that of a true solution of chlorophyll. He states that he was able to reproduce the spectrum of a leaf by adding an electrolyte to a colloidal solution of chlorophyll. With increasing size of the chlorophyll particles

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[17] Arnaud, *Compt. rend.*, **100**, 751 (1885). Tswett, *Trd. Obsc. jest Kasan.*, **35**, 86 (1901) (Russian). Palladin, *Ber. bot. Ges.*, **28**, 120 (1910); *Biochem. Zeit.*, **26**, 357 (1910). Lommel, *Pogg. Ann.*, **143**, 656 (1871).

[18] Lubimenko, *Compt. rend.*, **173**, 365 (1921).

[19] Iwanowski, *Ber. bot. Ges.*, **25**, 416 (1907); *Biochem. Zeit.*, **48**, 328 (1913).

the absorption spectrum shifts toward the red end of the spectrum. He considers that the absorption spectrum cannot give a definite answer regarding the state of the chlorophyll in the leaf. The proof of the colloidal state of the chlorophyll in the leaf is rather to be sought in the behavior of the pigments toward solvents.

Herlitzka,[20] apparently unfamiliar with Iwanowski's first publication, concludes that the similarity of the spectra of living leaves and colloidal chlorophyll solutions can be taken as evidence of the colloidal state of chlorophyll in the leaf; that the two, if not identical, are at least in a similar state. Willstätter and Stoll also compared the absorption spectra of leaves and of chlorophyll solutions. They found that the absorption bands of living leaves and of colloidal chlorophyll solutions were identical, although the intensity relations of the two were different. On the basis of these observations and because of the behavior of the chlorophyll in leaves toward solvents, they conclude that in the leaf the chlorophyll is in a colloidal state. The fact has already been mentioned that pure dry solvents as acetone, ether, benzol do not extract chlorophyll from dried leaves. On the addition of water these solvents immediately take up the chlorophyll. It is assumed that the water dissolves some of the salts in the dried leaves, that this salt solution precipitates some of the chlorophyll, and it should also be pointed out, that a colloidal solution of chlorophyll cannot be extracted with dry ether unless there is present a small amount of calcium chloride.

When leaves are put into boiling water for a short time the chloroplasts are ruptured, the chlorophyll diffuses out and the color of the leaves changes to a deeper green. This change of color is associated with a shifting of the spectral absorption bands toward the violet, and they are very close to the position of the absorption bands of a true chlorophyll solution. This Willstätter and Stoll interpret to mean that, due to the action of the hot water, the chlorophyll has passed from a colloidal solution to a true solution. With this conception, that the chlorophyll in the leaf is in a colloidal condition, the views of Stern, based upon his studies of fluorescence, are at variance. The reasons for this conclusion are given below.

The fluorescence of chlorophyll is one of its most striking properties and is especially noticeable in solutions of organic solvents. Brewster, as early as 1834, observed this, and through the studies of Stokes[21] the nature of the phenomenon, as well as the very important fact, that chlorophyll in the living leaf is also fluorescent, were discovered. Spectrometric determinations of the fluorescent light from solutions and living plants were made by Hagenbach[22] and by others, who used his method. This consists in projecting a beam of white light, filtered through a blue solu-

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[20] Herlitzka, *Biochem. Zeit.*, **38**, 321 (1912).

[21] Stokes, *Phil. Trans. Roy. Soc.*, 1852, 463. Reinke, *Ber. bot. Ges.*, **2**, 265 (1884). Simmler, *Pogg. Ann. Phys.*, **115**, 603 (1862).

[22] Hagenbach, *Pogg. Ann. Phys.*, **141**, 245 (1870); *Jubelb.*, 303 (1874). Lommel, *ibid.*, **143**, 568 (1871).

tion to remove red and yellow rays, onto the material containing the chlorophyll. In this manner, the blood-red fluorescent rays can be detected even in extremely dilute chlorophyll solutions. Tswett[23] states that he was able to detect methylphaeophorbid *a* in a concentration of 1 : 100,000,000 in his luminoscope.

The fluorescence of plants containing chlorophyll has also been studied by microscopic methods. Thus Tswett made use of the Reichert fluorescence microscope. As a source of light he used rays of $\lambda < 450$ μμ. In *Spirogyra* and *Elodea* he observed two lines in the fluorescent light: $\lambda =$ 685-670 and $\lambda =$ 660-650. The first line he considers is due to chlorophyll *a* while the second line, which is weaker than the first, is probably due to chlorophyll *b*. The fluorescence has also been observed in the ultramicroscope by Wilschke,[24] Giklhorn[25] and by Gaidukow.[26]

Lloyd[27] has devised methods of demonstrating the fluorescence of chlorophyll and other pigments microscopically by means of the dark field illuminator of wide aperture. He found that when blue green algae are heated enough to destroy the water soluble phycocyanin, the chlorophyll is unmasked and the fluorescence thereof may be seen with the microspectroscope, using appropriate filters to remove the red to green rays from the source of illumination. Lloyd gives the fluorescent rays as $\lambda =$ 650-700 μμ. He has made preparations of a large number of plants by this method. By using solutions of cane sugar or glycerine, which cause the cells to collapse, he was able to demonstrate the fluorescence of algae and higher plants. An interesting point noted by Lloyd is that on heating *Spirogyra* to the boiling point of water, "the chlorophyll is not rendered non-fluorescent, but it becomes more or less extruded into vacuoles which originate in the chloroplast, and these now contain the chlorophyll, or at least a derivative of it, which can be seen to be fluorescent without the aid of filters."

It will be recalled that Willstätter and Stoll prepared colloidal solutions of chlorophyll in water and found that these were non-fluorescent. Nevertheless, on account of the similarity in the position of the absorption bands between colloidal chlorophyll solutions and living leaves, they and Herlitzka were inclined to believe that in the living leaf the chlorophyll was in a colloidal state. It was found that leaves which had been immersed in boiling water had an absorption spectrum analogous to true chlorophyll solutions, and from this fact, it was concluded that the chlorophyll in such leaves, was no longer in colloidal solution. It is evident, however, that the observation of Lloyd just mentioned is not in entire accord with this conclusion.

Stern[28] bases his conclusion, that chlorophyll is not in a colloidal state

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[23] Tswett, *Ber. bot. Ges.*, **29**, 744 (1911); *Zeit. physik. Chem.*, **36**, 450 (1901).
[24] Wilschke, *Zeit. wiss. Mikros.*, **31**, 338 (1914).
[25] Giklhorn, *Sitzungsb. Akad. Wiss. Wien. Math.-nat. Kl. Abt.* 1, **123**, 1221 (1914).
[26] Gaidukow, "Dunkelfeldbeleuchtung," Jena, 1910.
[27] Lloyd, *Science*, **58**, 91, 229 (1923); **59**, 241 (1924).
[28] Stern, *Ber. bot. Ges.*, **38**, 281 (1920); *Zeit. Bot.*, **13**, 193 (1921).

in living leaves, on similar deductions. He examined in the spectroscope the fluorescence of solutions of chlorophyll of various degrees of dispersion. Neither solid chlorophyll nor colloidal solutions thereof show any fluorescence. Stern argues that if chlorophyll in the living plant is in the form of a colloidal solution, there must be present in the cells substances which affect the colloidal chlorophyll in a manner to produce fluorescence. He tested colloidal solutions of chlorophyll to which had been added various substances in order to determine whether these produce fluorescence. Proteins, carbohydrates, glycerine and lipoids were added. Stern concludes that chlorophyll fluoresces only when it is in true solution. By the addition of lipoids to colloidal chlorophyll solutions and shaking the mixture for a few minutes, an emulsion was produced in which some chlorophyll passed into the lipoid phase. These solutions showed the characteristic fluorescence. Thus lecithin, cholestrine, linseed oil, oleic acid, soap, etc., gave positive results. Stern concludes from this that in the living plant chlorophyll is not in a colloidal solution, but that in the intact chloroplast the chlorophyll is dissolved in a lipoid phase in true solution and consequently shows fluorescence. Photosynthesis, according to this view, would take place partly in a lipoidal and partly in an aqueous phase. The lipoidal solutions of chlorophyll also show a shifting of the absorption bands toward the red end of the spectrum as compared with true solutions. This Stern ascribes to the difference in the index of refraction between alcohol and the lipoids. This is in agreement with Kundt's law which states that the absorption maxima are displaced to the red in proportion as the refractivity of the solvent increases. The refractive index of alcohol may be taken as 1.36, that of the lipoid as about 1.46. The position of the fluorescent band of a solution of chlorophyll in alcohol is at 654 μμ, chlorophyll in lecithin at 677 μμ, and in the living *Chlorella* cells it is at 681 μμ; these are the positions of maximum intensity of the bands. It should, perhaps, be recalled that Iwanowski pointed out that the position of the absorption bands of a colloidal solution of chlorophyll and of a living leaf do not precisely coincide, but that the position of the absorption bands of a colloidal solution is between that of the leaf and that of a true solution. Liebaldt [29] also considers it probable that chlorophyll in the chloroplasts is in a lipoid solution.

The question of the fluorescence of chlorophyll solutions in different degree of dispersion is in need of further intensive study. It is a familiar fact that the fluorescence of chlorophyll solutions is greatly affected by the presence of finely divided particles. Whether this is due to dispersion of the fluorescent light or some other cause has not been definitely established.

Solutions of chlorophyll exhibit the Becquerel effect.[30] If two electrodes are placed in a solution of chlorophyll and one electrode is illumi-

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[29] Liebaldt, *Zeit. Bot.*, **5**, 65 (1913).

[30] Samsonow, *Zeit. wiss. Phot.*, **11**, 33 (1912).

nated, a difference in potential is set up between the electrodes. Dixon and Poole[31] and Dixon and Ball[32] have shown that the light of the frequency effective in photosynthesis does not produce a photo-electric effect with preparations of chlorophyll.

Solutions of chlorophyll exert a very decided photodynamic effect. When very dilute methyl alcohol solutions of chlorophyll are injected into the blood stream of animals and these exposed to light, an hemolysis of the red blood corpuscles takes place, in quite the same manner as that brought about by dilute solutions of fluorescent dyes. This action has been studied by Hausmann.[33]

Chlorophyll, in common with many fluorescent substances, also is capable of sensitizing photographic plates. From this fact the idea was developed that chlorophyll acts as a "sensitizer" in the photosynthetic reaction.[34]

Carotin can be crystallized from carbon bisulfide by the addition of absolute alcohol. Its solubility at 25°, as determined by Schertz,[35] is as follows: in absolute alcohol 15.5 mg. per liter, in petroleum ether (30-50°) 626 mg. per liter, in pure absolute ether 1,005 mg. per liter. In acetone carotin is but slightly soluble, easily in benzol, chloroform and carbon bisulfide. It melts at 174°, but this varies slightly with the rate of heating. In concentrated sulphuric acid carotin dissolves forming an indigo-blue color. Its solutions in alcohol have two absorption bands in the blue and an end absorption in the violet; there is also a faint absorption band in the violet ($\lambda = 425\ \mu\mu$). The absorption bands of a solution of carotin in carbon bisulfide are displaced toward the red end of the spectrum as compared with alcoholic solutions. The following absorption bands have been determined by Willstätter and Stoll with a grating. The limit of the end absorption in alcohol was determined with a spectrograph in 10 mm. layer as $\lambda = 430\ \mu\mu$.

TABLE 44

ABSORPTION SPECTRUM OF 0.005 G. CAROTIN IN 1 LITER OF ALCOHOL AND OF CARBON BISULFIDE. (Willstätter and Stoll.)

Solvent	Alcohol		Carbon Bisulfide	
Thickness of layer in mm.	5	10	10	20
Band I	492— —478	492—476	524...510	525— —508
Band II	459— —446	459—445	489 — 475	490 — 474
End absorption ...	415—	419—		

Xanthophyll can be crystallized in beautiful plates from saturated alcoholic solutions. Although solutions of carotin and xanthophyll are very

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[31] Dixon and Poole, Notes from the Botanical School of Trinity College, Dublin, 3, 90 (1920).

[32] Dixon and Ball, *ibid.*, 3, 199 (1922).

[33] Hausmann, *Biochem. Zeit.*, **12**, 331 (1908); *Jahrb. wiss. Bot.*, **46**, 599 (1909).

[34] Timiriazeff, *Compt. rend.*, **100**, 851 (1885); **102**, 686 (1886); **109**, 379 (1889). Liesegang, *Chem. Zentr.*, 1894, I, 636.

[35] Schertz, *Jour. Agri. Resear.*, **30**, 469 (1925).

similar in appearance the crystals can be distinguished by the fact that xanthophyll crystals appear red when a number of them are superimposed, while the carotin crystals are yellow. The solubility of these two yellow pigments differs considerably. When an alcoholic solution of xanthophyll is mixed with petroleum ether and a small quantity of water is added, most of the xanthophyll passes into the alcoholic layer. Schertz[36] has reported the following solubilities determined at 25° in 1 liter of the solvents: 9.5 mg. in petroleum ether (50-55°), 201.5 mg. in absolute alcohol, 134.9 mg. in absolute methyl alcohol, 952 mg. in pure anhydrous ether. It is easily soluble in chloroform, but not very soluble in carbon bisulfide. Its melting point is 173-174°, though this is not a good criterion of its purity.

There are two absorption bands in the blue and a faint band in the violet ($\lambda = 420$ μμ). In Table 45 are given the results of Willstätter and Stoll's determination of the absorption of xanthophyll.

TABLE 45

Absorption Spectrum of 0.005 G. Xanthophyll in 1 Liter of Alcohol and of Carbon Bisulfide. (Willstätter and Stoll.)

Solvent	Alcohol		Carbon Bisulfide	
Thickness of layer in mm.	5	10	10	20
Band I	484— —472	488—471	515...501	516— —501
Band II	454— —441	454—440	482 — 469	483 — 467
Band III				
End absorption ...	419—	420—	—	447 . 441

Fucoxanthin in the pure state is easily soluble in carbon bisulfide and alcohol. In 100 cc. of methyl alcohol 1.66 grams dissolve at boiling temperature and 0.41 gram at 0°. It can be crystallized from methyl alcohol, in red-brown, long prisms. It melts at 159.5-160.5°. The ether solution is orange-yellow, the alcohol and carbon bisulfide solutions appear more red. The absorption spectrum is given in Table 46.

TABLE 46

Absorption Spectrum of 0.005 Gram Fucoxanthin in 1 Liter of Alcohol. (Willstätter and Stoll.)

Thickness of layer in mm.	5	10
Band I	486—469	493—469 — —
Band II	455—440	454—
End absorption	440—	

c. The Chemical Properties of the Leaf Pigments.

The early efforts to determine the chemical composition and the constitution of chlorophyll met with some of the same obstacles which were encountered in similar investigations on proteins and other complex sub-

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[36] Schertz, *Jour. Agri. Resear.*, 30, 253, 575 (1925).

stances. There was, first of all, the problem of obtaining pure preparations of chlorophyll. For a long time investigators could not even come to an agreement as to what elements are contained in the chlorophyll molecule; thus it was held by some that besides carbon, hydrogen, oxygen, nitrogen and magnesium, chlorophyll also contained iron and phosphorus. It has, however now been quite definitely established that the last two elements are not components of the chlorophyll molecule. The methods for extracting chlorophyll, freeing it from other substances and making pure chlorophyll preparations have been carefully worked out, largely through the efforts of Willstätter and his collaborators. Besides the difficulties associated with freeing the pigments from other substances in the process of extraction and purification, the property characteristic of most leaf pigments, namely, that of great instability, must be dealt with. It must be realized that the oxygen of the air, certain enzymes and light, all cause decomposition to take place and that the substances thus formed interfere with procuring pure preparations.

The method of determining the constitution, which has been followed with considerable success in the investigation of highly complex substances, is that of breaking up the large molecule into a number of smaller ones. These splitting products are often crystalline substances of known composition and structure, which can be purified and identified by means of well established methods. This general method of analysis has been followed with many fatty substances, carbohydrates and proteins of plant and animal origin. A study of the chemical nature of the simpler splitting products and the establishing of their constitution are of much help in determining the constitution of the original complex substance and, in some cases, have made possible the synthesis of the latter from the simpler compounds. The general method of breaking up a complex molecule into simpler ones has also been utilized in the study of the leaf pigments. Many extensive investigations have been carried out by this method, notably by Hoppe-Seyler,[37] Fremy,[38] Gautier,[39] Tschirch,[40] Marchlewski [41] and Hartley.[42] At the present time most of these investigations have little more than an historical interest. In the hands of Willstätter and his collaborators the results of previous investigators were greatly extended, many of the contradictory results were clarified, errors were discovered and their cause determined; in brief, out of a generally chaotic condition, order was established. The complex molecular structure of many of the splitting products of chlorophyll was determined as well as the manner in which the different groups are bound together. This has constituted a great advance toward the final

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[37] Hoppe-Seyler, *Zeit. physiol. Chem.*, **3**, 339 (1879); **4**, 193 (1880); **5**, 75 (1881).

[38] Fremy, *Compt. rend.*, **50**, 405 (1860); **61**, 188 (1865); **84**, 983 (1877); *Ann. chim. phys.* (4), **7**, 78 (1866).

[39] Gautier, *Compt. rend.*, **89**, 861 (1879).

[40] Tschirch, *Ber. bot. Ges.*, **5**, 128 (1887).

[41] Marchlewski, "Die Chemie des Chlorophylls," Braunschweig, 1909.

[42] Hartley, *Jour. Chem. Soc.*, **59**, 106 (1891); **85**, 1607 (1904).

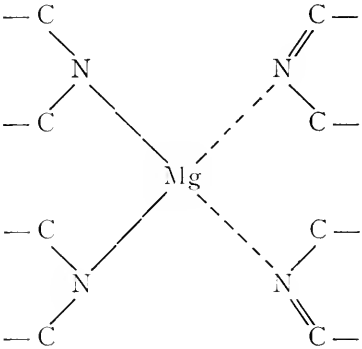
determination of the tremendously complex structure of chlorophyll itself. The value of the work of the earlier investigators should, however, not be underestimated, for this was of the nature of pioneer work and many of our present conceptions are based more or less directly upon these early studies.

Willstätter followed the method of breaking up the chlorophyll molecule into smaller, simpler molecules. This was accomplished in two ways, by the action of acids and by the action of alkalies on chlorophyll. These two reagents yield very different products; one of the most striking differences in the action of the two reagents is that the products resulting from the action of alkali contain magnesium, while the products of acid hydrolysis do not contain this element. Through the action of alkali on chlorophyll the molecule is broken down successively into simpler molecules containing a higher percentage of magnesium; this element is not separated from the rest of the molecule even by boiling with solutions of alkaline hydroxides. By the action of alkalies on chlorophyll, salts of chlorophyll-green color are formed, that is, the neutral chlorophyll has been converted into an acid which forms salts soluble in water. Thus, the action of the alkali has split off through hydrolysis, a component which in the original chlorophyll molecule was united to an acid group. The first products resulting from the action of alkali have optical properties which do not differ greatly from the original chlorophyll.

Chlorophyll is very sensitive to the action of acids, the magnesium is easily split out with oxalic acid and even with carbonic acid. Acid affects a different part of the molecule from that attacked by alkali. The former reagent does not produce an acid; hydrolysis therefore does not take place. Thus, those groups which are split off by alkalies are not affected by the action of acids and, *vice versa,* the products obtained by the action of alkali contain those characteristic groups which are easily destroyed by the action of the acid. The removal of magnesium from the chlorophyll molecule by acid produces drastic changes in the optical properties. The products formed by the action of acids are olive-green to brown in color and there is very little fluorescence. From a study of the products formed by the action of acids and alkalies Willstätter was able to establish the molecular composition of the chlorophyll components. For the details of these experiments reference must be made to the original publications of Willstätter and his collaborators or to the monograph of Willstätter and Stoll; "Untersuchungen über Chlorophyll—Methoden und Ergebnisse."

When chlorophyll ($a + b$) is hydrolyzed with methyl alcoholic potassium hydroxide, at the temperature of boiling methyl alcohol, there are formed, among other products, the so-called chlorophyllins. There are two series of these compounds, one arising from chlorophyllin, the other from isochlorophyllin. These are acids, which form salts with disodium phosphate, and the free acid can again be liberated by the action of monosodium phosphate. Chlorophyllins are complex magnesium compounds; the magnesium is not electrolytically dissociated in solution and does not give the usual ionic reactions for this metal. The group containing the magnesium is very stable toward alkalies, so much so, that the carboxyl groups in the molecule can be split off without affecting the magnesium linkages. This property enabled the fact to be established that magnesium was actually present, as well as the nature of the union of this metal with other groups in the molecule. By successive breaking down of the chlorophyllin molecule, finally by heating to 240° with alcoholic alkali, a series of beautifully colored and fluorescent crystalline compounds was obtained with, one, two and three carboxyl groups, the phyllins. These compounds, which have been given names descriptive of their color as glaucophyllin and rhodophyllin, and of which at least a dozen have been prepared are carboxylic acids containing magnesium, as e.g. $(MgN_4C_{31}H_{33})COOH$, pyrrophyllin and $(MgN_4C_{31}H_{32})(COOH)_2$ glaucophyllin. The removal of the last carboxyl group yields ætiophyllin, $C_{31}H_{34}N_4Mg$, giving an ash containing 8 per cent of magnesium oxide.

All phyllins contain four nitrogen atoms to one atom of magnesium. The oxygen atoms are not bound to magnesium and are not a part of the metal complex; only the nitrogen atoms are combined to the metal. Based upon Werner's conceptions of partial valences, of the structure of metal derivatives of acid imides, biuret and dicyandiamidine, Willstätter concluded that in ætiophyllin nitrogen is united to magnesium by primary and partial valences. The nitrogen is part of a pyrrol ring, so that the magnesium nucleus would have the following structure:

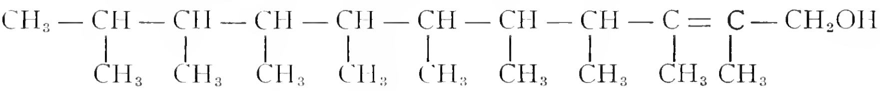


When treated with acids, phyllins break down with the loss of magnesium, forming mono- and polycarboxylic acids. These acids also have basic properties. The compounds thus formed are called porphorins and take the prefix of the phyllins from which they were obtained, e.g. cyanophyllin, $C_{31}H_{32}N_4Mg(COOH)_2 \longrightarrow$ cyanoporphyrin, $C_{31}H_{34}N_4(COOH)_2$, glaucophyllin $\longrightarrow$ glaucoporphyrin, isomeric with cyanoporphyrin, and phyllophyllin, $C_{31}H_{32}N_4Mg(COOH) \longrightarrow$ phylloporphyrin $C_{31}H_{35}N_4(COOH)$.

The decomposition of the phyllins in the manner just mentioned is of considerable importance in interpreting the behavior of chlorophyll toward acids. When chlorophyll is treated with an organic acid such as oxalic acid, the green color changes to an olive, the solution is only slightly fluorescent and magnesium is split out of the chlorophyll molecule. The compound formed when chlorophyll, in alcohol solution, is treated with oxalic acid in the cold, is called phæophytin. It contains no magnesium, is a dark waxy substance; in ether it is not very soluble, easily soluble in benzol and chloroform. It has no acid properties but is slightly basic. A remarkable property of this substance is that, while in solution it has a dark brown color, some metals can be introduced into the molecule, which results in the formation of brightly colored compounds. Thus copper and zinc in acetic acid solution react with alcoholic solutions of phæophytin. Especially interesting is the fact that when phæophytin is heated in a silver test tube with alcoholic potassium hydroxide and magnesium oxide a series of phyllins are formed. When these solutions are acidified very carefully and extracted with ether the brilliant green, blue and red fluorescent phyllins pass into the ether extract. On account of the alkali present the reformation of chlorophyll is prevented, however. Magnesium can also be introduced into the phæophytin molecule by means of the Grignard reagent, methylmagnesiumiodide:

$$\underset{\text{Phylloporphyrin}}{C_{31}H_{35}N_4(COOH)} \quad \overset{\text{acid}}{\underset{MgCH_3I}{\leftrightarrows}} \quad \underset{\text{Phyllophytin}}{C_{31}H_{33}N_4Mg\,.\,COOH}$$

On saponification with alkalies phæophytin yields, besides complex nitrogen compounds with 34 carbon atoms, a nitrogen-free alcohol with 20 carbon atoms. This compound which Willstätter called phytol, is an unsaturated, primary alcohol of open chain structure. It has the empirical formula $C_{20}H_{39}OH$ with the possible following structure:

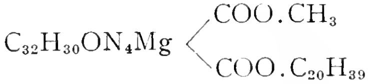
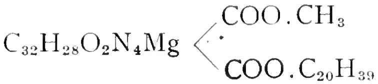
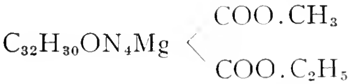
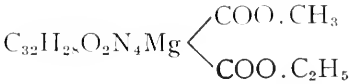
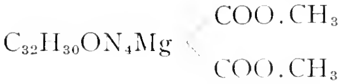
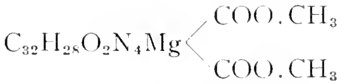


$$CH_3-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{CH}-\underset{\underset{\displaystyle CH_3}{|}}{C}=\underset{\underset{\displaystyle CH_3}{|}}{C}-CH_2OH$$

Phytol is a colorless, thick oil soluble in most organic solvents, boiling at 145° under 0.03-0.04 mm. pressure. It is easily oxidized, adds one molecule of bromine or of iodine. The capacity of phytol to take up oxygen may account for the reducing power of chlorophyll. The determination of the amount of phytol in a chlorophyll preparation, by treating this first with acid and then with alkali, has served as a method of determining the purity of the preparation.

The study of the products formed by the action of acids on chlorophyll has shown also what the action is of alkalies on the chlorophyll molecule; this is chiefly a saponification of the phytol ester. Besides the saponification of the phytol ester, the alkali attacks a $COOCH_3$ group which is contained in phæophytin and consequently is also a portion of the chlorophyll molecule.

Phytol has played a very important part in the investigations of chlorophyll; phytol is a constant component of chlorophyll and comprises one-third of the chlorophyll molecule. That is, the phytol content is never more than 33 per cent of the chlorophyll. It has been observed that some chlorophyll preparations contained very little phytol and it had been reported, that from the latter preparations crystalline chlorophyll was most easily obtained. It is now realized that this crystalline substance which was taken for chlorophyll is not chlorophyll as it exists in the chloroplast, but an altered chlorophyll-like compound produced by the action of an enzyme. When the chlorophyll is extracted rapidly from the leaves, the phytol-content is one-third of the chlorophyll. If, however, the extraction is slow and the leaf powder is in contact with alcohol for a long time, the amount of phytol is low. In the leaf there is an enzyme, chlorophyllase, which is capable of acting in an alcoholic medium. Chlorophyllase, in alcoholic solution, produces an alcoholysis of the chlorophyll with splitting out of phytol. The crystalline chlorophyll, just referred to, was not true chlorophyll, but a methyl- or ethylchlorophyllid, i.e., the methyl or ethyl alcohol had replaced the phytol. These methyl- and ethylchlorophyllids can be hydrolyzed to the corresponding free acids.

Chlorophyll *a*	 $C_{32}H_{30}ON_4Mg\left\langle\begin{array}{l}COO.CH_3\\COO.C_{20}H_{39}\end{array}\right.$
Chlorophyll *b*	 $C_{32}H_{28}O_2N_4Mg\left\langle\begin{array}{l}COO.CH_3\\COO.C_{20}H_{39}\end{array}\right.$
Ethylchlorophyllid *a*	 $C_{32}H_{30}ON_4Mg\left\langle\begin{array}{l}COO.CH_3\\COO.C_2H_5\end{array}\right.$
Ethylchlorophyllid *b*	 $C_{32}H_{28}O_2N_4Mg\left\langle\begin{array}{l}COO.CH_3\\COO.C_2H_5\end{array}\right.$
Methylchlorophyllid *a*	 $C_{32}H_{30}ON_4Mg\left\langle\begin{array}{l}COO.CH_3\\COO.CH_3\end{array}\right.$
Methylchlorophyllid *b*	 $C_{32}H_{28}O_2N_4Mg\left\langle\begin{array}{l}COO.CH_3\\COO.CH_3\end{array}\right.$
Chlorophyllid *a*	$C_{32}H_{30}ON_4Mg(COOH).(COO.CH_3)$
Chlorophyllid *b*	$C_{32}H_{28}O_2N_4Mg(COOH).(COO.CH_3)$

Starting with the chlorophyllid, it has also been possible to synthesize chlorophyll with phytol and chlorophyllase, according to:

$$(C_{32}H_{30}ON_4Mg)(CO_2CH_3)(CO_2H) + C_{20}H_{39}OH \longrightarrow \\ (C_{32}H_{30}ON_4Mg)(CO_2CH_3)(CO_2C_{20}H_{39}) + H_2O.$$

The discovery of the nature of the action of chlorophyllase and the chemical composition of the chlorophyllids and methyl- and ethylchlorophyllids aided greatly in determination of the constitution of chlorophyll.

It has already been mentioned that the saponification of phæophytin yields, besides phytol, a number of complex carboxylic acids containing nitrogen. The latter constitute a complicated mixture of substances the separation of which offered many severe obstacles. Willstätter and Mieg finally devised a method of separation which utilizes the basic nature of these substances. The chlorophyll derivative is differentiated by its "hydrochloric acid number," i.e., the concentration of hydrochloric acid which is necessary to extract it from an ether solution. Thus, an ether solution, containing a mixture of these carboxylic acids, can be fractionated by extraction with dilute hydrochloric acid of different concentrations. In this manner there were obtained two substances which were of much importance in the development of the chemistry of chlorophyll, *viz.*,

Phytochlorin *e* $C_{31}H_{32}N_4(CO)(COOH)_2$
Phytorhodin *g* $C_{30}H_{31}N_4(CO)(COOH)_3$.

Phytochlorin *e* is a tricarboxylic acid with two free carboxylic groups and one lactam; phytorhodin *g* is a tetracarboxylic acid in which two or three of the carboxyl groups may be free.

The hydrolysis of chlorophyll, first with acid and then with alkali thus leads to two products, the one green, the other red. This fact led to the conclusion that phæophytin as well as chlorophyll are both a mixture of two components each; one of these components yields phytochlorin *e*, the other phytorhodin *g*. This was finally established by separating chlorophyll by the method already described, into the two components, chlorophyll *a* and chlorophyll *b*, from which phytochlorin *e* and phytorhodin *g* respectively were obtained. The two chlorophyll components and the products derived from them by acid and alkali hydrolysis are very similar in chemical composition; they contain the same quantity of magnesium and of phytol; the basic nuclei of the two components, phytochlorin and phytorhodin, have very nearly the same composition. This relationship between the derivatives of the two components is maintained in parallel steps of the disintegration of the chlorophyll molecules. From analyses it would appear that the difference between the two series of derivatives from the *a* and *b* components lies chiefly in one oxygen atom, two atoms of hydrogen of chlorophyll *a* being replaced by one atom of oxygen in chlorophyll *b*, corresponding to the following probable formulæ:

Chlorophyll *a* $C_{55}H_{72}O_5N_4Mg$,
$(MgN_4C_{32}H_{30}O)COOCH_3 . COOC_{20}H_{39}$
Chlorophyll *b* $C_{55}H_{70}O_6N_4Mg$,
$(MgN_4C_{32}H_{28}O_2)COOCH_3 . COOC_{20}H_{39}$.

Chlorophyll *a*, $C_{31}H_{29}N_3Mg(NH . CO)(CO_2CH_3)(CO_2C_{20}H_{39})$, with acid ⟶ phæophytin *a*, $C_{32}H_{32}ON_4(CO_2CH_3)(CO_2C_{20}H_{39})$, with alkali ⟶ phytochlorin *c*, $C_{32}H_{32}ON_4(CO_2H)(CO_2H)$.

Chlorophyll *b*, $C_{32}H_{28}O_2N_4Mg(CO_2CH_3)(CO_2C_{20}H_{39})$, with acid ⟶ phæophytin *b*, $C_{32}H_{30}O_2N_4(CO_2CH_3)(CO_2C_{20}H_{39})$, with alkali ⟶ phytorhodin *g*, $C_{32}H_{30}O_2N_4(CO_2H)(CO_2H) . H_2O$. It has not been possible to convert one component into the other by oxidation or reduction.

The determination of the nature of the products obtained by the disintegration of the chlorophyll molecule necessitated a tremendous amount of work and it is impossible to describe here the methods which are used to separate the products or the physical and chemical properties of these substances. For this information reference must be made to the very voluminous original literature. It may aid, however, in obtaining a survey of the subject, to outline the relation between the different substances and the formulae which have been ascribed to these.

Mention has already been made of three methods which have been used to break down the chlorophyll molecule, all of which yield different products: 1, alkali hydrolysis, 2, acid hydrolysis and 3, the action of the enzyme chlorophyllase. The latter, when acting on chlorophyll in alcohol solution, splits off phytol forming the methyl- or ethylchlorophyllids from which in turn the free chlorophyllid may be obtained. These compounds can be obtained in no other way. By elimination of magnesium from these compounds through the action of acids, phæophorbids are formed. The latter, in turn, may be formed from phæophytin with methyl or ethyl alcohol and hydrogen chloride, thus replacing the phytol by a methyl or ethyl group. The relationship of these various compounds and the manner in which they are obtained becomes evident from the diagram describing the reactions of chlorophyll *a*. The reactions of chlorophyll *b* follow similar steps (see page 362).

The action of alkalies on chlorophyll is somewhat more complex than that of acids. Both chlorophyll *a* and *b*, on treatment with alkali give two series of products; the members of one fluoresce like chlorophyll, the members of the other do not. Thus chlorophyll *a* with alkali yields chlorophyllin *a* and isochlorophyllin *a*. Chlorophyllin *a*, $[MgC_{31}H_{29}N_3](NH . CO)(COOH)_2$, alkali 140° ⟶ glaucophyllin, $MgC_{31}H_{32}N_4(COOH)_2$, with alkali 165° ⟶ rhodophyllin, $MgC_{31}H_{32}N_4(COOH)_2$, with alkali 200° ⟶ pyrrophyllin, $MgC_{31}H_{33}N_4(COOH)$. Isochlorophyllin *a* with alkali in the same manner yields compounds which are

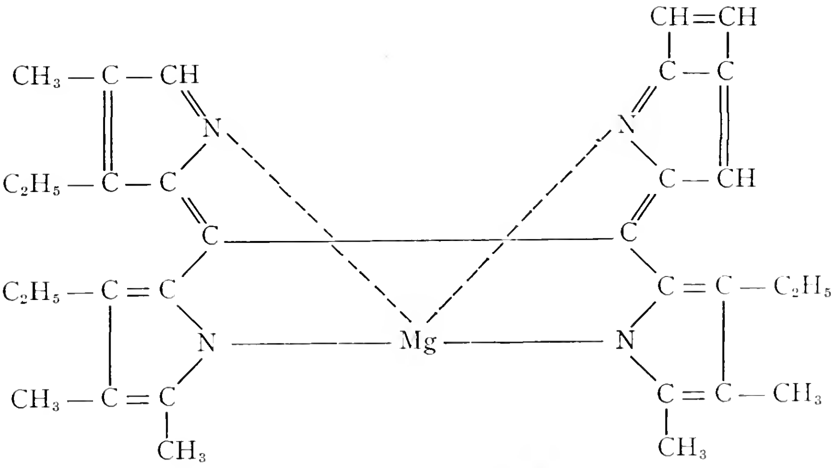
$$\underset{\text{Chlorophyllid } b}{(MgN_4C_{32}H_{28}O_2)COOH_3.COOH} \longrightarrow \underset{\text{Phæophorbid } b}{(N_4C_{32}H_{30}O_2)COOCH_3.COOH}$$

$$\begin{tikzcd}[column sep=huge, row sep=huge]
\begin{array}{c}\text{Chlorophyll } a\\ (MgN_4C_{32}H_{30}O)COOCH_3.COOC_{20}H_{39}\end{array} \arrow[r, shift left, "\text{with dilute acid}"] \arrow[d, "\text{with}"', "\text{enzyme}"] & \begin{array}{c}\text{Phæophytin } a\\ (N_4C_{32}H_{32}O)COOCH_3.COOC_{20}H_{39}\end{array} \arrow[l, shift left, "\text{with } MgCH_3I"] \arrow[d, "\text{with}"', "CH_3OH + HCL"] \arrow[dd, bend right=60, "\text{with conc. acid}"'] \\
\begin{array}{c}\text{Methylchlorophyllid } a\\ (MgN_4C_{32}H_{30}O)COOCH_3.COOCH_3\end{array} \arrow[r, "\text{with dilute acid}"] \arrow[d, "\text{with}"', "\text{enzyme}"] & \begin{array}{c}\text{Methylphæophorbid } a\\ (N_4C_{32}H_{32}O)COOCH_3.COOCH_3\end{array} \arrow[d, "\text{with}"', "\text{conc. acid}"] \\
\begin{array}{c}\text{Chlorophyllid } a\\ (MgN_4C_{32}H_{30}O)COOCH_3.COOH\end{array} \arrow[r, "\text{with dilute acid}"] & \begin{array}{c}\text{Phæophorbid } a\\ (N_4C_{32}H_{32}O)COOCH_3.COOH\end{array}
\end{tikzcd}$$

isomeric with the phyllins from chlorophyllin, *viz.*, cyanophyllin, erythrophyllin and phyllophyllin. The splitting off of the last carboxyl group is accomplished with soda-lime; this leads to the formation of ætiophyllin, $MgC_{31}H_{34}N_4$. The isomeric phyllins, just mentioned, differ in the position of the carboxyl group. Finally, ætiophyllin, on treatment with acid, loses magnesium with the formation of ætioporphyrin $C_{31}H_{36}N_4$.

$$\begin{tikzcd} \begin{matrix}\text{ætiophyllin}\\ C_{31}H_{34}N_4Mg\end{matrix} \arrow[r, shift left, "\text{with acid}"] & \begin{matrix}\text{ætioporphyrin}\\ C_{31}H_{36}N_4\end{matrix} \arrow[l, shift left, "\text{with } MgCH_3I"] \end{tikzcd}$$

The structures of ætioporphyrin and of ætiophyllin are of great interest for the chemistry of chlorophyll; these are in all probability the groups which are responsible for the characteristic properties of chlorophyll. It is also of interest because of the possible relation of chlorophyll to the pigments of blood. That an intimate chemical relationship may exist between these two pigments has been suggested by Marchlewski and others.[43] These suggestions are based largely upon the similarity in constitution of compounds derived from chlorophyll and those derived from hemoglobin. Both chlorophyll and hemin may be regarded as derivatives of ætioporphyrin. The constitution of these compounds has not been definitely established; the following have been proposed by Willstätter and Stoll:



Ætiophyllin $C_{31}H_{34}N_4Mg$

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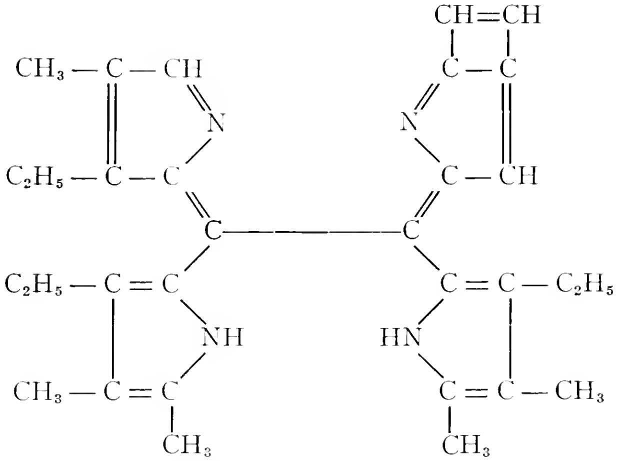
[43] Marchlewski, "Die Chemie des Chlorophylls," 1909. Nencki, *Ber. chem. Ges.*, **29**, 2877 (1896); **34**, 997 (1901). Burgi and Tracewski, *Biochem. Zeit.*, **98**, 256 (1919). Gregoriew, *ibid.*, **98**, 284 (1919).



Ætioporphyrin $C_{31}H_{36}N_4$

These formulæ indicate that the compounds under discussion are complex aggregates of substituted pyrrole. The efforts to determine whether there exists a chemical relationship between the chlorophyll and blood pigments have been directed largely toward establishing the structure of these complex compounds containing several pyrrole rings. (See page 365.)

The photo-oxidation of solutions of chlorophyll and consequent change of color is, as far as we know, the only effect which light has on these substances. The red rays seem to exert the most powerful effect.[44] Light which has been filtered through a solution of chlorophyll has very little effect on a second solution of chlorophyll. Wager[45] and Warner[46] have reported that chlorophyll is oxidized in the light with the formation of aldehyde. It is doubtful, however, whether this reaction can be interpreted as constituting a step in the course of photosynthesis. In fact, Willstätter and Stoll are of the opinion that no aldehyde is formed in the photo-oxidation of chlorophyll, but rather that the aldehyde which was found arose from the oxidation of impurities in the chlorophyll preparations.

Wurmser[47] studied the rate of decolorization of chlorophyll solutions in light of $\lambda = 680$ μμ. He found that colloidal substances such as gelatine, gum arabic and casein, when added to the chlorophyll solutions, greatly retard the rate of their decolorization; starch did not produce a retardation. Wurmser demonstrated that the decolorization of chlorophyll solutions is due to the presence of oxygen; solutions from which the last traces of oxygen have been removed remained unchanged after

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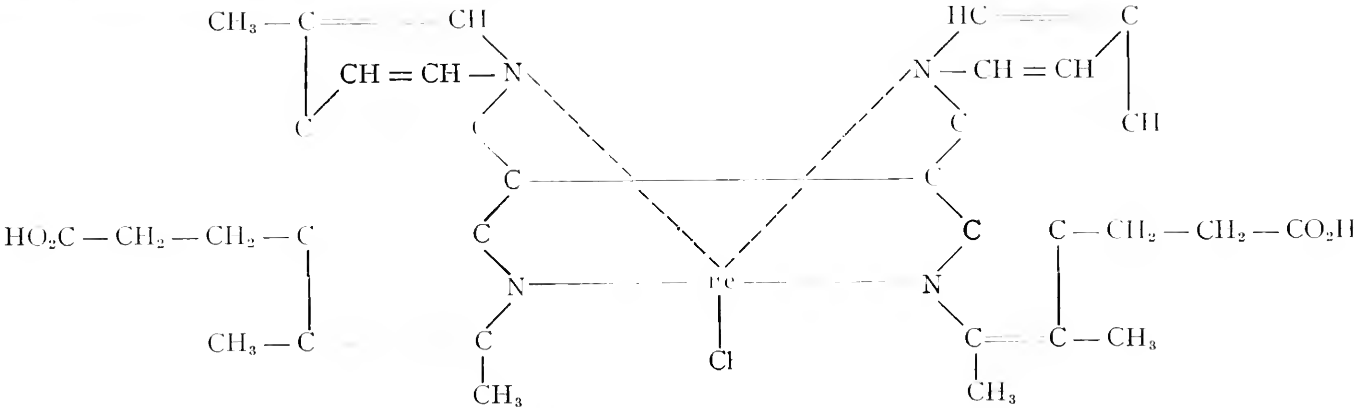
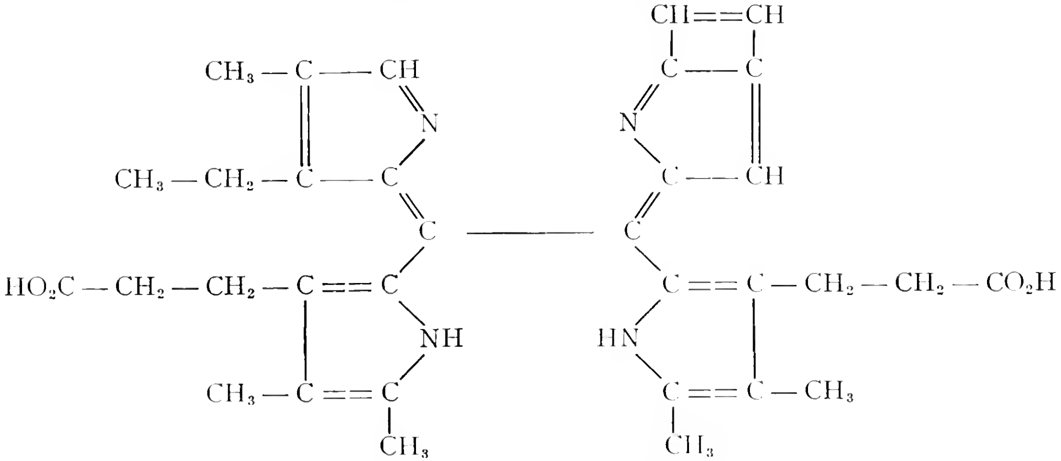
[44] Dangerard, *Compt. rend.*, **151**, 1386 (1910).
[45] Wager, *Proc. Roy. Soc.*, **87** B, 386 (1914).
[46] Warner, *ibid.*, 378 (1914).
[47] Wurmser, "Récherches sur l'assimilation chlorophylienne," Paris, 1921, p. 52.



Hemin $C_{33}H_{32}O_4N_4FeCl$



Hemoporphyrin $C_{33}H_{36}O_4N_4$

exposure to light for months. Wurmser considers that the protective action of the colloidal substances mentioned is due to the fact that these envelop the particles of chlorophyll and thus protect them against the access of oxygen. He found that the initial rates of decolorization in light of different wave length have the following relation: $\lambda > 560\ \mu\mu = 45$; $\lambda = 580 - 460\ \mu\mu = 125$; $\lambda < 400\ \mu\mu = 30$.

Carotin is an unsaturated hydrocarbon of the formula $C_{40}H_{56}$. It is easily oxidized by the oxygen of the air, thereby it loses its color and increases in weight by 35 per cent in dry air and by 41 per cent in moist air. The crystals do not change form in this process. When perfectly pure, carotin is not oxidized so readily by air. Carotin reacts with halogens; iodine is easily taken up with the formation of a dark violet crystalline compound, $C_{40}H_{56}I_2$. Under anhydrous conditions carotin forms a bromine compound, $C_{40}H_{36}Br_{22}$. In concentrated sulfuric acid, carotin dissolves forming an indigo-blue solution.[48]

Xanthophyll, $C_{40}H_{56}O_2$, like carotin is unsaturated, it is oxidized in the air with an increase of 36.5 per cent of its original weight, corresponding to the absorption of 13 atoms of oxygen or the formula $C_{40}H_{56}O_{15}$. When the oxidized compound is recrystallized from ether a compound having the formula $C_{40}H_{56}O_{18}$, m.p. 140, is obtained. The structure of xanthophyll has not been established. It has been suggested that xanthophyll is an oxidation product of carotin; this has, however, not been found to be the case.[49] It is probable that the oxygen in xanthophyll is present in an ether like combination, for it gives no reactions for the carbonyl, alcohol or carboxyl groups. Xanthophyll, like carotin, is also bleached on oxidation. Schertz[50] reports that it is unstable in ether solution, slightly unstable in petroleum ether solution but very stable in absolute ethyl alcohol solution. It adds iodine to form $C_{40}H_{56}O_2I_2$, a dark violet colored substance. Xanthophyll crystals when dissolved in concentrated sulphuric acid also form a deep blue solution; on diluting this with water, green flakes are precipitated. In warm ethyl alcohol containing concentrated hydrochloric acid it dissolves with a green color which changes to blue. This reaction has been used to distinguish xanthophyll from hydrocarbon carotinoids. Only strong alkalies seem to affect xanthophyll. It is absorbed by dry calcium carbonate, by the Tswett method, from petroleum ether solutions. However, even traces of alcohol interfere greatly with the adsorption.

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[48] Kohl, "Untersuchungen über das Carotin und seine physiologische Bedeutung in der Pflanze.," Leipzig, 1902. Willstätter and Mieg, *Ann. Chem.*, **355**, 1 (1907). Willstätter and Escher, *Zeit. physiol. Chem.*, **64**, 47 (1910). Escher, *ibid.*, **83**, 198 (1913). Palmer, "Caratinoids and Related Pigments," 1922. Schertz, *Jour. Agri. Res.*, **30**, 469 (1925).

[49] Willstätter and Mieg, *l. c.* Willstätter and Escher, *l. c.* Wisselingh, *Flora* (7), **107**, 371 (1915).

[50] Schertz, *Journ. Agr. Res.*, **30**, 575 (1925). Ewart, *Proc. Roy* [illegible]. *Victoria* **30**, 178 (1918).

d. The Quantitative Estimation of Leaf Pigments.

The early realization that the leaf pigments were an essential part of the photosynthetic apparatus naturally led to efforts to determine whether a quantitative relation exists between the chlorophyll-content of plants and their photosynthetic activity. For such investigations quantitative methods of determining the leaf pigments were necessary. Various efforts were made to devise such methods; the principle of these was usually the extraction of the pigment and the determination of the absorption thereby of light of a given intensity, thus enabling a comparison to be made between two sets of leaves. The great advance in our knowledge of the leaf pigments, contributed by the investigations of Willstätter, made possible the development of methods for the estimation of these substances in fresh plant material on a far more rational and accurate basis than had hitherto been possible. Through the investigations of Willstätter it was made evident not only what the essential leaf pigments are, but also how these can be extracted without undergoing alteration and how they can be separated from one another. Willstätter's investigations thus constitute an exceedingly valuable contribution to our knowledge of photosynthesis. At the same time, it should be mentioned that the methods for estimating the quantities of leaf pigments, devised by Willstätter and Stoll are by no means simple. The pigments themselves are exceedingly labile, many operations are involved, numerous special precautions must be observed, so that considerable experience is necessary in order to obtain results which, at best, are not of the highest degree of accuracy.

Willstätter and Stoll [51] have given a detailed description of their method of determining the leaf pigments. A rehearsal in more general terms of their statements would be of very little value. Before endeavoring to use this method the original publications should be consulted and a thorough knowledge of the chemical reactions involved in each step must be gained.

The principle of the method is given here very briefly. The pigments are extracted from the fresh, macerated leaves with acetone in the cold after a preliminary treatment with 40 and 35 per cent acetone. The pigments are then transferred to ether and the acetone is removed completely by washing with water. One portion of the pure ether solution is used for the determination of the two chlorophyll components, another portion for the estimation of carotin and xanthophyll. The two chlorophyll components are determined by converting them into decomposition products through hydrolysis with acid and alkali. Chlorophyll *a* thus yields phytochlorin *e* and chlorophyll *b*, phytorhodin *g*:

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[51] Willstätter and Stoll, "Untersuchungen über die Assimilation der Kohlensäure," Berlin, 1918, p. 11.

$$\begin{tikzcd}
 & \text{(Iso-)chlorophyll } (a, b) \arrow[r, "\text{with acid}"] & \text{Phytochlorin } e \\
\text{Chlorophyll } (a, b) \arrow[ur, "\text{with hot alkali}"] \arrow[dr, "\text{with acid}"'] & & \\
 & \text{Phæophytin } (a, b) \arrow[r, "\text{with hot alkali}"] & \text{Phytorhodin } g
\end{tikzcd}$$

The manipulations involved in the hydrolysis and saponification reactions and the separation of the products by fractionation of the ether solutions with hydrochloric acid must be carried out with great care.

Another portion of the original ether solution containing all the leaf pigments is used for the determination of carotin and xanthophyll. This is saponified with methyl alcoholic potash to remove the chlorophyll, the yellow pigments remain in the ether. These are transferred to petroleum ether solution and the xanthophyll removed with methyl alcohol; the carotin remains in the ether.

The quantities of the four pigments are determined colorimetrically by comparison with standard solutions. For the determination of the chlorophyll components known quantities of different pure chlorophyll preparations, e.g., crystalline ethyl chlorophyllid or the methyl phæophorbids are used and these are treated in exactly the same manner as the extracts of unknown concentration; the solutions finally obtained are then compared in a colorimeter. The standard solutions for the determination of carotin and xanthophyll are prepared from pure crystalline preparations of these pigments or solutions of potassium bichromate can also be used. In the hands of a skillful manipulator the method of Willstätter and Stoll can doubtless yield valuable comparative results. It need, however, hardly be emphasized that considerable experience in handling solutions of the pigments and in the various chemical reactions involved is an absolute requisite for the successful operation of the method.

The method has probably not the accuracy of spectrophotometric measurements. Schertz [52] has shown that the spectrophotometric method for xanthophyll has an accuracy of 2.8 per cent while the colorimetric method has an accuracy of 17 per cent. The same author has also described results of the spectrophotometric determination of carotin.

The chlorophyll-content of leaves, as determined by the method of Willstätter and Stoll, is fairly constant in normal leaves: 0.15 to 0.35 gram per 100 grams fresh weight, 0.6 to 1.2 gram per 100 grams dry weight and 0.3 to 0.7 gram per one square meter of leaf surface. The same authors found that for one mole of chlorophyll *b* there were present, on an average, 2.9 moles of chlorophyll *a*. On the basis of dry weight,

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[52] Schertz, *Jour. Agri. Res.*, **26**, 383 (1924); **30**, 254 (1925).

shade leaves have a relatively higher chlorophyll-content than leaves grown in direct sunlight. This relationship may not hold, however, on the basis of leaf surface, for shade leaves are often very thin. The ratio of the two chlorophyll components, designated by Willstätter and Stoll [53] as $Q\frac{a}{b}$, undergoes no appreciable change during the course of the day.

The carotinoid-content ranges from 0.07 to 0.2 per cent of the dry weight and 0.03 to 0.07 gram per square meter of leaf surface. The ratio of carotin to xanthophyll, $Q\frac{c}{x}$, exhibits no great variations; it is on the average 0.60 with a maximum variation of ± 0.1; to each mole of carotin there are 1.5 moles of xanthophyll. In general leaves contain more chlorophyll than carotinoids, though the ratio of the chlorophyll to the yellow pigments, $Q\frac{a+b}{c+x}$, may exhibit considerable variation, from 0.15 in yellow autumn leaves to 4.68 in shade leaves. It should be noted that the carotin and xanthophyll-content of leaves does not change greatly during the course of their development, and that the yellow color of autumn leaves is, in part at least, due to water soluble yellow pigments, of unknown chemical composition.

e. The Rôle of Chlorophyll in Photosynthesis.

That the presence of chlorophyll is essential for photosynthesis was recognized by the earliest investigators of the phenomenon, Senebier, Ingen-Housz and de Saussure. This fact has been repeatedly verified and there exists no evidence which could raise much doubt regarding the necessity of chlorophyll for photosynthesis. Certain bacteria perhaps constitute an exception to this, though the nature of the entire respiratory functions as well as the manner of carbon dioxide reduction in these organisms is probably different from that of chlorophyllous plants. The well-established facts are these: that photosynthesis has been observed to occur only in cells containing chlorophyll, that in albino portions of a leaf no photosynthesis occurs. The latter portion of the argument should perhaps be accepted with some caution, for it is possible that the stroma of the chloroplasts of albino cells may differ from that of chlorophyll-bearing cells in other respects besides chlorophyll-content. It is true that the chloroplasts of albino and chlorotic cells are capable of forming starch from sugar, but this fact does not entirely meet the objection raised to the argument. The problem cannot be studied by the use of etiolated chloroplasts, because in these chlorophyll is formed very rapidly on exposure to light. Many theories have been suggested to describe the function of chlorophyll in photosynthesis, some of these are not worth discussing and few of them have been tested experimentally. In fact, the problem

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[53] Willstätter and Stoll, "Untersuchungen über Chlorophyll," p. 109.

has received very little thorough study and constitutes one of the least explored fields in the whole subject of photosynthesis.

The theories which have been advanced to explain the function of chlorophyll in photosynthesis are of two types. In one type the physical properties of chlorophyll, such as the absorption of light and fluorescence are chiefly responsible for its action; in another type, certain chemical properties or reactions of chlorophyll are also made use of.

The discovery of the fact that chlorophyll "sensitized" a photographic plate to the longer wave lengths lead Timiriazeff [54] to propose the theory that chlorophyll plays a similar rôle in photosynthesis. Carbon dioxide as well as carbonic acid and its salts do not absorb light of the visible spectrum; photochemical reaction in this portion of the spectrum is therefore excluded, unless the system can be "sensitized" to these rays. Although this theory does not throw much light on the kinetics of the photosynthetic reaction, it has been accepted by many. One of the chief difficulties with the theory is that it merely supplies a term and, in the absence of preciser knowledge regarding the mechanism of optical sensitizers, has not aided greatly in elucidating the chemical reactions of photosynthesis.[55]

Tswett [56] considers that the fluorescent light of chlorophyll is the main source of energy in the photosynthetic process. He assumes that some compound of carbon dioxide is capable of absorbing these rays. He also assumes that fluorescence is due to a reversible reaction of tautomeric molecules. Light is absorbed by some one of the chlorophyll components which is thereby changed into another form. When the original molecule is reformed, the energy taken up in the first change is radiated as photoluminescence or phosphorescence and it is this light which is used in photosynthesis. The function of the chlorophyll would thus be the conversion of polychromatic light into monochromatic red light. Tswett assumes that it is really phosphorescence which is the immediate source of energy for photosynthesis, that there is an interval between the excitation (first tautomeric change) and the emission of light (reversal of first tautomeric change). In this connection it is interesting to note that Warburg [57] noticed a higher rate of photosynthesis with intermittent light than with the same light intensity of continuous radiation. This latter observation is in accord with Tswett's predictions. While the theory of Tswett has some very interesting features, it has got us little farther, for it offers nothing as to the mode of action of the phosphorescent rays on the carbonic acid. Stern [58] has pointed out that if Tswett's theory is correct and

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[54] Timiriazeff, *Compt. rend.*, **96**, 875 (1883); **100**, 851 (1885); **102**, 686 (1886); **109**, 379 (1889); *Ann. Sci. Nat. Bot.* (7), **2**, 99 (1885). Hoppe-Seyler, *Zeit. physiol. Chem.*, **3**, 339 (1879). Reinke, *Ber. bot. Ges.*, **1**, 419 (1883). Englemann, *Bot. Zeitg.*, **46**, 717 (1888).

[55] Kayser, *Handb. der Spektroskopie*, **4**, 1078 (1907).

[56] Tswett, *Zeit. physik. Chem.*, **76**, 413 (1911).

[57] Warburg, *Biochem. Zeit.*, **100**, 262 (1919).

[58] Stern, *Ber. bot. Ges.*, **38**, 33 (1920).

the red phosphorescent rays are used in photosynthesis, the intensity of these ought to increase when photosynthesis is artificially inhibited. The results of investigations of this point are still incomplete.

Dixon and Poole[59] endeavored to determine whether a photo-electric effect was produced on illuminating various chlorophyll preparations. They found that the wave lengths of light which are effective in photosynthesis do not expel electrons from chlorophyll in sufficient quantity to account for the formation of carbohydrates external to the chlorophyll. It is possible that the electrons are not ejected, but are merely displaced within the molecule. This displacement may possibly cause a rearrangement within the chlorophyll molecule or within a chlorophyll-carbonic acid complex. Dixon and Ball[60] showed that the sensitization of photographic films with chlorophyll is effective at the temperature of liquid air. They are of the opinion that the absorbed visible light causes a displacement of electrons within the chlorophyll molecule and suggest the following scheme:

$$\begin{array}{ccc} C_{55}H_{72}O_5N_4Mg + CO_2 & \longrightarrow & C_{55}H_{70}O_6N_4Mg + CH_2O \\ \text{chlorophyll } a & & \text{chlorophyll } b \\ C_{55}H_{70}O_6N_4Mg + H_2O & \longrightarrow & C_{55}H_{72}O_5N_4Mg + O_2 \\ \text{chlorophyll } b & & \text{chlorophyll } a \end{array}$$

A cycle of reactions of this nature would account for the constant proportions of the two chlorophyll components which have been found to exist in most leaves before and after a period of active photosynthesis.

Czapek[61] pointed out that if chlorophyll acted as a sensitizer it would probably be decomposed by the light as fluorescent dyes are which act as optical sensitizers. This led to the conclusion that the well-known decomposition of chlorophyll in the light, which takes place quite rapidly in solutions *in vitro,* may play a rôle in the normal photosynthetic process. Similar expressions were made by other investigators.[62] Thus, basing his opinions upon experiments on the action of light on chlorophyll preparations and upon published reports of the decomposition of chlorophyll in illuminated plants by Ewart and Lubimenko, Wager concluded that "the carbon dioxide before it can be used is built up independently into the chlorophyll molecule, and it is possible that the production of sugars and starch may be initiated by photo-oxidation of the chlorophyll rather than by direct photosynthesis of carbon dioxide and water."

However, the theory that in the photosynthetic process chlorophyll is constantly being decomposed and reformed is now untenable.[63] Much

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[59] Dixon and Poole, Notes from Bot. School, Trinity College, Dublin, **3**, 90 (1920).

[60] Dixon and Ball, *ibid.*, **3**, 199 (1922).

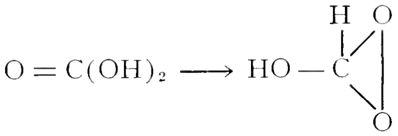
[61] Czapek, *Ber. bot. Ges.*, **20**, 52 (1902).

[62] Wager, *Proc. Roy. Soc.*, **87** B, 405 (1914). Ewart, *Journ. Linnéan Soc.*, **31**, 554 (1897). Askenasy, *Bot. Zeitg.*, **33**, 498 (1875).

[63] Jorgensen and Kidd, *Proc. Roy. Soc.*, **89** B, 342 (1916). Ewart, *ibid.*, **89** B, 1 (1915).

of the experimental data upon which this theory was based have been found to be faulty, because impure preparations of chlorophyll were used. Wurmser's[64] observations on the photo-oxidation of chlorophyll also indicate that in the plant the chlorophyll is protected against photo-oxidation.

The opinion that photo-oxidation plays a rôle in photosynthesis has also been developed by Noak.[65] He regards chlorophyll as a photodynamically active substance which due to its fluorescence has the power of forming a peroxide from oxygen and an acceptor under the influence of light. The mode of reduction of carbonic acid is, however, not elucidated. Noak showed that benzidine in the presence of chlorophyll is oxidized in the light, in solutions as well as in the chloroplasts. Noak, as well as Woker[66] stress the significance of peroxide formation under the influence of light and it is conceivable that peroxides may play an important part in the photosynthetic reactions. The nature of these peroxides is still undetermined, but these may be either of the type of Willstätter and Stoll's formaldehydeperoxide, an isomeric form of carbonic acid,



, or

other peroxides which may split off oxygen or themselves act as reducing agents. When a fluorescent substance produces a photodynamic action it is apparently destroyed. This, in the case of photosynthesis, would lead to the destruction of chlorophyll. Whether the idea of a continuous destruction and reformation of chlorophyll during photosynthesis is tenable is doubtful.

Perhaps the strongest argument against the theory that chlorophyll is decomposed during photosynthesis is offered by the investigations of Willstätter and Stoll.[67] They made careful determinations of the amount of chlorophyll in various leaves before and after very active photosynthesis. There were no differences observed in the amount of chlorophyll. This is illustrated in Table 47. These investigators established this fact in an extended series of experiments.

Willstätter and Stoll also showed that there was no appreciable conversion of one chlorophyll component into the other during the course of photosynthesis. At least, after very active photosynthesis the ratio of chlorophyll *a* and *b* in the leaves is very nearly the same as before illumination. On the other hand, a change in the amounts and ratio of the two yellow pigments, carotin and xanthophyll, was observed after active photo-

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[64] Wurmser, "Récherches sur l'assimilation," p. 18.

[65] Noak, *Zeit. Bot.*, **17**, 481 (1925); **13**, 389 (1921); Review of Warburg, *ibid.*, **12**, 273 (1920).

[66] Woker, *Arch. ges. Physiol.*, **176**, 11 (1919).

[67] Willstätter and Stoll, "Untersuchungen ü. Kohlensäureass.," pp. 11-166.

TABLE 47

CHLOROPHYLL IN 10 GRAMS OF VARIOUS LEAVES BEFORE AND AFTER PHOTOSYNTHESIS. 5 PER CENT CO_2 AND ABOUT 75,000 LUX. (From Willstätter and Stoll.)

Plant	Temp.	Duration of Experiment	Chlorophyll, mg. Before	Chlorophyll, mg. After
Prunus Laurocerasus	30°	6 hours	12.2	12.4
" "	30°	22 "	9.4	9.5
Hydrangea opulodes	30°	6 "	9.2	9.1
Pelargonium zonale	40°	6 "	12.5	12.8

synthesis. Englemann[68] as well as Kohl[69] thought that carotin played a rôle, along with chlorophyll, in photosynthesis, but there has been little evidence to support this view. Willstätter and Stoll consider that the yellow pigments play no rôle in photosynthesis. They base this opinion upon the fact that the rate of photosynthesis is altered by very little when a screen of a solution containing the yellow pigments is interposed between the leaf and the source of light. This evidence may be of significance in connection with the optical properties of the yellow pigments, but can hardly be brought to apply to a possible chemical function of carotin and xanthophyll in the photosynthetic process. In Table 48 are given the results of Willstätter and Stoll's analyses of cherry laurel leaves before and after a period of photosynthetic activity, showing the unaltered chlorophyll-content and the change in the yellow pigments. The experiments were carried out with 5 per cent carbon dioxide, about 68,000 Lux and at 31-32°.

TABLE 48

EFFECT OF PHOTOSYNTHESIS ON THE CHLOROPHYLL COMPONENTS AND ON THE CAROTINOIDS. (Willstätter and Stoll.)

Duration of Illumination	Content in mg. of 10 grams Fresh Leaves						
	Chlorophyll *a*	Chlorophyll *b*	Carotin	Xanthophyll	$\underset{\sim}{Q}\frac{a}{b}$	$\underset{\sim}{Q}\frac{c}{x}$	$\underset{\sim}{Q}\frac{a+B}{c+x}$
Unilluminated	7.2	2.2	0.87	1.65	3.3	0.56	2.3
22 hours	7.1	2.4	0.63	2.29	3.1	0.39	2.0

It is still to be determined whether a change in the carotinoid-content during photosynthesis, as reported by Willstätter and Stoll, is of significance in the photosynthetic reaction. Under the conditions of the experiment, there was a high rate of photosynthesis, so that conditions may have been favorable for the oxidation of the carotin. Willstätter and Stoll also showed that during active respiration in the dark (30-37° for 48 hours) there was no change in the $\underset{\sim}{Q}\frac{a}{b}$ and $\underset{\sim}{Q}\frac{c}{x}$ ratios.

Wlodek[70] has examined various leaves spectroscopically and found

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[68] Englemann, *Bot. Zeitg.*, **45**, 393, 409, 425, 441, 457 (1887).
[69] Kohl, *Ber. bot. Ges.*, **24**, 222 (39) (1906).
[70] Wlodek, *Bull. Acad. Polonaise Sci. et Let. Ser. B. Sci. Nat.*, 1920, 20; 1921, 143.

that the limits of the absorption bands undergo a change after illumination, following fertilization with certain mineral salts and with age. He considers that this displacement of the absorption bands is due to a change in the proportion of the two chlorophyll components and believes to have found evidence for the theory that the ratio of the two components alters during photosynthesis. It is a question, however, whether the observed displacements of the absorption bands cannot be ascribed to a number of other causes as well.

It will be remembered that Baeyer, in formulating the theory that formaldehyde is an intermediate product in the photosynthesis of carbohydrates, assumed that the function of chlorophyll was to combine with carbon monoxide, which had been formed by the action of light on carbon dioxide. He thus assumed that chlorophyll had properties similar to hemoglobin in its power to combine with carbon monoxide and that the latter was then reduced to formaldehyde. This was probably the first time that it was suggested that chlorophyll may enter directly into the chemical reactions of photosynthesis. Various modifications of this idea have appeared from time to time. Hoppe-Seyler[71] considered the possibility of a loose union of chlorophyll and carbon dioxide in which condition the latter was reduced and subsequently chlorophyll liberated again.

Willstätter and Stoll[72] have developed a theory of the function of chlorophyll based upon their studies of the action of carbon dioxide on chlorophyll. When solutions of chlorophyll in organic solvents, such as ether, are treated with carbon dioxide, there is no evidence of chemical reaction. There is no difference in the absorption of carbon dioxide between pure alcohol and an alcoholic solution of chlorophyll. On the other hand, colloidal solutions of chlorophyll in water, show an absorption of carbon dioxide and chemical reaction occurs. The final evidence of this reaction is the splitting out of magnesium from the chlorophyll, as occurs with other acids, with the formation of phæophytin: $C_{55}H_{72}O_5N_4Mg + CO_2 + H_2O \longrightarrow MgCO_3 + C_{55}H_{74}O_5N_4$. On comparing the solubility of carbon dioxide in water and in colloidal chlorophyll solutions, it was found that the latter was considerably higher. In similar experiments carried out by Kremann and Schniderschitsch[73] no absorption by chlorophyll could be observed. This, Willstätter and Stoll maintain, was due to the use of impure preparations in which the chlorophyll had been precipitated.

The reaction of chlorophyll with carbonic acid is complete when the chlorophyll has been entirely decomposed according to the equation just given. However, Willstätter and Stoll are of the opinion that in this reaction an intermediate product is formed, a compound of chlorophyll and carbonic acid. This intermediate product is dissociable and is capable

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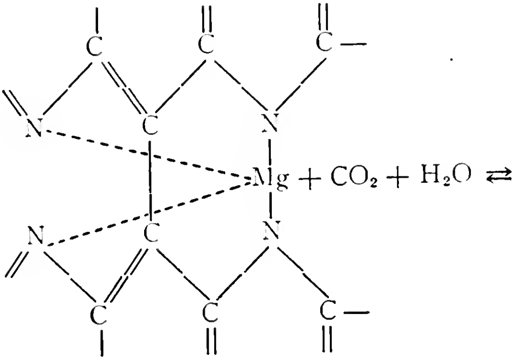
[71] Hoppe-Seyler, "Physiologische Chemie," Berlin, 1881.

[72] Willstätter and Stoll, "Untersuch. ü. Ass. der Kohlensäure," p. 226.

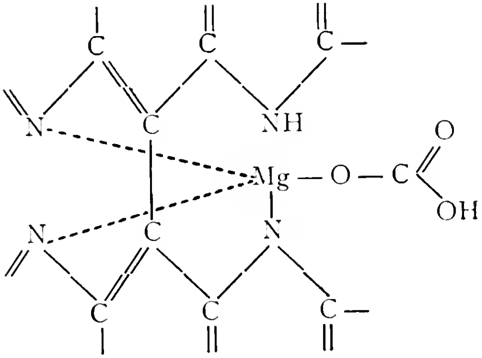
[73] Kremann and Schniderschitsch, *Monatsh.*, **37**, 659 (1916).

of reforming carbonic acid and chlorophyll. Some evidence is presented which supports this conclusion and it would be highly desirable to have further investigation of this point. Willstätter and Stoll showed that when a colloidal solution of chlorophyll has been allowed to absorb carbon dioxide (the reaction not having gone far enough to liberate magnesium), the gas thus absorbed and the chlorophyll can be recovered by adding the chlorophyll-carbonic acid solution to alcohol at 0°. It was shown that in this process the chlorophyll had lost no magnesium.

Willstätter and Stoll have shown that when chlorophyll is treated with weak acids both bonds of magnesium to nitrogen are broken. They suggest that in the chlorophyll-carbonic acid addition product only one of these bonds is broken and a primary magnesium compound of phæophytin is formed. The following formulae, in abbreviated form, illustrate the hypothetical reaction of the magnesium and carbonic acid:



Chlorophyll

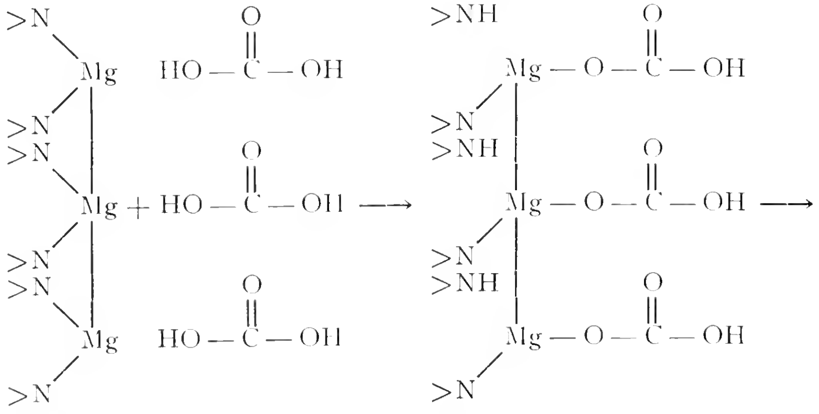


Chlorophyll-carbonic acid

The hypothetical chlorophyll-carbonic acid may break down in either of two ways: 1, by a reversal of the above reaction into chlorophyll and carbonic acid (in alcoholic solution) and 2, in water solution, into phæophytin and magnesium carbonate or bicarbonate (the usual reaction of acids on chlorophyll).

From these facts Willstätter and Stoll conclude that chlorophyll, which in their opinion is also in a colloidal state in the leaf, may, as the first step in photosynthesis, unite with carbon dioxide, and thus also play a chemical rôle. It is possible, however, that carbon dioxide first reacts with some other substance in the leaf which acts as an absorbent, thus producing a high concentration of carbon dioxide in the chloroplast which then enables the chlorophyll to take up the carbon dioxide. The next step in the photosynthetic reaction, as suggested by Willstätter and Stoll is the rearrangement of the chlorophyll-carbonic acid, through the action of light, into chlorophyll-formaldehyde peroxide and the splitting off of an oxygen atom from the latter. This latter step has already been described in the chapter on the Chemistry of Photosynthesis.

Maquenne [74] has made some further theoretical deductions on the basis of Willstätter and Stoll's theory. He also assumes that in the leaf the chlorophyll is in a colloidal state, and that in this state the chlorophyll molecules are held together in aggregates by "supplementary valencies." These valencies are so feeble that they are easily broken and as a consequence the chlorophyll passes into the molecularly dispersed state. These valencies are secondary valencies of magnesium or of nitrogen; for illustration Maquenne assumes that in the colloidal state three chlorophyll molecules are linked through the secondary valencies of the magnesium. The same reactions occur with this trichlorophyll which Willstätter and Stoll assume to take place between chlorophyll and carbonic acid. The difference is that instead of a single chlorophyll molecule and one carbonic acid molecule being involved, three (or more) of each are involved. We thus have the following steps showing the reactions between three chlorophyll molecules and three molecules of carbonic acid; only the magnesium and two of the nitrogen atoms of the chlorophyll are given in the formulae:



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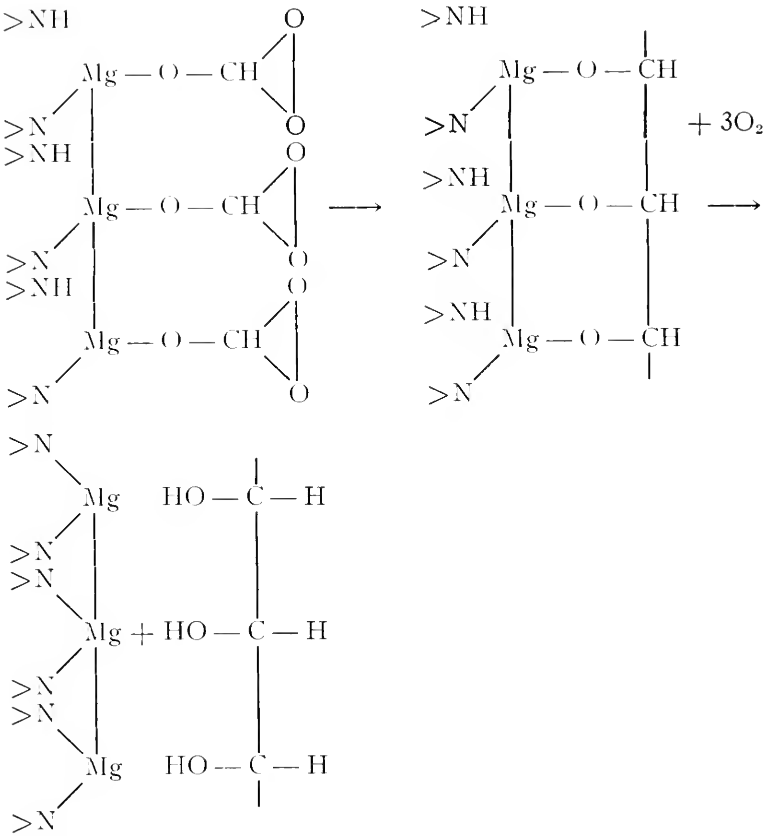
[74] Maquenne, *Bull. soc. chim.* (4), 35-36, 649 (1924).



From these formulae it is evident that Maquenne assumes the final formation of a compound containing a chain of carbon atoms, in the illustration glyceric aldehyde or dioxyacetone, instead of formaldehyde. The formation of a straight chain of carbon atoms would occur in the chlorophyll complex, i.e., by inference, as a result of the colloidal state of the chlorophyll. The theory is essentially a modification of the Baeyer conception; the primary formation of formaldehyde and the subsequent condensation of this to hexose are avoided in Maquenne's conception of the reaction. The assumption of an intermediate product such as formaldehyde, the proving of which has been the source of so much dispute, is thus obviated. In order to conceive of the direct formation of a hexose sugar, it suffices to admit the possibility of a complex of six chlorophyll molecules which absorbs six molecules of carbonic acid.

That this theory of Maquenne is highly speculative needs no comment. There are many steps in the reasoning which are pure assumption or based upon facts which are not well established. It is evident that the whole theory rests upon the opinion that chlorophyll in the leaf is in a colloidal state. While this has been widely accepted, the work of Stern has cast some doubt upon the validity of this conception. It, moreover assumes that the colloidal state is due to the union of secondary valencies and accepts the possibility of a chlorophyll-carbonic acid compound. Nevertheless, the theory is not without interest and is also suggestive of possible mechanisms of the action of other colloidal substances which behave like catalysts, as for example, some of the enzymes. The theory suggests that the condensing or synthesizing action of enzymes may be due to the fact that these are colloidal substances or aggregates of molecules which absorb or adsorb molecules of simple structure, A. The adsorption compound undergoes change, e.g., loss of water, and subsequently the enzyme and a complex compound, the product of the condensation of several molecules of A, are formed.

2. The Chloroplasts

The extreme difficulties which are associated with any endeavor to interpret the reactions of a living organism in terms of the concepts of physics and chemistry become evident when the finer anatomical structure of the organisms is considered. Our knowledge of chemistry has been obtained largely from intensive study of the properties of matter under known and well defined conditions. One of the purposes of laboratory investigation has been, as it were, to isolate and confine single reactions and to study these under control of all imaginable conditions. It has thus been possible to determine what conditions influence the course and rate of the reactions. This has been a comparatively simple task, though it has led to a prodigious mass of information regarding the properties of matter and the conditions under which this changes form. There are many chemical reactions, of inorganic and carbon compounds, taking place in a homogeneous system, which are now fairly well understood, and while in heterogeneous systems more complexities are introduced, nevertheless in most of these reactions the properties of the substances involved are either already well known or can eventually be determined.

The chemical reactions in living organisms, on the other hand, occur in minute compartments, the cells. In no case are the chemical changes taking place in a cell confined to one reaction or a group of similar reactions. In the leaf of a plant, at least, each cell is a whole chemical laboratory in itself. The cells are microscopic units of complex physical structure, which can be discerned only under the microscope. Many of the reactions in cells are apparently catalytic, i.e., enzymatic or surface reactions. The agents inducing such reactions are usually present in very small amounts, are exceedingly complex substances, easily disintegrated and therefore not amenable to study by the same means applicable to other chemical reactions. There is an enormous difference between a clean glass vessel into which substances can be introduced at will and which can be subjected to almost any conditions, and a microscopic living cell of the most complex structure, containing a large number of substances and rendered inactive by relatively slight alterations in external conditions.

In reactions taking place in a living organism, as for example, in photosynthesis, there are substances which play a very important rôle about which, from the chemical viewpoint, we know virtually nothing and for the study of which no adequate means have been found. There is some evidence that in the photosynthetic process a reaction of enzymatic character plays an important part. This enzyme is presumably a product of "protoplasmic activity." We also know that photosynthesis apparently does not take place in the entire cell, but centers in or about small, well defined bodies within the cytoplasm of the cell. These minute bodies, the chloroplasts, contain the chlorophyll and are the centers of photosynthetic activity. The chloroplasts consist of a colorless portion, the stroma, and a colored portion containing the pigments. They have been the subject of much micro-anatomical study and much attention has been given to their origin and development as far as can be observed under the microscope. The results of those studies have proved to be of only limited value for an understanding of the chemistry of photosynthesis and as yet we can ascribe to the chloroplasts no definite chemical function.

The size, form and number of chloroplasts in each cell varies considerably in different species. In some of the simplest forms each cell contains one large chloroplast, often the shape of a disc or saucer. With higher differentiation of the plants, the number of chloroplasts in each cell increases. Haberlandt[75] attempted to show that there was a close relationship between the number of chloroplasts in a leaf and its rate of photosynthesis. He counted the chloroplasts in the mesophyll and calculated the number of chloroplasts per unit area of leaf surface. For example, in a leaf of *Ricinus communis* he found an average of 36 chloroplasts in the palisade cells and 20 in each cell of the spongy mesophyll. There are about twice as many palisade cells, so that there may be ascribed about 403,200 chloroplasts to the palisade tissue and 92,000 to the spongy parenchyma per square millimeter. That is, about 82 per cent of the chloroplasts are in the palisade tissue and 18 per cent in the spongy mesophyll. The ratio of the number of chloroplasts in the two portions of the mesophyll differs with the species. The total number of chloroplasts per unit area also varies, e.g., according to Haberlandt, *Tropæolum majus* has 383,000 chloroplasts per square millimeter, *Phaseolus multiflorus* 283,000, *Ricinus communis* 495,000, *Helianthus annuus* 465,000. Haberlandt endeavored to establish a proportionality between the number of chloroplasts and the rate of photosynthesis or the "specific photo-

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[75] Haberlandt, *Jahr. wiss. Bot.*, 13, 74 (1882); "Physiologische Pflanzenanatomie," 1924, p. 256.

synthetic energy." Thus, if both values are placed at 100 in *Tropæolum majus,* the following results are obtained for other plants:

	"Specific Photosynthetic Energy"	Number of Chloroplasts
Tropæolum majus	100	100
Phaseolus multiflorus	72	64
Ricinus communis	118.5	120
Helianthus annuus	124.5	122

Although the results show a general direct proportionality there is little doubt that other factors also control the photosynthetic rate.

In the lower plants containing only one or a few chloroplasts these are frequently quite large, while in the higher plants they are smaller and do not show great variation in size. Of the 215 species examined by Möbius [76] 50 per cent had chloroplasts which measured 5 μ and 75 per cent 4-6 μ. The size varied from 3-10 μ, but only very few species had chloroplasts over 7 μ.

The chloroplasts exhibit a variety of form. They are often compact bodies ranging in form from spherical, ellipsoidal, lens and disk-shaped to rough-edged plates. Küster [77] has also described chloroplasts of fluid or ameboid consistency found in the orchids. The physical structure of the chloroplasts is apparently variable. They have been described on the one hand as perfectly homogeneous bodies and, on the other hand, as possessing a granular or spongy appearance containing droplets of oil. This granular appearance may be due to the accumulation of starch or oil, formed by photosynthesis, and held as reserve food material. Meyer [78] has developed the idea of the structure of the chloroplasts as consisting of a porous colorless mass which is filled with droplets of an oil or lipoidal substance, the grana, in which the pigments are dissolved. It has also been suggested that the oil is a direct product of photosynthesis, while some consider the oil as a degradation product of the chloroplasts.[79] The ultramicroscopic observations of Price [80] indicate that the chloroplasts are slightly opaque, heterogeneous bodies of a gel structure. In some plants crystals, supposedly of proteins, have been observed as inclusions in the chloroplasts. Ullrich [81] has also demonstrated that the chloroplast may play an important rôle in protein synthesis in the leaf, though all of the protein in the chloroplast can evidently not function as temporary reserve food material. The composition of the chloroplasts is not definitely known, though doubtless the colorless portion, the stroma, contains pro-

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[76] Möbius, *Ber. bot. Ges.,* **38**, 224 (1920).

[77] Küster, *ibid.,* **29**, 362 (1911).

[78] Meyer, "Das Chlorophyllkorn in Chemischer, Morphologischer und Biologischer Beziehung," Leipzig, 1883. Meyer, *Ber. bot. Ges.,* **35**, 658 (1917); **36**, 235 (1918).

[79] Liebaldt, *Zeit. Bot.,* **5**, 65 (1913). Rothert, *Bull. Acad. Sci. Cracovie. Class Math. Nat. Sci.,* 1911, p. 189. Lepeschkin, *Ber. bot. Ges.,* **28**, 91 (1910); **29**, 181 (1911). Ponomarew, *ibid.,* **32**, 483 (1914).

[80] Price, *Ann. Bot.,* **28**, 601 (1914).

[81] Ullrich, *Zeit. Bot.,* **16**, 513 (1924).

tein as well as lipoid substances. There has been considerable discussion as to whether the chloroplasts possess a membrane and opinions are still at variance regarding this point.[82]

There is a phase boundary at the surface of the chloroplast between this and the surrounding cytoplasm. Senn [83] is of the opinion that the chloroplast is bounded by a peripheral layer, the peristromium, while Meyer [84] is of the opinion that the latter is a part of the cytoplasm.

The manner in which the pigments are distributed or held in the chloroplasts is not clearly understood. According to Meyer's views the pigments are dissolved in the oil of the grana which are imbedded in the colorless stroma. It has also been stated that the pigments are uniformly distributed through the chloroplast while some investigators have concluded that the chlorophyll is on the surface of the chloroplasts.[85]

Of considerable interest is the fact that chloroplasts have been removed from the cells in which they were contained, and it has been found that thus isolated, the chloroplasts continue their photosynthetic activity for some time.[86] While Kny [87] has maintained that in these preparations some cytoplasm was still adhering to the chloroplasts, which made possible their activity, there is no doubt that the method offers a splendid means of studying their behavior. With modern cytological technique it is possible that much valuable information could be gained by the development of this method.

Finally, another property of the chloroplasts which may exert an influence on the rate of photosynthesis should be mentioned. It has been observed that the chloroplasts orient themselves in the cell in response to the intensity of light. Owing to differences in the number of chloroplasts in a single cell and other physiological and morphological properties, the form which this orientation takes varies in different species. These phototactic reactions of the chloroplasts have been extensively studied and the results have been recorded diagrammatically. We shall here only indicate the principle of this orientation. Thus, for example, in light of moderate intensity, the disk-shaped chloroplasts may be so arranged within the cell that their flat surface is at right angles to the incident radiation. In this manner maximum absorption of the light is attained. When the illumination intensity is high, the position of the chloroplasts is changed; under these conditions the edge of the disk is in line with the incident rays. Many variations of this orientation of the chloroplasts resulting from alterations in the intensity of light have been

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[82] Zacharias, *Bot. Zeitg.*, **41**, 209 (1883). Schmitz, "Die Chromatophoren der Algen," Bonn, 1882.

[83] Senn, "Die Gestalts- und Lageveränderungen der Chromatophoren," Leipzig, 1908.

[84] Meyer, *Ber. bot. Ges.*, **40**, 161 (1922).

[85] Priestley and Irving, *Ann. Bot.*, **21**, 407 (1907).

[86] Englemann, *Bot. Zeitg.*, **39**, 446 (1881). Ewart, *Jour. Linnéan Soc.*, **31**, 364 (1896); *Bot. Centralb.*, **72**, 289 (1897); **75**, 33 (1898).

[87] Kny, *Ber. bot. Ges.*, **15**, 388 (1897); *Bot. Centralb.*, **73**, 426 (1898). Kostytschew, *Ber. bot. Ges.*, **39**, 333 (1921).

described. It is apparent that considerable differences in the surface exposed to the light may result according as to whether the flat side or the profile of the chloroplast is in the direct line of the incident rays. The same result is attained in some plants through the chloroplasts in each cell forming one or more congregate masses under conditions of intense illumination. Through mutual shading the total surface exposed to illumination is reduced. All these alterations in the position of the chloroplasts may also be brought about by changes in external conditions other than light. Mechanical injury, removal of water and changes in temperature induce the same alterations. However, no accurate determinations have been made of the influence on the rate of photosynthesis of these alterations in the position of the chloroplasts.[88]

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[88] Frank, *Jahrb. wiss. Bot.*, **8**, 216 (1872). Stahl, *Bot. Zeitg.*, **38**, 297, 321, 345, 361, 377, 393, 409, 868 (1880). Haberlandt, *Jahrb. wiss. Bot.*, **13**, 74 (1882). Schimper, *ibid.*, **16**, 1 (1885). Senn, "Die Gestalts- und Lageveränderung der Pflanzenchromatophoren," Leipzig (1908). Liese, *Beitr. allg. Bot.*, **2**, 323 (1922).

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