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STUDIES IN PLANT RESPIRATION AND PHOTOSYNTHESIS

BY

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PREFACE.

The experiments on photosynthesis which were begun at the Desert Laboratory in Tucson, Arizona, about eight years ago yielded, among other things, the conclusion that prerequisite to an understanding of the nature of the energy transfer in photosynthesis was a more extensive knowledge of the metabolism of chlorophyllous organs. It became clear that, before attempting to follow the course of the synthesis of carbohydrate material in illuminated leaves, it was essential to know more about the conditions governing the equilibria and mutual transformations of the various groups of carbohydrates, quite independent of the photosynthetic process.

In publication No. 287 some of the more essential of these conditions were described. A further prerequisite to an understanding of the carbohydrate economy of chlorophyllous leaves is more precise information regarding the nature of the carbohydrate catabolism and the conditions governing this phenomenon. In the present publication are described the results of experiments on the relation of the amino-acid and carbohydrate content to the respiration of leaves. The information gained from these studies, as well as from those of a number of other workers in this field, helps to emphasize the fact that photosynthesis is an exceedingly complex process. The present status of the problem is, however, exceedingly encouraging in that refinement of methods of observation and experimentation and the utilization of the results and conceptions of allied physical sciences are contributing greatly to a better understanding of the phenomenon of photosynthesis.

This publication comprises the results of investigations carried out during 1919-1922. It is a pleasure to acknowledge here the assistance rendered in the preliminary part of this work by Dr. Frances Long.

H. A. SPOEHR.

COASTAL LABORATORY,
Carmel, California, June, 1922.

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STUDIES IN PLANT RESPIRATION AND PHOTOSYNTHESIS.

By H. A. SPOEHR AND J. M. MCGEE.

I. THE CARBOHYDRATE-AMINO-ACID RELATION IN THE RESPIRATION OF LEAVES.

INTRODUCTORY DISCUSSION.

Already at the beginning of the nineteenth century it had been realized that the materials which support the respiratory activity in plants are carbohydrates and fats. The observations of Lavoisier and of De Saussure that the respiration coefficient, CO_2/O_2 , in mature higher plants is usually very close to unity were even at that time accepted as evidence of the preponderance of carbohydrates as the fuel material. Since then a continually increasing number of chemical compounds has been discovered in plants. When derived from the higher, autotrophic plants, these multifarious products must be considered as coming primarily from the carbohydrates through the various intricate channels of plant metabolism. So while the substances which are used as sources of energy by the lower plants and bacteria are of great variety, the higher, mature chlorophyllous plants are in general consumers of carbohydrates.

It would, however, be drawing a very incomplete picture of plant respiration if this were confined to the carbohydrates and fats and the more elusive and complex nitrogenous components were to be omitted. But unfortunately our knowledge of the function of nitrogen compounds in the metabolism, particularly of the higher plants, is most fragmentary. It is our belief that a rational conception of the function of nitrogen in the economy of the higher plants can be gained only through an analytic study of definite groups of nitrogen compounds. This belief has its foundation first of all in the fact that nitrogen is capable of forming such a variety of compounds of basically different physiological properties, and furthermore because our chemical knowledge of the nitrogen compounds derived from protoplasmic proteins is fairly well organized and related and so can serve as a most valuable experimental guide.

Even a cursory study of the carbohydrate economy of the higher plants must emphasize the old conclusion that physiology is essentially a study of dynamics. Thus far organic chemistry has dealt largely with the properties and reactions of carbon compounds. The recent tendency to enter into various phases of energetics and the fundamental causes of chemical reactivity will make chemistry an even more valuable adjunct to physiology than heretofore.

It would seem, then, that the true province of plant chemistry should be the unraveling of the tangle of chemical reactions which give rise to this mass of carbon compounds and ultimately to determine the function of these reactions in the economy of the living plant. Historically, what is now termed organic chemistry had its origin in such a task. But the chemist discovered that he could find a large number of short cuts; that the intermediary action of living things was not essential to the production of most of the substances found in nature; that, in fact, he could greatly improve on nature in the production of the multifarious substances of use in industry and the arts.

In some respects the present has its points of similarity with the time of Woehler and Liebig. Much sober thought is now being given to the fundamental difference between the living and the non-living. The first flush of success of the purely mechanistic interpretation of life processes has paled as the enormous difficulties of applying this conception to the highly intricate and manifold manifestations of life have been more clearly recognized. The methods and modes of transforming matter followed by the chemist and by the living organism are perhaps not as similar as appears at first glance. At first we were primarily interested in the final goal, the products of material transformations; but, in chemistry as well as in physiology, as we follow more closely the energetics of such transformation, many almost irreconcilable differences arise which, before the two can be brought together, necessitate sweeping compromises to the unknown and indefinite. And it is this question of energy which is forcing us to a deeper study of living things. The scientific world is awakening to the necessity of taking stock of our available sources of energy. Repeatedly attention has been called to the inexhaustible floods of solar energy and to the extensive use of plant products to drive the machinery of our civilization. Carbohydrates are to prevent the too-rapid exhaustion of our oil and coal resources. As the realization of the limits of our energy resources has become more general, reliance on the magic accomplishments of the chemist has increased, and the chemists, with cool theoretical nonchalance, have pointed to the untold possibilities of solar energy. But the living chlorophyllous plant still remains the only converter of solar energy!

The chemical reaction most fundamental to all living things, the photosynthetic process, the bridge between the inanimate and the animate, the source of most of our energy, has stubbornly resisted all attempts at solution by physico-chemical methods. Here it has become plainly evident that while physiology is rife with chemical possibilities, physiological experience alone is the guide to the interpretation of biological processes. Furthermore, it has become clear

that physiology is a great deal more than applied physics and chemistry, although we must rely upon these disciplines in order to form conceptions of the various vital phenomena as operations of known causes. With complete disregard of biological facts, chemists have continued to evolve theories of the chemistry of photosynthesis and to supplement and modify existing theories. The fundamental fallacy in these speculations has been that photosynthesis has been regarded—to quote a recent technical article—as “simply a manufacture that provides material used in the process of living,”¹ that because a process can be suspended for hours and months it is not a process or function of the living. The many attempts which have been made to reproduce the photosynthetic process apart from the living cell have not been successful.² This also applies to the experiments in which various chlorophyll preparations have been used, as well as to those in which ultra-violet light was employed as a source of radiant energy, although the latter conditions have no direct bearing on photosynthesis, because normally the plant makes use of only the radiations in the visible spectrum.

That the photosynthetic process is intimately associated with the protoplasmic activity of the living cell was recognized a long time ago. Boussingault,³ in his studies of the effect of gases such as hydrogen, nitrogen, and methane on photosynthesis, came to this conclusion. The same conclusion was reached and somewhat elaborated by Pringsheim.⁴ Interesting and valuable as these older investigations are, as well as the later ones of Ewart,⁵ little precise information can be gained from them, because the methods of experimentation and observation are naturally not in accord with present standards of accuracy.

It has become evident that further insight into the complex phenomenon of photosynthesis is to be gained only by means of intensive study of the process under very carefully controlled external conditions and the elimination of secondary factors. Unfortunately, however, in the study of photosynthesis, internal conditions, such as available nutritional material, have been totally neglected and little or no regard has been paid to the previous history of the plant. It is highly probable that this neglect in many instances accounts

¹ *Scientific American Supplement*, No. 2257, 223 (1918).

² WILLSTÄETTER, R., and A. STOLL. Untersuchungen ueber die Assimilation der Kohlensaure. 1918, 391-415.

SPOHR, H. A. The theories of photosynthesis in the light of some new facts. *Plant World*, 19, 1-16 (1916).

³ BOUSSINGAULT, J. B. Étude sur les fonctions des feuilles. *Compt. rend.*, 60, 608; 1865, *Agronomie*, 4, 359-397 (1868).

⁴ PRINGSHEIM, N. Ueber die Abhaengigkeit der Assimilation Gruener Zellen von ihrer Sauerstoffathmung, und den Ort, wo der im Assimilationsacte der Pflanzenzelle gebildeter Sauerstoff entsteht. *Sitzber. Preuss. Akad. Wiss.*, 763-777 (1887).

⁵ EWART, A. J. On assimilatory inhibition in chlorophyllous plants. *Jour. Linnean Soc.*, 31, 364-461 (1896); 556 (1897).

for the contradictory observations and reports which have been made by different investigators on various phases of the phenomenon of photosynthesis. Furthermore, wherever consideration must be given to the respiratory activity of plants, it is essential that all possible information be obtained regarding the internal conditions, as, for instance, available carbohydrate supply. Such data can be obtained only by rather laborious analytical methods.

If such an interdependence between photosynthesis and respiration actually exists, a better understanding of the nature of respiratory activity is an absolute prerequisite to further investigation of this relationship.

While any consideration of the relation of photosynthesis to respiration must take into account the carbohydrate economy of the plant, it is evident that respiratory activity itself can not be interpreted solely on the basis of carbohydrate balance and supply. In fact, it has been known for some time that there is no direct relation between the amount of available carbohydrates and the rate at which the organism uses this material in its respiratory activity. Although carbohydrates constitute the major portion of the material used by the plant in its process of respiratory combustion, the other agencies and factors which play a role in this process are still but vaguely known. Our knowledge of the chemical possibilities which would find application here is still very incomplete.

Plant physiologists have generally accepted the dictum that, given an adequate supply of carbohydrates, water, certain inorganic salts, and the proper temperature, the rate of life processes in the higher plants depends upon the active mass of protoplasm. To the activity of protoplasm have been ascribed all those functions and reactions which could not be described in the terms and through existing conceptions of physics and chemistry. The attempts of MacDougal, Loeb, and others to describe the physical behavior of protoplasm on the basis of colloidal phenomena have done much to break away from this tendency and to determine to what extent protoplasmic activity is amenable to physical simulation. Similarly, attempts have been made by Palladin to correlate certain components of the protoplasm with the respiratory activity of the plants.

The carbohydrate-nitrogen relation has of late found application in the investigations of Kraus and Kraybill,¹ who have formulated

¹KRAUS, E. S., and H. R. KRAYBILL. Vegetation and reproduction with special reference to the tomato. *Oregon Agr. Exp. Sta. Bull.* 149 (1918).

KRAUS, E. S. The modification of vegetative and reproductive functions under some varying conditions of metabolism. *Amer. Jour. of Bot.*, 7, 400-416 (1920).

HARVEY, E. M., and A. E. MURNECK. The relations of carbohydrates and nitrogen to the behavior of apple spurs. *Oregon Agr. Exp. Sta. Bull.* 176 (1921).

HOOKE, H. D., JR. Seasonal changes in the chemical composition of apple spurs. *Missouri Agr. Exp. Sta. Research Bull.* 40 (1920).

a general theory which has met widespread success in its practical bearing on agriculture. Their investigations deal in the main with the relation between vegetation and fruit setting and nitrate fertilization. They have been able to differentiate the carbohydrate-nitrogen relations of certain plants on the basis of their vegetative and sexual activities.

There exists very little exact information regarding the general course of formation, synthesis, or fate in the general metabolism of any of the organic nitrogen compounds in higher plants. The number and variety of different types of nitrogenous substances found in plants are enormous, and it appears an almost hopeless task to unravel the tangle of chemical reactions which lead to this multiplicity of compounds. It seems, however, quite erroneous to class together the various types of nitrogen compounds, such as the proteins and related substances, the amino-acids, amides, etc., and regard their physiological functions as being the same or even very intimately connected. An insight into this complex of chemical reactions comprising metabolic energetics can, of course, be obtained only through laborious experimental investigation involving much observational and chemical-analytical work with living plants. Chemistry can offer no short cuts, but rather supplies the instruments with which to illuminate these hidden activities. Nor can the conceptions of chemistry always find immediate application to reactions in living organisms. Thus the many theoretical speculations regarding the chemistry of the photosynthetic process have contributed very little to the solution of that problem. This is true also of the chemical speculations regarding the formation of the amino acids and the proteins, although there are a number of elaborate theories which have been formulated to explain this complex process.¹ To these the warning of Pfeffer applies very aptly, which is 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 supported by very little experimental physiological evidence. Moreover, plant chemistry can hardly claim to be an independent science. The great advances which have been made in protein chemistry have been stimulated largely by animal physiology, and progress in our understanding of plant proteins has been made, in the main, by following the methods previously worked out for proteins of animal origin. These studies seem to indicate that the

¹ LOEB, W. *Ber. d. deutsch. chem. Ges.*, **46**, 684-697 (1913).

FRANZEN, H. *Jour. Prakt. Chem.*, **86**, 133 (1913).

BAUDISH, O. *Jour. Biol. Chem.*, **48**, 489-502 (1921). *Ber. d. deutsch. chem. Ges.*, **46**, 115-125 (1913); **49**, 1159-1167 (1916); **50**, 652-660 (1917); **51**, 793-805 (1918); **52**, B 35-43 (1919).

LOEW, O. *Ibid.*, **50**, 909-910 (1917).

proteins of plant and animal origin are very similar in composition. It is, nevertheless, an open question as to what degree we are justified, on the basis of this analogy, in drawing conclusions as to the function of the proteins in the two types of organisms.

The extraction of proteins from leaves is associated with considerable difficulty. Winterstein¹ demonstrated this by the use of different methods on a variety of plants. These difficulties arise from the fact that the proteinaceous material is quite indiffusible and clings tenaciously to the structural elements of the plant, and that any methods of extraction or expression yield complex mixtures from which the protein can be freed only by means of a variety of substances which in some degree also affect the proteins. Very little systematic work has been done on the proteins with a view to determining which are present in green leaves. A good beginning has been made by Chibnall and Schryver,² who attempted to work out methods for isolating and identifying the proteins in the leaves of some of the higher plants. Osborne, Wakeman, and Leavenworth³ have also outlined a procedure for the separation of various protein constituents from the alfalfa plant.

Mature leaves are relatively high in proteins. The mesophyll, freed from leaf-veins, often shows values for protein of over 30 per cent of the dry material. Lakon,⁴ by means of the Molish reaction, was able to demonstrate decided differences in the amount of protein in variegated leaves. The chlorophyllous portions gave a very intense protein reaction, while the albescent portions were poor in proteins. This seems to confirm the opinion that in leaves the principal masses of protein are located in or about the chloroplasts. In fact, Meyer⁵ found that as leaves become older the reduction of the green color runs parallel with a reduction in the protein-content of the leaves. According to these investigations the leaf proteins are located chiefly in the chloroplasts, while the nucleus and cytoplasm seem but very slightly affected by variations in protein-content. Thus the size of the chloroplasts is greatly influenced by the amount of protein in the leaf, in that, as the proteins disappear when the leaf is kept in the dark, the chloroplasts become smaller and contain less chlorophyll, while with an increase in proteins the

¹ WINTERSTEIN, E. Ueber die Stickstoffhaltigen Bestandtheile greuner Blaetter. *Ber. d. deutsch. bot. Ges.*, 19, 326-330 (1901).

Also HAMILTON, T. S., W. B. NEVENS, and H. S. GRINDLEY. The quantitative determination of amino-acids of feeds. *Jour. Biol. Chem.*, 48, 249-272 (1921).

² CHIBNALL, A. C., and S. B. SCHRYVER. Investigations on the nitrogenous metabolism of higher plants. *Biochem. Jour.*, 15, 60-75 (1921).

³ OSBORNE, T. B., A. J. WAKEMAN, and C. S. LEAVENWORTH. The proteins of the alfalfa plant. *Jour. Biol. Chem.*, 49, 63-91 (1921).

⁴ LAKON, G. *Biochem. Zeitschr.*, 78, 145-154 (1916).

⁵ MEYER, A. Eiweisstoffwechsel und Vergilbung der Laubblaetter von *Tropaeolum majus*. *Flora*, 11, 85-127 (1918).

Cf. also CZAPEK, F. *Biochemie der Pflanzen*, 2d ed., 3, 293. Jena (1920).

reverse occurs. In this connection it is interesting to note that according to the experiments of Deleano¹ it is claimed that when leaves (*Vitis vinifera* at 18° to 20°) are kept in the dark, these utilize only carbohydrates during the first hundred hours; thereafter drastic disturbances in the proteinaceous components of the leaf occur. The carbohydrates thus act in the nature of protein-sparers. Now, Meyer points out that when leaves are kept in the dark for a long time, the chloroplast apparatus becomes so greatly impaired through loss of protein that, on subsequently placing the leaves in the light, photosynthesis is no longer sufficiently active to produce enough material to cover the nocturnal loss through respiration. Meyer's observation of decreasing protein-content with advancing age of the leaves is especially interesting when compared with the studies of Nicolas,² who found that on the basis of respiration coefficients the energy release in young leaves is considerably higher than in old ones.

According to Emmerling, there is with advancing age only a reduction of amino nitrogen and total nitrogen but not of protein nitrogen. This latter observation is in even closer accord with the hypotheses of a causal connection between amino-acid content and respiration. Also, the carbohydrate-content shows a general increase as the leaves grow older.³ These facts indicate that carbohydrate-content alone can not be taken as an index of the rate of respiration; nor, obviously, can the carbohydrate-content serve as a measure of the photosynthetic activity.

The following analyses taken from Czapek (*Biochemie der Pflanzen*, 1st ed., Vol. II, p. 202) indicate the amounts of protein which have been found in leaves. These values have, however, only a limited significance, because in the analysis neither the age of the leaves, their history, nor the environic conditions were taken into consideration.

TABLE 1.

Plant.	Water-content.	Crude protein N × 6.25.	Plant.	Water-content.	Crude protein N × 6.25.
	<i>p. ct.</i>	<i>p. ct.</i>		<i>p. ct.</i>	<i>p. ct.</i>
<i>Lactuca sativa</i>		31.75	<i>Ilex paraguayensis</i>	9.41	4.51
<i>Plantago major</i>	81.44	2.65	<i>Zea mays</i>		6.28
<i>Quercus cerris</i>	10.10	10.20	<i>Allium porrum</i>		21.23
<i>Spinacia oleracea</i>		33.06	<i>Cichorium endiva</i>		38.77
<i>Vicia cracca</i>		27.37	<i>Coffea arabica</i>	10.29	5.10

¹ DELEANO, N. *Jahrb. f. wiss. Bot.*, 51, 541-592 (1912).

² NICOLAS, G. *Rev. Gén. Bot.*, 30, 210-225 (1918).

MICHEL-DURAND, E. *Ibid.*, 30, 337-345, 377-382 (1918); 31, 10-27, 53-60, 143-156, 196-204, 251-268, 287-317 (1919).

³ EMMERLING, E. *Landw. Versuch. Stat.*, 34, 113 (1880).

The relation of protein nitrogen to non-protein nitrogen can be seen from Pigorini's¹ results of the analysis of *Morus* leaves (table 2).

The true function of the leaf proteins is a question on which there exists very little definite information, although it has been considered one of the most vital problems in plant physiology. In the nitrogen economy of animals it is apparent that a portion of the assimilated proteins serves to replace the "wear and tear" on the protoplasmic machinery. For this Rubner introduced the conception "repair quota" of protein. Also a "growth quota" of protein is necessary to supply material for the development of protoplasm in new cells in growing organisms. When protein is available in such amounts beyond the requirements of "repair quota" and "growth quota," it can be converted into glucose and fatty acids and thus serve as fuel, very much as though these substances had been ingested as food. To such material Rubner gave the term "dynamic quota." Under conditions of copious protein-supply the animal can to a degree store or deposit the protein in the tissue cells. It seems to be an undecided question whether such protein becomes a part of the living tissue or is stored by the cells very much as glycogen is. The

TABLE 2.

	Morning.		Evening.	
	Dry material.	Fresh material.	Dry material.	Fresh material.
Total N.....	<i>p. ct.</i> 2.445	<i>p. ct.</i> 0.772	<i>p. ct.</i> 2.534	<i>p. ct.</i> 0.884
Protein N.....	2.309	.729	2.368	.824
Non-protein N.....	.1105	.043	.166	.059

animal organism, unlike the plant, is constantly losing a certain quantity of nitrogen, and it appears that if this quantity is not replaced by ingested protein-food the proteinaceous body-tissue is drawn upon.

In the autotrophic plant the condition seems to be quite different. Here also, however, it is first of all essential that, in the discussion, well-defined conditions be established and that generalizations be not made too inclusive. This has been an unfortunate feature of the contributions to protein metabolism in plants. It should hardly need emphasis that results with the fungi may not find immediate application to autotrophic plants, nor that the behavior of germinating seeds corresponds to mature chlorophyllous leaves. In general, it appears as though plants were exceedingly economical with their

¹ CZAPEK, F. *Biochemie der Pflanzen*, vol. II, 294; 2d ed., 1920.

nitrogen compounds. There are no nitrogenous excreta, and the plant possesses the power, to a very highly developed degree, of resynthesizing the decomposition products of proteins. The exception to these statements should be mentioned at once. Thus there are the denitrifying actions of certain bacteria and the behavior of the lower non-chlorophyllous plants. These organisms exhibit a behavior toward the proteins which seems to differ in many fundamental aspects from that of the higher plants.¹

In this study we are concerned primarily with the metabolism of mature autotrophic leaves. *A priori*, the nitrogen metabolism of such organs would seem to have but little similarity to that of germinating seeds, in which it is primarily a question of the resorption of stored proteinaceous material. In view of its great importance it is surprising how very little work has been done on the protein metabolism of leaves.

Deleano's² investigations show that the total nitrogen-content of leaves of *Vitis vinifera* did not change appreciably during 493 hours of respiration in the dark. He also found but very slight variation in the protein nitrogen up to 87 hours. Thereafter these values decreased. These determinations were made by means of Stutzer's method, so that they represent coagulable protein, and no information can be gained therefrom regarding the amino acid-protein relations.

Whether a sharp differentiation can be drawn between living and inanimate protein, or better, perhaps, between protoplasmic and non-protoplasmic protein, in the leaf appears exceedingly difficult. It has also been impossible to determine in plants a factor corresponding to the "protoplasmic mass," which has an important role in the discussions of animal physiologists in considering the phenomenon of respiration. What, finally, the function is of the total protein or of the protoplasmic proteins in plants is a question on which we have no direct experimental evidence. As has been stated, one of the chief difficulties in this problem has been the inadequate information that exists regarding the nature of the proteins of leaves.

Palladin³ has attempted to establish a relation between the rate of carbon-dioxid emission and of the proteins in wheat seedlings. Based upon the old observations of Reinke,⁴ Zacharias,⁵ and Schwartz,⁶ Palladin assumed that the protoplasmic proteins are

¹ MAYER, A. *Agriculturchemie, Gaerungschemie*, 138-139 (1902).

Cf. also IRVING, A., and R. HANKINSON. *Biochemical Jour.*, 3, 87, 1908. IVANOFF, N. N., *Biochem. Zeitschr.*, 120, 1-80 (1921).

² DELEANO, N. *Jahrb. f. wiss. Bot.*, 51, 587 (1912).

³ PALLADIN, W. *Recherches sur la corrélation entre la respiration des plantes et les substances azotées actives. Rev. Gén. de Bot.*, 8, 222-284 (1896).

⁴ REINKE, J. *Studien ueber das Protoplasma* (1881).

⁵ ZACHARIAS, E. *Bot. Zeitung* (1881) 169; (1883) 209.

⁶ SCHWARTZ, F. *Morphologische und chemische Zusammensetzung des Protoplasmas*. 1887.

not digested by gastric juice. He thus finds that in germinating seeds the rate of carbon-dioxid emission is proportional to the amount of protein indigestible in gastric juice when the supply of carbohydrates is ample. While these studies are of preliminary nature and the methods¹ arbitrary and quite lacking in precision, they are nevertheless suggestive of many interesting relations.

The fundamentally important questions in both respiration and photosynthesis are those relating to the dynamic aspect of these phenomena. Both of these processes are primarily of interest because they represent the energy transfer of life phenomena. Without such energy transfers no manifestations of life could be possible. Just as in physics and chemistry, energetics is being recognized as the key to both the structure and behavior of matter, so in physiology further development awaits a more comprehensive conception of energetics. For a long time physiological chemistry consisted in the study of the substances which enter into this device of energy transformation and the products which leave it in the form of excreta; it is now more and more attempting to study the chemical phenomena which underlie the activity of such energy changes. By tradition the behavior of protoplasm is associated with the complex reactions of the proteins. The most complex group of carbon compounds, it is also by inference the medium for the intricate reactions of life processes. Etymologically, proteins are the substance of "first importance." Perhaps there is here a fundamental difference between plants and animals, but, at any rate, it is very doubtful whether in plants a preponderant role can be ascribed to the proteins. From the chemical viewpoint the conception of *living matter* presents many insurmountable difficulties, while, on the other hand, the idea of a complex of coordinated chemical reactions taking place in a material medium falls within the domain of modern physical-chemical reasoning. If "living matter" is possessed of properties and forms of energy *sui generis*, the disciplines of physics and chemistry can be of very little aid in interpreting the behavior of living things. As long as we are working on a basis of physics and chemistry it is of little value to introduce conceptions into physiology which have no sound foundation nor direct analogues in these sciences. Thus to ascribe the energy of life processes to "biotic energy" is certainly not clarifying our conceptions of these processes on the basis of physical science. The fact that physiologists are frequently having recourse to the coining of new phrases, not found in the physical sciences, to describe life processes ought

¹ For the action of pepsin and trypsin on plant protoplasm, cf. W. BEIDERMAN, *Microchemische Beobachtungen an den Blattzellen von Elodea*. *Flora*, 11-12, 604 (1918). WALTER, H. *Biochem. Zeitschr.*, 122, 86-99 (1921).

to serve as a modulator to the mechanists and bring the realization that pronouncements on the mechanistic view of life processes can be made only with reserve and as partial truths.

The term "living matter" is in a sense anthropomorphic, in that we ascribe to the matter of living things attributes and qualities which we recognize in our own behavior. This is in a large measure responsible for the confusion in our conception of the essential nature of the processes in living things, we having superimposed on the matter of vegetable organisms the behavior of our own tremendously complex system, including the maze of mental and spiritual experiences. The differences in the two conceptions at first seem insignificant or subtle, the one of "living matter," the other of "life in matter"; but from the experimental physical-chemical viewpoint the differences are fundamental.

The gap between living and non-living substances in a cell has never been bridged nor have the differences ever been clearly defined. To the proteins has been ascribed the function of life, partly because of their complex structure and reactions and also because they have been found wherever vital phenomena occur. That the proteins form an essential part of the medium in which these complex chemical reactions, called "life," take place, and that the proteins or their derivatives contribute directly or through catalytic action to these reactions, embodies a conception which is compatible with our modern physical-chemical thinking. This conception of a relatively inert substance or medium in which the interplay of chemical reactions takes place, and through which these always manifest themselves as life activities, is advanced, of course, not as describing the actual state of affairs, but as a hypothesis which lends itself to experimental investigation. Thus in the plant-cell the carbohydrates and fats serve as the fundamental sources of energy in respiration. The nitrogen derivatives, with which the plant deals most economically as proteinaceous compounds, constitute an essential portion of the medium in which the multiplicity of reactions occur. Furthermore, these proteins, of themselves inert, are of fundamental importance because of their ability, through their decomposition products—the amino-acids—to influence the enzymatic reactions. The differences in protoplasm are thus to be ascribed to the differences in the media in which the various reaction complexes occur. Protoplasm is to be regarded as a colloidal mass of varying composition, which serves as a medium for the manifold chemical reactions involving the breakdown of many molecules, thus releasing energy which may be used for the synthesis of new compounds, which in turn may be incorporated in the colloidal mass of the substratum. Nor can such a hypothesis explain or define life. It simply attempts

to ascribe more directly the final forces which induce changes in living things to the same causes which activate the inorganic world. This is the only hope for a mechanistic conception of life processes.

The question naturally arises whether in metabolic processes which involve the liberation of energy a continual decomposition of protein serves the plant (1) to contribute to the total energy release of decomposition by material other than carbohydrates and fats, or (2) to provide material essential to the proper functioning of enzymes and catalysists, or (3) whether such a continued decomposition is an unavoidable accompaniment of metabolic energetics. Concerning the first possibility, it is evident that the amount of energy obtained by the decomposition of proteins would be very small, for this is usually a cleavage into amino-acids, and, furthermore, the leaf is capable of re-forming these amino-acids into proteins without the direct utilization of an extraneous source of energy, i. e., through the chemo-synthetic energy derived from the oxidation or the breakdown of sugars.¹ The total gain in energy for the plant from the decomposition of proteins would therefore be *nil*; and unless the decomposition of proteins yields chemical energy of a certain form or at a particular rate, it is difficult to see what advantage would result or what the mode would be of deriving energy by a continual decomposition and subsequent resynthesizing of protein material. Of the possible different forms of chemical energy referred to we know at present practically nothing, so that this is quite beyond the ken of physical-chemical reasoning. That the rate of energy release from protein decomposition differs fundamentally from that of carbohydrate-oxidation can not be assumed; judging, however, from the rate of accumulation of protein-splitting products, it would appear that the carbohydrate breakdown proceeds considerably faster, mol for mol.

As to the second possibility of the function of protein decomposition—the providing of material essential to the functioning of enzymes—the experiments hereinafter described may contribute some information. This aspect is of interest on account of the stimulating action which amino-acids exert on certain enzymes, as well as on account of the fact that amino-acids, being amphoteric electrolytes, i. e., capable of uniting with both acids and bases, have the power of maintaining the hydrogen-ion concentration within definite limits.

That amino-acids accumulate in seedlings left in the dark has been known since the time of Hartig and Boussingault. This

¹ It is quite well established that leaves are capable of synthesizing proteins from amino-acids and even nitrates in the dark in the presence of an abundant carbohydrate supply. Protein decomposition in the presence or absence of oxygen differs primarily in the proportion of the various amino-acids, principally asparagine, tyrosine, and leucine. SUZUKI, *Bot. Centbl.*, 75, 289 (1898); ZALESKI, W., *Ber. d. deutsch. bot. Ges.*, 15, 536-542 (1897); SAPOZNIKOW, W., *Bot. Centbl.*, 63 (1893); PALLADIN, W., *Ber. d. deutsch. bot. Ges.*, 6, 205-212, 296-304 (1888).

accumulation of amino-acids has been the subject of numerous investigations and the basis of much speculation regarding the function and fate of proteins in plant respiration.¹ Suzuki² is also of the opinion that during the night the proteins of the leaf are broken down to amino-acids and that these migrate to other parts of the plant. This seems to be very near to the actual state of affairs, although the extent to which this takes place is still an undecided question. The solution thereof is made very difficult because other processes, such as photosynthesis during the day and respiration at night, may often mask any definite results observable in the analytical data.

Since the development of protein chemistry the improved methods of isolating and determining the various amino-acids have made it possible to establish what compounds are formed in the decomposition of the proteins of plants when these are left in the dark. Most of the investigations have been carried out on germinating seeds, so that many of the results are not altogether applicable to mature leaves. However, Schulze for many years has been working on the amino-acid content of plants and he has obtained some very valuable results. Seedlings of *Lupinus* grown for 14 days in the open were reported by Schulze and Castoro³ to contain nitrogen compounds, as follows:

TABLE 3.

	Total nitrogen.	Proteins.	Asparagine.	Arginine.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Cotyledons (dry material).....	7.83	14.64	17.59	0.14
Stems (dry material).....	6.77	9.56	21.12	
Roots (dry material).....	5.40	11.22	10.23	
Leaflets (dry material).....	6.5	24.66	6.65	.006

Schulze attributes the great difference in the asparagine content between the stems (21.12 per cent) and the leaves (6.65 per cent) to the photosynthetic action of the leaves, which in the presence of carbohydrates convert the asparagine into proteins. In harmony with this it is pointed out that the protein-content of the leaves is 2.5 times as great as in the stems.

¹ PFEFFER, W. Untersuchungen ueber die Bedeutung des Asparagins beim Keimen der Samen. *Jahrb. f. wiss. Bot.*, 8, 429-574 (1880).

MEUNIER, F. Étude sur l'asparagine. *Ann. Agron.*, 6, 275-281 (1880).

BORODIN, J. Ueber die physiologische Rolle und die Verbreitung des Asparagins im Pflanzenreiche. *Bot. Zeit.*, 36, 801-832 (1878).

² SUZUKI, W. *Bull. Coll. Agr. Tokyo* 2, 458 (1896).

³ SCHULZE, E., and N. CASTORO. Beiträge zur Kenntniss der Zusammensetzung und des Stoffwechsels der Keimpflanzen. *Zeit. physiol. chem.*, 38, 244 (1903).

Also KIESEL, A., *Zeit. physiol. chem.*, 49, 72-80 (1906); ANDRÉ, G., *Compt. rend.*, 148, 1685-1687;

THOMPSON, S. G., *Jour. Amer. Chem. Soc.*, 37 230-235 (1915).

In our own experiments with mature leaves of *Helianthus annuus* we found that the amino-acid content of the leaves was higher than that of the stems, while the carbohydrate-content was considerably higher in the stems.

Comparing seedlings which had developed in the light with those grown in the dark, Schulze and Castoro found that the amount of amino-acids in etiolated seedlings 7 days old was higher than in the green plants twice as old, while the amount of asparagine was about the same in both. In general, it would seem from these investigations that the amount of amino-acid formation and protein breakdown is considerably reduced when there are carbohydrates present or the power of carbohydrate formation through photosynthesis is available to the seedlings. This substantiates the general principle that carbohydrates are protein-sparers, which has been found by other

TABLE 4.—*Distribution of nitrogen compounds in leaves and stems according to Schulze's analysis.*

	In leaves.	In stems.
Proteins.....	62.56	23.04
Phosphotungstic-acid precipitate.....	8.06	6.20
Asparagine.....	21.46	66.17
Other compounds....	7.92	4.59

investigators.¹ These studies by Schulze have, however, only an indirect bearing on our investigations, because Schulze was dealing largely with the germination of seeds in which reserve proteinaceous material was in the course of the metabolic activity of the plant resolved into simpler compounds, and the plants were not mature self-supporting organisms. Schulze² has made some extensive investigations of the amino-acid transformations in young plants. In seedlings of the *Vicia sativa*, *Pisum sativum*, *Lupinus luteus*, and *Lupinus albus* grown in the dark there occurs a marked accumulation of amino-acids. The nature of the amino-acids changes with the length of time the plants are allowed to remain in the dark. Seedlings left in the dark 6 to 7 days contain leucin, asparagine, arginine, histidine, lysine, and tyrosine in small quantities. After remaining in the dark for longer periods, 2 to 3 weeks, leucine, tyrosine, and arginine decrease, while the amount of asparagine is greatly increased. Schulze considers leucine, arginine, histidine, and lysine as the primary products of the protein breakdown in leaves, and that these substances in turn are converted into asparagine and glutamine

¹ DELEANO, N. T. *Jahr. f. wiss. Bot.*, 51, 541-592 (1912).

² SCHULZE, E. Ueber den Umsatz der Eiweissstoffe in der lebenden Pflanze. *Zeit. physio. chem.*, 30, 241-312 (1900).

after longer periods of darkness. He considers that asparagine is used in the leaves for protein synthesis. This is concluded from the smaller quantities of asparagine found in the leaves.

In our own experiments with germinating beans (see page 40), some in the dark and some in the light, we found but slight difference between the "light" and "dark" plants. However, these experiments were run only 120 hours, while in the investigations of others, already referred to, the analyses were made after periods of 1 to 4 weeks.

The experiments with mature leaves which are described hereafter show clearly that in the dark there is an accumulation of amino acids. This is true not only of the excised leaves but also of those attached to the plant. How intimate a causal interrelationship exists between amino-acid accumulation and carbohydrate depletion it is impossible to state from the experimental data as yet available. This is, of course, an extremely complicated problem, and requires for solution much deeper knowledge of the nature and function of plant proteins than we are now in possession of. This point, however, does become evident, that the amino-acids have a profound influence on the rate of respiratory activity of the leaves, in the sense that an increase in amino-acid content is accompanied by an increase in carbon-dioxid emission and carbohydrate consumption.

In compendium, then, it appears that in mature leaves:

1. Amino-acids accumulate in the dark as carbohydrates are consumed. There is some evidence that proteins also diminish in this process.

2. The rate of carbohydrate consumption is accelerated by amino-acids. At the same time the fact must not be neglected that respiratory activity is a product (within limits) of mass action, the rate depending as one factor upon the supply of available carbohydrates.

The questions then presented are: In what manner can amino acids influence the rate of respiratory activity? What is the influence of these substances on the general carbohydrate metabolism? Are there any chemical relations between amino-acids and sugar which can account for this behavior, or is this influence exercised through the operation of enzymes? Contributing to the elucidation of these questions are a number of facts which can be drawn from allied sciences.

Of direct bearing on the question of the influence of amino-acids on the respiratory activity is the phenomenon of the specific dynamic effect of proteins, which, observed in animals, has been the subject of extensive experimental investigation and much controversy. Rubner and others established that, in the diet of animals, fats and carbohydrates are mutually interchangeable on a calorific basis. Thus 100 grams of fat can be replaced by 232 grams of starch and by 234 grams of saccharose. In attempting to apply this principle of

isodynamic values to proteins, a number of difficulties were encountered. It was found that an increase of protein in the diet stimulates the total metabolism, so that actually more food is utilized and more heat emitted from a diet rich in protein than from one containing little protein. Rubner¹ subjected this phenomenon to a very thorough and painstaking investigation and gave it the name of specific dynamic action of proteins. He found that the increase in metabolic activity was greater with proteins than with any other class of foods and that dogs fed on meat respired more actively without doing any external work. After establishing the relation of environmental temperature to metabolic rate and excluding the reflex increase in metabolism through chemical regulation, Rubner was able to determine the true values of the specific dynamic action of proteins. The ingestion of the starvation requirement for the various forms of food raised the metabolism as shown in table 5.²

TABLE 5.

Diet.	Heat increase.
	<i>p. ct.</i>
Meat.....	36.7
Fat.....	12.7
Sugar.....	5.0
10 per cent meat, 90 per cent fat...	13.4
Meat, fat, sugar.....	4.0
Meat, fat, starch.....	4.0
20 per cent meat, 80 per cent fat...	21.5

A great advance in the understanding of the specific dynamic action of proteins was made by the investigations of Lusk.³ Instead of using proteins Lusk fed amino-acids. The same action is observable with these splitting products of the proteins. It was found that glycocoll and alanine greatly increase the metabolic activity, that leucin and tyrosine exert but a slight effect, and that glutamic acid is without effect. These findings are especially interesting in view of the following facts: It is known that glycocoll and alanine are completely convertible into glucose in the diabetic organism, whereas glutamic acid is so converted that three of its carbons go to form glucose, while the other two carbon atoms are oxidized. When glycosuria is artificially produced by giving phlorihizin, and the

¹ RUBNER, MAX. *Die Gesetze des Energieverbrauchs bei der Ernährung* (1902).

² RUBNER, MAX. *l. c.*, 325.

³ LUSK, GRAHAM, and S. A. RICHE. Animal calorimetry. V. Influence of the ingestion of amino-acids upon metabolism. *Jour. Biol. Chem.*, 13, 155-183 (1912).

LUSK, GRAHAM. The cause of the specific dynamic action of protein. *Arch. Intern. Med.*, 12, 485-487 (1914). Animal calorimetry: XI. An investigation into the causes of the specific dynamic action of the foodstuffs. *Jour. Biol. Chem.*, 20, 555-617 (1915).

dog is given glycocoll, there is no oxidation of this material, the energy content of the glycocoll is eliminated in the urine as glucose and urea, nevertheless the metabolism is greatly increased. When corresponding or even larger quantities of glucose are given there is no stimulation of metabolism. From this it follows that the immediate cause of the specific dynamic action is not in the oxidation of the amino-acids and liberation of energy therefrom, but that the amino-acids in some manner stimulate the activity of the cells and thus cause them to metabolize more food material.

The specific dynamic action of proteins, especially with the insight gained through Lusk's investigations, throws considerable light on the general problem of the function of amino-acids in engeresis. It does not, however, enlighten us on the fundamental causes of this stimulating action of the amino-acids.

Our first inclination was to search for a purely chemical explanation which could account for this remarkable behavior. A natural supposition seemed to be that the amino-acids affected the sugars in such a manner as to make them more easily broken down and oxidized. As Nef¹ has shown, glucose is a relatively stable sugar, while fructose is more completely oxidized. Thus, in order to reduce completely a mixture of 382.5 grams CuSO_4 and 163.8 grams NaOH (Fehling's solution), the amounts of the various sugars shown in table 6 were required:

TABLE 6.

Sugar.	Grams required for reduction.	Corresponding to atoms of oxygen.
Arabinose. . . .	55.0	2.13
Mannose.	56.4	2.45
Dextrose.	58.0	2.39
Levulose.	64.0	2.16

Similarly, Lusk² demonstrated that fructose was metabolized more actively than glucose, so that the percentage of increase over the indirect basal metabolism for the various sugars was found to be: glucose 30, fructose 37, sucrose 34, galactose 22, and lactose 3. In view of these facts it was possible that the amino-acids acted as isomerizing agents, converting the glucose into the more active fructose, just as had been shown to happen with a great variety of substances, e. g., $\text{Ca}(\text{OH})_2$, $\text{Pb}(\text{OH})_2$, Na_2CO_3 , etc.³ All of our experiments directed to establish such an isomerizing action of the amino-acids on various hexose sugars yielded negative results.

¹ NEF, J. U. *Annalen der Chemie* (Leibig), 357, 219 (1908).

² LUSK, GRAHAM. *Jour. Biol. Chem.*, 20, 555 (1915).

³ NEF, J. U. *Annalen der Chemie* (Liebig), 357, 294-312 (1908); 403, 240-383 (1914).

Furthermore, while fructose fed to mature leaves showed relatively high respiratory activity, we were surprised to find that the respiratory activity of the leaves fed fructose was apparently depressed when amino-acids were also given.

A more encouraging possibility for an explanation of the stimulatory action of amino-acids was offered by the consideration of the influence of these substances on enzyme activity. That some amino acids have a decided effect on the rate of activity of certain enzymes has been known for a long time. Effront¹ in 1893 reported that asparagine accelerates the hydrolysis of starch by takadiastase and later² showed that this accelerating action was also accomplished by other amino-acids, while amines and their salts and acid amides and their salts showed no such action.

The observation that the saccharification of starch by pancreatic juice was accelerated by amino-acids was reported by Terroine and Weill.³ Even very low concentration of glycocoll, 1:10,000, increases the velocity of the saccharification 30 to 40 times. Dakin⁴ observed that many condensations which have their analogues in the metabolism of living organisms are induced by amino-acids, peptones, albuminose, and some proteins, while the same reactions do not take place without these substances. Jacoby and Umeda⁵ also observed that the activity of soja-urease is greatly accelerated by glycocoll, analine, tyrosine, leucin, and glutamic acid. The activity of pine diastase was investigated by Ujhara,⁶ who found that this action was increased 48.5 per cent by very small quantities of glutamic acid and by glycocoll 45.1 per cent. Similar results were obtained with dog-serum diastase. Sherman and Walker⁷ have subjected the accelerating action of amino-acids on diastase to thorough investigation. They have found the acceleration to take place with diastase derived from a variety of sources as well as with starches of widely different origin. Also, the acceleration is not due to any change in the hydrogen-ion concentration nor to a more favorable concentration of the electrolytes.

Finally, Burge⁸ has investigated the influence of introducing various amino-acids into the intestine and stomach on the catalase activity of the blood. While this is stimulated apparently by a large variety of substances, it would seem that the amino-acids

¹ EFFRONT, I. *Mon. Sci.*, **41**, 266 (1893).

² *Idem.* *Ibid.*, (4), **18**, 561 (1904).

³ TERROINE, E., and J. WEILL. *Chem. Abstracts*, **4**, 1535 (1913).

⁴ DAKIN, H. D. *Jour. Biol. Chem.*, **7**, 49-55 (1910).

⁵ JACOBY, M., and N. UMEDA. *Biochem. Zeitschr.*, **68**, 23-47 (1918).

⁶ UJHARA, K. *Chem. Abstracts*, **12**, 1971 (1918).

⁷ SHERMAN, H. C., and FLORENCE WALKER. Influence of aspartic acid and asparagine upon the enzymatic hydrolysis of starch. *Jour. Amer. Chem. Soc.*, **41**, 1866-1873 (1919); **43**, 2461-2476 (1921).

⁸ BURGE, W. E. *Amer. Jour. Physiol.*, **47**, 351-355 (1918); **48**, 133-140 (1919); *Science*, n. s., **49**, 594-595 (1919).

are more effective in this respect than any of the other substances tried, i. e., sodium acetate, acetamide, glycerine, oleine, and dextrose.

It must be realized, of course, that the evidence regarding the behavior of amino-acids toward enzymes, here introduced, does not serve as a direct explanation of the influence of amino-acids on the rate of respiratory activity nor of the specific dynamic action of proteins. However, in the present very incomplete state of our knowledge of this subject, the information of these relations may serve as a valuable guide in developing further our conceptions of the respiratory processes. So, while there is as yet no simple explanation of the role of amino-acids in respiration, it seems also at present exceedingly doubtful whether such a process is amenable to simple treatment.

We are obliged to recognize more and more that not only are the various activities of a living organism intimately interrelated, but that the proper functioning of any one process depends upon the coordination of various enzymatic activities. Thus in the oxybiosis of carbohydrates there are naturally a great many steps between the stored starch and the formation of carbon dioxid and water. Plant physiologists have very generally fallen into the habit of overemphasizing and reasoning as to the *modus vivendi* of reactions in living things from evidence of the substances found in such organisms. It must be borne in mind that, applying the principles of the kinetics of step reactions, the rate of the whole reaction is determined by the rate of the step progressing with the lowest speed, and the more rapidly a total reaction progresses the fewer intermediate products are there possible to detect. Thus, often the most important and reactive products are not detectable by our present chemical methods.

Fundamentally a better understanding of respiration depends upon a knowledge of the nature of enzyme activity, and this, of course, brings us to the very frontier of scientific thought. While the activity or mode of action of enzymes has its analogy in more or less well-defined behavior of catalysis, the composition of the enzymes themselves is an open question. Briefly, there appear to be two schools—the one conceiving of an enzyme as a definite complex substance with catalytic properties, the other regarding enzymes not as chemical individuals but rather as mixtures or complex systems. Although the former conception has received very little advancement through the efforts of synthetic chemistry, it has its strong adherents, as, for instance, Willstaetter,¹ who hopes by means of preparation work to obtain constantly purer products until their chemical nature can be established. On the other hand, following the idea of enzymes as complex mixtures, some very suggestive

¹ WILLSTAETTER, R. *Zeitsch. f. angew. chem.*, 33, 209 (1920).

results have recently been obtained which are pertinent to the general subject of the role of amino-acids in respiration.

Various claims have been advanced from time to time regarding the artificial production of enzymes, and a number of catalytic reactions have been produced by means of these products—reactions which are very closely analogous to those produced by enzymes. In the extensive literature on this subject a very serious error has been allowed to enter—a matter of inference originally rather than of direct statement. It is a very debatable question whether the results with these “artificial enzymes,” i. e., mixtures of substances which induce certain reactions, which are also produced by true enzymes, are of such great value in revealing the composition of the enzymes as has been claimed. It appears rather that these reactions can be induced by a variety of catalyses, as has been found to be the case for many inorganic and organic reactions, and simply that it has been possible for chemists to produce one of these catalysts. However, it does not now follow that this is necessarily the same catalyst which is produced and used by the organism in the form of an enzyme. The point is essentially one of interpretation of experimental results.

Nevertheless, the results obtained with these various “artificial enzymes” are of great value in explaining catalytic action, and therefore are of direct bearing on enzyme activity. Herzfeld¹ has shown that the action of the proteolytic enzymes, pepsin and trypsin, can be simulated by a prepared mixture of amino-acids and polypeptides. According to this author's views, autolysis is simply an autocatalysis which is started by the introduction into the system of some of the products of decomposition. Baur and Herzfeld² have carried these researches further, and announce a splitting of glucose into alcohol and carbon dioxid by means of carefully prepared mixtures containing peptone, bile salts, dextrin, and sodium bicarbonate. Although the proportion of carbon dioxid and alcohol formed in these reactions does not correspond to that obtained in zymase fermentation, the authors explain this by the fact that their catalyst also produces other sugar decompositions. Provided the toluene employed to prevent infection by living organism was thoroughly efficacious, which is open to some doubt, these experiments point the way to a very fruitful field in the investigation of enzyme activity.

¹ HERZFELD, E., *Biochem. Zeitschr.*, 64, 103-105 (1914); 68, 402-435 (1915); 88, 260 (1918).

² BAUR, E., and E. HERZFELD. *Biochem. Zeitschr.*, 117, 96-112 (1921).

METHODS AND APPARATUS.

1. *The Experimental Material.*

For the study of respiration and photosynthesis the leaves of all plants are not equally well suited. That is, in some plants, due to structural peculiarities, these processes exhibit complications and complexities which make the interpretation of observational data extremely difficult. The fundamental feature of both processes is that they depend upon the ingress and egress of gases. The most satisfactory methods of measuring the rate of respiration and photosynthesis are based upon the gaseous exchange, and any factor which at all influences the easy diffusion of these gases interferes with the accuracy of the determinations. For this reason, thin leaves are more easily worked with than those which are succulent or possess protective devices against the loss of water.

Moreover, in a study of respiration, analytical data are of great importance and the leaf material should not present undue difficulties for the chemical determination of the materials intimately associated with the respiratory activity. The plant material should also be capable of being easily grown in quantity and should not be susceptible to infection or disease. Further insight into the mechanism of the processes of respiration and photosynthesis can be gained only by means of experimentation, that is, by subjecting the plant to a variety of external conditions and at will altering some of the internal constituents. For such a procedure the plant must not be too sensitive nor easily affected by slight variations of external conditions. In view of the fact that these studies were directed primarily toward an attack on the problem of photosynthesis, it was desirable to work with leaves in which the chlorophyll apparatus was well developed, and that these leaves should be autotrophic in the sense that, aside from mineral nutrients, they should be entirely capable of producing their own food material.

It would, of course, be desirable to gain information relative to the respiratory and photosynthetic processes of a large variety of species. We are fully aware that different species exhibit great variation in their behavior. Similarly, a knowledge of the behavior of these plants in the field, under natural conditions, would probably be of greater significance than the information gained under such highly artificial conditions. However, these ideals are not amenable to the experimental method and can be attempted only at too great a sacrifice of precision and intensity of investigation.

We were therefore constrained to confine our studies to a very limited number of plants which fulfilled the requirements mentioned. The wild sunflower of southern Arizona, *Helianthus annuus*, and

the "Canada Wonder" bean have answered the requirements admirably, and we have been able to grow this material in the greenhouse during most of the year. Naturally, care was exercised to use only perfect leaves, free from infections and other imperfections. By thus concentrating our efforts we hoped to be able to gain deeper and more detailed information which might serve to elaborate some general principles of plant metabolism.

For most of the experiments leaves which had been cut from the plant were used. The method of cutting is described later.

Although experiments with excised leaves apparently represent highly artificial conditions, this method possesses many distinct advantages. An entire plant usually requires so much space that it is exceedingly difficult and cumbersome to construct respiration chambers which will accommodate the plant and at the same time permit accurate control of the temperature. By using excised leaves the space can be very materially reduced and the requirements of temperature control adequately met. A relatively small volume of the respiration chamber has another advantage in that with a given rate of the air-stream it permits the more perfect removal of the gases. By cutting the leaf from the plant the factor of translocation is also eliminated. Thus materials which are formed in the leaf during the course of respiration do not migrate to other parts of the plant, and the only source of supply of food material is that which is stored in the leaf itself. The general effect of these conditions as compared with the attached leaves is, as it were, to shorten the time of certain reactions or accentuate their intensity. By removal from the rest of the plant, the leaf has thus been severed from its base of supplies as well as from the receiver of its surplus products.

The use of excised leaves also offers the only satisfactory method of feeding to leaves substances the behavior of which it is desired to study. In comparison with animals, physiological work with plants is at a greater disadvantage in this respect. It is not possible to ingest into plants a definite quantity or kind of food material, as can be done with animals. However, it has been established that many organic substances are taken up into the leaves in a short time when the petioles are placed in solutions of these substances. On the other hand, under the conditions of our experiments there was no migration of materials, such as sugars, from the leaf into the nutrient solution.

The plants used in these investigations were grown in a greenhouse in a loam soil to which no fertilizer had been added. Care was taken to use leaves of about the same age, size, and development. It was endeavored to study the behavior of mature, well-functioning leaves; so both rapidly growing and old leaves were discarded.

2. *The Apparatus.*

The determinations of the rate of respiration were made on the principle of drawing air free from carbon dioxide over the leaves and absorbing the carbon dioxide given off by the leaves in a standardized solution of barium hydroxide. The apparatus was so arranged that the leaves were always under the pressure of the atmosphere. A small piston-pump driven by an electric motor drew the air through the entire apparatus; a large reservoir and a pressure regulator assured the regularity of the stream. The air was drawn from out of doors in order to avoid the possibility of deleterious effects from impurities in the air of the laboratory. It was passed first through a train of soda-lime tubes to remove the carbon dioxide and then through a coil of metal tube, 10 feet long, which was immersed in the water thermostat. A large Freas electric thermostat was employed and set at exactly 25°. From the metal tube the air passed to the upper opening of the respiration chamber.

A drawing of the respiration chamber is given in figure 1. This consists essentially of a galvanized-iron can 40 cm. deep and 18 cm. in diameter. A heavy lead base permits the entire chamber to be submerged in the water of the thermostat. To the top of the chamber is soldered a collar which forms a trough. Into this trough fits loosely

FIGURE 1.

Respiration chamber with mercury seal and cover, with broken out section showing container for nutrient solution and device for holding petioles of leaves.

an inverted glass crystallizing dish and over this a galvanized-iron cover. As a seal, the trough contains mercury about 3 cm. deep and over this is placed water. In order to protect the trough and metal cover against the action of the mercury, these were first electroplated with copper, then covered with nickel, and finally given a coat of waterproof lacquer. When the respiration chamber is in use it is entirely covered by the water of the thermostat and quickly attains the temperature of the bath. The metal cover

FIGURE 2.

Apparatus for automatically changing the course of the air-stream from one absorbing tube to another. The bell-valve with mercury seal is opened by means of the electromagnetic coil and closed when the current is cut out of the coil. One valve is attached to each coil. In the cut, one complete valve connected with the electromagnet which controls it is shown; the three other coils are indicated.

can be removed during the course of an experiment to observe the leaves in the chamber or to admit light, as the glass cover in the mercury makes an absolutely tight seal.

On the bottom of the chamber is a glass dish containing a large number of short pieces of glass tubing set on end. This dish contains the nutrient solution and the glass tubes are of a size to admit easily

the petioles of the leaves; one leaf in position is shown in the drawing. Fresh sterilized solutions are of course used for each experiment, the entire chamber and dish being sterilized each time.

The air-stream enters the chamber through the upper tube and leaves through the lower one. To the latter a trap is attached to catch the water-vapor which is carried out and condenses in the tubes. By means of glass tubing the air-stream is distributed through four glass stopcocks to the four mercury bell-valves. These valves are electrically controlled by means of a clock and require detailed description.

Our preliminary studies on the respiration of leaves soon revealed the necessity of making carbon-dioxid determinations over a longer period of time than had usually been done. From observations of a few hours or a whole day only limited information can be gained as to the true nature of this process, especially if the leaves are to be fed various substances. Moreover, too long periods, as during an entire night, are for obvious reasons to be avoided. These requirements necessitated attention being given to the experiments during the entire 24 hours of the day. Therefore, in order to obviate the night work, an apparatus was devised which automatically changed the course of the air-stream from one absorbing tube to another at definite times.

As was stated, the air-stream passes from the distributing tubes to the mercury valves. The construction of these valves, together with the electric coils which control them, is pictured in figure 2; a detail of the mercury valve is shown in figure 3. In figure 2 the valve is shown open, giving free passage of the air-stream from the distributing tube through the valve to the absorbing tubes. The valve is connected by means of a heavy wire to a piece of laminated iron which forms the core of an electric magnet. When the current passes through the wire coil, the core is drawn up and the valve remains open as long as the current passes through this coil. As soon as the current stops passing through the coil, the iron core drops and the valve is closed by means of the glass tube which is immersed

FIGURE 3.

Detail of bell-valve, made of glass and sealed with mercury. The upper portion of the glass tube is attached to the core of the electromagnet; the lower end is immersed in mercury when the valve is closed and drawn out of the mercury by a magnet to permit the air-stream to pass into the absorbing tubes.

in the mercury in the small bottle. The detail in figure 3 shows what is essentially a bell-valve made of glass and sealed with mercury.

As is shown in figure 2, the apparatus contains four of these valves, each with its own electromagnet and each connecting with a separate absorption tube. The time during which each valve was held open represents one period of carbon-dioxid determination.

The manner in which the electromagnets are controlled can be seen by referring to figure 4. This apparatus consists essentially

FIGURE 4.

The clock which makes electrical contact through the mercury trough and thus controls the valves by means of the electromagnets.

of a circular piece of wood 5 cm. thick and 30 cm. in diameter. A circular trough has been cut in the upper face. The whole is mounted on three wooden legs. In the center hole is a 24-hour cylinder clock, *C*. This carries a metal arm, on one end of which is a mercury cup, and through this cup connection is made with the electric current of the line through the wire and binding post *L*. From the other end of the arm is suspended a looped piece of copper

wire. This copper wire floats on the mercury in the trough. The sides of the trough and the rest of the wood were covered with several coats of Bakelite paint to insure insulation. The trough is partitioned off with thin pieces of mica, dividing the whole trough into quadrants which are completely insulated from each other. If necessary the trough can be divided into a larger number of sections. The mercury in each section (in fig. 4 there are four) is connected by means of a short wire to a binding post *V*. A wire from each one of these binding posts connects to one of the electromagnetic coils, the other end of the coil connecting to the line. Thus the circuit is completed, the current running from the line to binding post *L*, in turn through the mercury cup on the clock, the arm (not through the clock!), through a portion of the mercury in the trough, through a sectional binding post *V*, to a coil, and back to the line.

As the clock moves, the arm is carried over the trough, making contact in one section of the mercury. When the arm reaches a mica intersection it breaks the connection with one section and makes contact with another. Thus the current is broken from one coil and run through another coil, which process closes one valve and opens another. In this manner a 24-hour clock with four intersections makes possible four 6-hour periods. The periods can be increased or shortened at will by changing the number of intersections or by using a clock of different rotation. For this work commercial 110-volt A. C. current was used; the coils were constructed with a resistance of about 80 ohms and heated very little. Sparking can be avoided by insertion in the circuit of suitable condensers, although no trouble was experienced from this source.

From the mercury valves the air-stream passes to the absorbing tubes. These were 10-bulb Meyer tubes which were supported by wooden racks. The glass tubes were connected by means of heavy rubber tubing and all rubber connections were wired and covered with Bakelite paint. Beyond the absorbing tubes a pressure regulator was inserted. This was patterned after the well-known Palladin regulator¹ and modified so that a cylinder containing a saturated solution of calcium chloride was used as the liquid. This permitted more accurate adjustment of the pressure than mercury. A water manometer was also inserted through the rubber stopper in the cylinder, so that any change in the pressure in the system could be detected at once. The pressure regulator was immersed in the water of the thermostat to avoid the influence of changes of temperature. With this device the pressure was kept quite constant, never exceeding 2 cm. of water, and the air-stream was perfectly regular. Connection was made from the pressure regulator

¹ ABDERHALDEN, E. *Handbuch der Biochemischen Arbeitsmethoden*, vol. III, 1, 481 (1910).

to a large carboy and from this to the electric pump. The rate at which air is drawn through the apparatus is of minor importance as long as it allows no accumulation of carbon dioxide in the respiration chamber and as long as the carbon dioxide is completely absorbed in the barium-hydroxide solution.

The carbon dioxide absorbed by the barium-hydroxide solutions was calculated from determinations of the changes in concentration of this solution. At first this was done by titration with standard acid; later this method was supplanted by the more rapid and more accurate method of determining the electrolytic conductivity of the solution.

For the titration method a solution of about 0.1 normal strength was used. This was made up in 18-liter lots, and to each 1,000 c. c. 1 gram of barium chloride was added. The barium-hydroxide solutions, through which carbon dioxide had passed, were transferred to narrow bottles, the barium carbonate was allowed to settle, and three aliquot portions of the clear supernatant solution were used for titration with 0.1 normal hydrochloric acid. In order to attain very accurate results with this method it is necessary to realize that barium carbonate is slightly soluble in the barium-hydroxide solution. From the work of Vesterburg¹ and of Weissenberger² it is evident that the addition of barium chloride to suppress the hydrolysis of the barium carbonate is essential in order to attain satisfactory results with this method. This is a precaution frequently neglected in determining rates of respiration by this procedure.

On account of the labor and time involved in making the many titrations required by the foregoing method, an electrolytic method was devised which gave exceedingly satisfactory results. For this purpose a pure barium-hydroxide solution was used, prepared with CO₂-free water. Considerable preliminary experimentation was necessary to work out a satisfactory and simple apparatus for the electrolytic determinations. It was found quite impracticable to incorporate the electrodes for the conductivity determinations within the absorption tubes. The chief difficulty encountered here was, in brief, that the electrodes became covered with barium carbonate and thus greatly increased the resistance of the cell. Hence the cell-constant at the beginning and end of the determination was not the same; even a very slight deposit of barium carbonate, such as is formed by wetting the electrodes with a barium-hydroxide solution and allowing this to remain in the air for a few minutes, gave spurious results. The procedure which was followed, therefore, was to transfer the entire contents of the absorption tube into a glass vessel with a wide mouth. This was carefully stoppered and the barium car-

¹ VESTERBURG, A. *Zeit. physik. chem.*, 70, 550-568 (1910).

² WEISSENBERGER, G. *Ibid.*, 88, 257-270 (1914).

bonate allowed to settle out completely. When the supernatant liquid was perfectly clear, this vessel was placed in the water thermostat kept at 25°. The stopper was replaced by a dip-conductivity arrangement, as shown in figure 5. The electrodes are essentially the same as those used in the Cain method¹ for the electrometric determination of carbon in steel. A Leeds & Northrup combination bridge and galvanometer operating on a 110-volt A. C. 60-cycle current gave highly satisfactory results.

For the successful operation of this method a number of facts had to be determined which were not available in satisfactory form in the chemical literature. It was necessary to choose a barium-hydroxide solution of a concentration such as would completely absorb the carbon dioxide in the air-stream at the maximum rate emitted by the leaves and for the longest period of observation. Such a solution should also show the maximum change of resistance for a given change in concentration. After preliminary experiments in which these limits were established, a solution was decided upon with an initial concentration of 0.12 normal, which could with safety be decreased, through the precipitation of barium carbon-

FIGURE 5.

Conductivity cell used to determine the strength of the barium-hydroxide solution. The barium carbonate is allowed to settle and the resistance of the clear supernatant solution is determined at 25°.

ate, to 0.05 normal. It was necessary then to obtain experimental data from which a curve of specific resistance could be drawn for definite concentrations of the barium-hydroxide solution. The concentrations of the solutions used for these determinations were obtained by means of titration with hydrochloric acid, and the resistances were determined at 25° by means of the cell shown in figure 5. The values thus obtained are given in table 7. Fortunately there were available two determinations of the specific conductivity of barium hydroxide by A. A. Noyes² at concentrations very close to both extremes of the concentrations used by us. Cal-

¹ CAIN, J. R., and L., C. MAXWELL. *Jour. Ind. and Eng. Chem.*, 11, 852 (1919).

² NOYES, A. A. *Tables annuelles constantes et données numériques de chimie, de physique et de technologie*, vol. 1, p. 463 (1912).

culating the specific resistance from these values, those of Noyes are for 0.10 normal 48.97 and for 0.05 normal 92.98, while our results were respectively 49.80 and 92.20.

In view of the fact that our determinations were not made for the purpose of obtaining physical-chemical constants, and we required only relative values for the determination of respiration-rates, the agreement is quite satisfactory.

TABLE 7.—*Specific resistance of various concentrations of barium-hydroxide solution, determined at 25°.*

Cell constant = 1.2255.

No.	Normal concentration.	Observed resistance.	Specific resistance.	No.	Normal concentration.	Observed resistance.	Specific resistance.
Stock	0.11690	53.2	43.40	6	0.08297	72.3	59.00
1	0.11028	55.8	45.53	7	0.07771	76.5	62.42
2	0.10758	57.0	46.51	8	0.06940	84.6	69.03
3	0.10337	59.2	48.30	9	0.06111	95.1	77.60
4	0.09787	62.4	50.92	10	0.05717	100.7	82.17
5	0.08899	67.8	55.32	11	0.05221	108.9	88.86

The curve plotted from these determinations is given in figure 6. On the basis of these values it was possible to calculate the amount of carbon dioxide represented by any given reduction in the concentration of the absorbing barium-hydroxide solution. The manner in which this was employed is given in the following section.

3. *The Procedure of the Respiration Experiments.*

Before using the apparatus just described, it was of course tested for small leaks, the absorption capacity of the soda-lime train at the required rate of the air-stream was determined, and the limits of complete absorption by the barium-hydroxide solution were established. While the apparatus seems to be rather complex, for continuous use for an extended series of determinations the various appliances proved very reliable and efficient.

Before each experiment the respiration chamber was washed out with a solution of formaldehyde and then with distilled water. Thereafter filtered air was drawn through the chamber until the last trace of formaldehyde was removed. All nutrient solutions were also sterilized by heating twice in an autoclave.

Only the purest chemicals available were used. The sugars were Pfanzstiehl brand. Some inconvenience was occasioned by the difficulty of procuring reliable amino-acid. At the time these experiments were begun amino-acids were not procurable or only at an exorbitant price and of a quality utterly unfit for physiological work. We were therefore obliged to devote about three months

to the preparation of amino-acids. The laboratory at Carmel, California, had not yet been completed, and we are greatly indebted to Professor G. N. Lewis for extending to us the opportunity of carrying out a large part of this preparation work in the chemical laboratories of the University of California. We were thus enabled to prepare adequate quantities of a variety of amino-acids of the highest purity.

The mineral nutrient solution, recommended by Dr. B. M. Duggar, was the following: Solution No. 1: 3 grams CaSO_4 in 3,000 c. c. H_2O . Solution No. 2: 6 grams $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1,000 c. c. H_2O . Solution No. 3: 3 grams Merck's "soluble ferric phosphate" in 1,000 c. c. H_2O . Solution No. 4: 12 grams KCl in 1,000 c. c. H_2O . The complete nutrient solution was made by mixing in the following proportions: 30 c. c. No. 1; 10 c. c. No. 2; 10 c. c. No. 4; diluted to 200 c. c.; and then 40 c. c. of No. 3 was added. This mixture gave excellent results. A number of other commonly used nutrient solutions employed in the preliminary experiments were unsatisfactory because the leaves, after being in the dark for some time, were wilted at the tips, had dark spots, appeared rather yellow, or showed other abnormal conditions.

All the solutions were thoroughly sterilized before using by twice heating in the autoclave to 15 pounds pressure. The mineral nutrient, sugar, and amino-acid solutions were heated separately before mixing. The dish and the glass tubes which held the leaf petioles were also sterilized.

Great care was exercised in selecting the leaves in order to use those of the same age and development. Consideration was also given to the conditions to which the plants had been exposed previous to the cutting of the leaves, viz, temperature and illumination. Where this was of importance the leaves were taken only when these conditions were as nearly the same as can be obtained in a greenhouse. The petioles of the leaves were cut under distilled water. They were immediately taken to the laboratory and about an inch of the petiole cut off under water. In the meantime the nutrient solution had been prepared and placed in the respiration chamber. The leaves were then quickly transferred to the solution in the chamber, care being taken that a drop of water adhered to the end of the petiole. The leaves were placed as loosely as possible, with the petioles well submerged in the nutrient solution. The glass cover was then placed over the chamber and then the metal cover. The level of the water in the thermostat was raised so that it covered the entire chamber. Before cutting the leaves the absorption tubes had been filled and connected, so that as soon as the chamber was closed the air-stream could be started through the apparatus. For about the first hour no account was kept of the carbon-dioxid emis-

sion, as results obtained during this period would be spurious, owing to the carbon dioxide of the air in the chamber, etc. Thereafter, however, the determinations of the rates appear to be quite concordant, depending upon nutritional conditions, and no evidence of traumatic effects was noticeable. We did not find it advisable to run these experiments over 100 hours, as the danger of infection developing after this time became great, and this naturally would introduce serious error.

To return to the barium-hydroxide solution: 125 c. c. of this solution was introduced into each absorption tube by means of a pipette. After the air-stream had passed through one of these tubes for a definite period, it was disconnected from the apparatus, and the solution, containing the suspended barium carbonate, was transferred to a glass vessel (see fig. 5) and tightly stoppered. By

FIGURE 6.

Specific-resistance curve of barium hydroxide at concentrations from 0.05 to 0.12 normal. The ordinate represents the specific resistance, while on the abscissa the CO₂ gram equivalents of 125 c. c. barium-hydroxide solution are plotted for concentrations of 0.05 to 0.12 normal.

rapid manipulation there is no appreciable error introduced from the carbon dioxide of the air. The barium carbonate was allowed to settle until the supernatant liquid was perfectly clear. The vessel was placed in the thermostat kept at 25° and the resistance determined by means of the apparatus already described. At frequent intervals the electrodes were dipped in dilute hydrochloric acid and while not in use kept in distilled water. With these precautions the cell-constant remained unchanged for a long time.

As was described in the previous section, a curve was drawn with the ordinate as specific resistances and on the abscissa the normal concentration. From this data the carbon-dioxide equivalent for

125 c. c. of solution (the amount used in the absorption tubes) can easily be calculated and used as the abscissa of the curve just mentioned. Thus, after the observed resistance value of a barium-hydroxide solution had been converted into the correct value of specific resistance by correcting for the cell-constant, the CO_2 equivalent could be directly read from the chart. The difference between this and the CO_2 equivalent of the original solution gives the amount of carbon dioxide emitted during the period the air passed through the barium-hydroxide solution. This chart is reproduced in figure 6. By this means the respiration rates were determined with very satisfactory accuracy and consumption of a minimum amount of time.

4. *The Analyses.*

For each experiment 15 to 20 mature leaves were cut. One-half of these were taken for immediate analysis; the rest of the leaves were analyzed after the respiration experiment. Both sets were of course treated exactly alike. Dry-weight determinations were made in duplicate. The leaves were folded up and placed in wide-mouth weighing bottles, the covers placed on the bottles, and weighed. The weighing bottles, with the covers on, were then placed in an electric oven at 98° to insure rapid killing. After about 30 minutes the covers were removed and the material was dried at the same temperature for 24 hours. The weighing bottles were then placed in a desiccator and weighed. This dry material was then ground in an agate mortar to a fine powder, after which it was ready for analysis.

For the determination of the total sugars, 0.5 gram of the leaf material was hydrolyzed for 3 hours in 25 c. c. of 1 per cent hydrochloric acid, filtered, neutralized with sodium bicarbonate made up to 100 c. c., and the sugars determined with an alkaline copper-sulphate solution by a method the details of which have already been described.¹ Although this method entails more work than some of the other methods in common use, it was found that these precautions were justifiable in order to obtain accurate results with plant material.

The determination of total sugars seemed to yield sufficient information relative to the carbohydrate-supply of the leaf, especially in the light of previous investigations which showed that with ample water-supply the carbohydrate balance was in favor of the simpler sugars, hexoses and disaccharides. Special experiments showed that the method of hydrolysis used (1 per cent hydrochloric acid) affected only the reserve starch and not the structural elements, the cellulose of the leaf. It could therefore be assumed that the values for the total sugars give an indication of the amount of carbohydrate material which was available to the leaf for respiratory purposes.

¹ SPOHR, H. A. Carnegie Inst. Wash. Pub. No. 287, 31 (1919).

For the determination of amino-acids, 1 gram of the dry material was heated for 3 hours with 25 c. c. of water on the boiling water-bath. This was filtered and thoroughly washed with hot water. The filtrate and washings were concentrated on the water-bath to 25 c. c. It was found inadvisable to try to remove the small amount of soluble protein from this extract, because any procedure designed to accomplish this also affected the amino-acid content, and this amount of protein exerted an exceedingly slight influence on the amino-acid determinations. These determinations were made by means of the Van Slyke micro apparatus. Deleano¹ stated that the nitrous-acid method of determining amino groups was unreliable when there were carbohydrates and nitrates present in the mixture. Special tests made with the Van Slyke apparatus with pure amino-acids to which nitrates and sugars were added did not confirm this statement of Deleano. With some of the plant extracts there was considerable frothing, but this was obviated by the use of a drop of caprylic alcohol added to the reaction mixture in Van Slyke apparatus.

The results of the respiration-rate determinations are reported on the basis of the amount of carbon dioxid emitted per gram of dry material. This is of course an arbitrary method and of only relative value. However, there exists as yet no rational basis in plant physiology of connecting the respiratory activity of vegetable organisms, and the establishment of such a basis must await a clearer understanding of the nature of the process itself. The basis of fresh weight has the great disadvantage that the alterations in water-content, which are easily effected in plants, very materially change the results. The use of a surface standard, as has been done in some work in animal physiology, has little to recommend it in work with plants.

In the interpretation of analytical data obtained from living material great care must be exercised to refrain from accepting the apparent results as the true state of affairs. Very rarely is a single chemical change unaccompanied by other changes which may run in the same or opposite directions. Especially is this difficulty encountered in the interpretation of analytical data of leaves calculated on the basis of percentage of original dry or fresh material. Thus the increase in one component very often simply means the decrease of another component which goes to make up the total mass of the original material.

In the tabulation of the experimental data some liberty was also taken in expressing the time in decimals of hours instead of in hours and minutes. However, this method facilitates greatly the work of calculating and avoids a common source of error.

¹ DELEANO, N. *Jahrb. wiss. Bot.*, 51, 552 (1921).

EXPERIMENTAL RESULTS.

1. *The Normal Course of Respiration.*

It has been known for a long time that as the supply of carbohydrates in a plant diminishes the rate of carbon-dioxid emission also decreases. This in general may be conceived of as a matter of mass action, in that as the concentration of the available material to be oxidized becomes less the rate of the reaction is decreased. It has also been recognized, however, that these relations are not as direct and as simple as was supposed at first, for there are upper and lower limits where other complications or limiting factors enter and where the general complex of reactions takes other courses and makes use of different material.¹ But in order to form some conception of the behavior of chlorophyllous leaves apart from the photosynthetic process it is necessary to know what might be termed the normal course of respiration, meaning thereby the rate at which the stored material is converted into carbon dioxid and water with the liberation of energy. In many investigations on the photosynthetic process the assumption that respiration proceeds at the same rate in the light as in the dark has been accepted as definitely established, or the possibility of any variation of this factor has been totally neglected. In a practical sense we are interested in photosynthesis as a process in which the amount of energy stored exceeds that liberated by the organism. Nevertheless, if we are to formulate any sort of conception of the synthetic processes or make a quantitative measure thereof, the reverse action, going on simultaneously, can not be neglected.

No conception such as the basal metabolism in animals has been introduced into plant physiology, and, on account of fundamental differences in the two organisms, it is improbable that such relations can be worked out. But the normal course of respiration, although of necessity a rather arbitrary value, offers a base-line, as it were, on which subsequent respiratory activity can in a sense be superimposed. Another feature of such respiration rates is that with sufficiently short periods and accuracy of CO₂ determinations it becomes evident that these rates show irregularities which emanate from the internal workings of the leaves and are independent of any external conditions. We shall call attention to one such irregularity in the form of a sudden rise in the respiration rate after the leaves had been in the dark for about 40 hours.

In order to give a comparison of excised leaves with the normal plant, the rate of respiration of an entire plant was determined. As the analytical data in table 13 indicate, the plant has in the stems

¹ SPOEHR, H. A. The carbohydrate economy of cacti. Carnegie Inst. Wash. Pub. No. 287 (1919).

and roots a large reserve of carbohydrates on which it can draw for the maintenance of its respiratory activity. Any changes in respiration due to carbohydrate exhaustion do not, therefore, in the case of the attached leaves, appear for a considerable time, while in the case of excised leaves such changes become apparent after relatively short periods of darkness. For this experiment an entire *Helianthus annuus* plant was used. The plant was growing in soil in a glass jar. Before the respiration rates were determined, the soil was covered with tinfoil and the edges and portions around the stem of the plant were sealed with cocoa-butter. The experiment was allowed to run for 94.25 hours, after which the plant was still in perfectly healthy and vigorous condition. The results of the determination are given in table 8.

TABLE 8.—Rate of emission of CO_2 by a small potted plant of *Helianthus annuus*.
Soil covered with tinfoil and sealed with cocoa-butter. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution,
0.11835 normal, 125 c. c. of which has the equivalent of 0.3254 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Grams CO_2 equivalent of 125 c.c. $\text{Ba}(\text{OH})_2$ solution.	Grams CO_2 ab- sorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	11 ⁴⁵ a.m. to 1 p.m.	1.25	1.25					
1	1 p.m. to 4 p.m.	3.00	4.25	54.85	0.3140	0.0114	3.80	4.51
2	4 p.m. to 10 ³⁰ p.m.	6.5	10.75	56.6	.3024	.0230	3.53	4.19
3	10 ³⁰ p.m. to 5 a.m.	6.5	17.25	57.5	.2970	.0284	4.37	5.18
4	5 a.m. to 10 a.m.	5.0	22.25	56.6	.3024	.0230	4.60	5.46
5	10 a.m. to 4 p.m.	6.0	28.25	57.3	.2982	.0272	4.53	5.37
6	4 p.m. to 10 ³⁰ p.m.	6.5	34.75	58.0	.2942	.0312	4.80	5.69
7	10 ³⁰ p.m. to 5 a.m.	6.5	41.25	57.9	.2950	.0304	4.67	5.54
8	5 a.m. to 10 a.m.	5.0	46.25	56.9	.3008	.0246	4.92	5.84
9	10 a.m. to 4 p.m.	6.0	52.25	57.5	.2970	.0284	4.73	5.61
10	4 p.m. to 10 ³⁰ p.m.	6.5	58.75	57.8	.2954	.0300	4.61	5.47
11	10 ³⁰ p.m. to 5 a.m.	6.5	65.25	57.5	.2970	.0284	4.36	5.17
12	5 a.m. to 10 a.m.	5.0	70.25	56.3	.3040	.0214	4.28	5.08
13	10 a.m. to 4 p.m.	6.0	76.25	56.7	.3016	.0238	3.96	4.70
14	4 p.m. to 10 ³⁰ p.m.	6.5	82.75	57.1	.2992	.0262	4.03	4.78
15	10 ³⁰ p.m. to 5 a.m.	6.5	89.25	56.9	.3004	.0250	3.84	4.55
16	5 a.m. to 10 a.m.	5.0	94.25	55.9	.3070	.0184	3.68	4.36

These results are graphically represented by the curve in figure 7. An initial drop in the respiratory rate is noticeable here; it is very much more prominent in the experiments with excised leaves. The striking feature of this curve is that during about the first 48 hours there is a gradual increase in the rate of respiration. During all this time the available supply of fuel material is diminishing and there are no alterations in temperature or other external conditions. It therefore seems justifiable to conclude that some internal factor comes into play which acts in a stimulatory manner. From the experiments hereinafter recorded it would seem that the amino-acids

are themselves this factor, or are very intimately associated therewith. The amino-acids exert this stimulating action on the respiratory activity; they behave very much like a catalyst, accelerating the rate of carbohydrate catabolism and resulting in an increased release of energy when the supply of material is decreasing. Thus the

FIGURE 7.

Rate of respiration of an entire plant of *Helianthus annuus*. Soil covered with tinfoil and sealed with cocoa-butter. The ordinate represents mg. CO₂ per hour per gram dry material, and the abscissa the time in hours.

catabolic activity of the plant tends to be maintained, though the store of fuel is ebbing, until photosynthetic activity again replenishes the fuel supply and the light diminishes the amino-acid content. True to the nature of catalyst, the amino-acids do not yield the energy, but when the concentration of the *substrat* (the carbohydrates) is sufficiently decreased, the rate of the general reaction also decreases. This accounts for the dropping in the latter portion of the curve.

TABLE 9.

	Dry weight.	Total sugars in dry material.	Amino nitrogen in dry material.
Original condition	<i>p. ct.</i> 10.59	<i>p. ct.</i> 5.93	<i>p. ct.</i> 0.102
After 71.25 hours in dark.....	12.45	2.39	0.380

These respiration curves of necessity show in but a gross manner the course of respiratory activity. They represent the resultant of forces. The reversal of the rate (a drop in the curve) simply indicates when an influence opposed to the stimulatory one is the more potent; it does not show when these various forces started. As the analytical data given in tables 12 and 13 show, leaves attached to the plant draw heavily upon the carbohydrate

supply in the rest of the plant. This undoubtedly accounts for the fact that the plant is able to continue its initial respiratory rate for so long a time, which is in decided contradistinction to the behavior of the excised leaves when these are not supplied with carbohydrates.

TABLE 10.—Rate of CO_2 emission of 8 leaves of *Helianthus annuus* at 24° .
Petioles in nutrient solution containing no organic substances.

No.	Time.	Hours.	Total hours.	Cubic centimeters 0.1/N HCl.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry material.
0	3 ^h 45 ^m p.m. to 4 ^h 30 ^m p.m.....	0.75	0.75			
1	4 ^h 30 ^m p.m. to 8 ^h 45 ^m p.m.....	4.25	5.00	27.40	14.190	3.337
2	8 ^h 45 ^m p.m. to 9 ^h 15 ^m a.m.....	12.50	17.50	60.30	11.088	2.607
3	9 ^h 15 ^m a.m. to 3 p.m.....	5.75	23.25	20.80	7.964	1.873
4	3 p.m. to 9 ^h 15 ^m p.m.....	6.25	29.50	18.50	6.512	1.531
5	9 ^h 15 ^m p.m. to 9 a.m.....	11.75	41.25	30.80	5.764	1.355
6	9 a.m. to 3 ^h 45 ^m p.m.....	6.75	48.00	19.60	6.380	1.500
7	3 ^h 45 ^m p.m. to 8 ^h 45 ^m p.m.....	5.0	53.00	13.15	5.786	1.360
8	8 ^h 45 ^m p.m. to 9 a.m.....	12.25	65.25	29.60	5.224	1.229
9	9 a.m. to 3 p.m.....	6.00	71.25	14.65	5.268	1.239

In table 10 are given the results of the rate of carbon-dioxid emission from sunflower leaves which had been cut from the plant. The petioles of the leaves were immersed in a sterilized nutrient solution which contained only inorganic salts. The following analytical data give an idea of the changes of material in the leaves during the period of respiration.

FIGURE 8.

Solid line represents rate of respiration of 8 leaves of *Helianthus annuus* at 24° ; petioles in a nutrient solution containing no organic substances. Values taken from table 10. Broken line represents rate of respiration of 15 leaves of Canada Wonder bean; petioles in nutrient solution containing no organic substance. Values taken from table 11. The ordinate gives mg. CO_2 per hour per gram dry material, the abscissa the time in hours.

In table 11 similar results are given for excised bean leaves. From the curves of these respiratory rates, given in figure 8, it is evident that in the excised leaves the rate of CO_2 emission declines rapidly when the only supply of carbohydrates is the material stored in the leaf, and that these respiratory rates decrease with but a slight variation, except at about the forty-eighth hour. These graphs represent the normal course of respiratory activity of excised leaves.

The course of the changes of material was followed by placing five vigorous potted *Helianthus* plants in the dark at 20° and every 24 hours cutting 10 to 12 leaves, which were then analyzed. After the plants had been in the dark for 96 hours they were again placed in sunlight in the greenhouse for 4 hours and another sample of leaves analyzed. The results are given in table 12.

TABLE 11.—Rate of CO₂ emission of 15 leaves of "Canada Wonder" bean at 24°. Petioles in nutrient solution containing no organic substances.

No.	Time.	Hours.	Total hours.	Cubic centimeters 0.1/N HCl.	Mg. CO ₂ per hour.	Mg. CO ₂ per gram dry material.
1	3 ^h 30 ^m p.m. to 9 ^h 30 ^m p.m.	6	6	33.95	12.40	2.793
2	9 ^h 30 ^m p.m. to 3 ^h 30 ^m a.m.	6	12	23.10	8.40	1.892
3	3 ^h 30 ^m a.m. to 9 ^h 30 ^m a.m.	6	18	25.10	9.10	2.049
4	9 ^h 30 ^m a.m. to 3 ^h 30 ^m p.m.	6	24	24.10	8.70	1.959
5	3 ^h 30 ^m p.m. to 9 ^h 30 ^m p.m.	6	30	22.60	8.30	1.869
6	9 ^h 30 ^m p.m. to 9 ^h 30 ^m a.m.	12	42	35.60	6.50	1.464
7	9 ^h 30 ^m a.m. to 3 ^h 30 ^m p.m.	6	48	19.10	7.00	1.576
8	3 ^h 30 ^m p.m. to 9 ^h 30 ^m p.m.	6	54	18.10	6.63	1.495
9	9 ^h 30 ^m p.m. to 9 ^h 30 ^m a.m.	12	66	30.25	5.50	1.238

The analyses given in table 12 indicate a gradual depletion of the carbohydrates available to the leaves when the plants are left in the dark. This depletion can not be made up entirely by drawing upon the reserve material in other parts of the plant. In spite of this depletion it is noteworthy, as has been pointed out, that the rate of respiration of the entire plant rises and then decreases slowly, as is

TABLE 12.—Analysis of leaves of *Helianthus annuus*, after exposure to light, then in dark for 96 hours, and again in light for 4 hours.

Leaf samples taken.	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
After 5 hours sunlight..	17.45	0.230	1.824
24 hours in dark.....	15.61	0.200	0.983
48 hours in dark.....	15.15	0.271	0.736
72 hours in dark.....	15.47	0.254	0.921
96 hours in dark.....	15.21	0.286	0.777
4 hours in sunlight....	16.52	0.203	1.373

shown in figure 7. After 4 hours of photosynthetic work the leaves have again accumulated considerable sugar. The amino-acids, however, increase in the dark and after subsequent exposure of the plant to light again decrease.

In table 13 are given the results of plants similarly treated in which the leaves, stems, and roots were analyzed separately. Two

similar *Helianthus* plants of the same age and size, growing in large pots, were used; the one was analyzed at once, the other after being in the dark at 20° for 96 hours.

TABLE 13.—Analyses of *Helianthus annuus* plants, leaves, stems, and roots separately after exposure to light for 5 hours and after being in the dark for 96 hours.

	After exposure to light for 5 hours.			After being in dark for 96 hours.		
	Leaves.	Stems.	Roots.	Leaves.	Stems.	Roots.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Dry weight.....	18.10	20.24		15.93	17.70	
Amino nitrogen in dry material....	.219	.101	.116	.294	.075	.104
Total sugar in dry material.....	1.61	12.17	5.28	.81	11.36	3.92

The analyses in table 13 show a general decrease in the carbohydrate-content of the plant kept in the dark. Here also the leaves of the plant kept in the dark show an increase in amino-acids. The stems and roots, however, show a very slight decrease in amino compounds.

The experiments of Schulze and Castoro¹ on the accumulation of amino-acids have already been mentioned. In this work the seedlings were kept in the dark for 2 weeks and the amino-acid content in these plants was very much higher than in the ones exposed to the

TABLE 14.—Amino nitrogen in beans (*Canada Wonder*) sprouted on sawdust.

No. of hours.	In light.		In dark.	
	Amino nitrogen.	Dry weight.	Amino nitrogen.	Dry weight.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
0	0.252		0.252	
24			.146	41.40
72	.357	36.41	.274	39.81
96	.343	35.90	.399	36.42
144	.368	32.89	.372	29.22
192	.411	30.13	.403	26.04

light. During this length of time the seedlings were capable of developing well-formed chlorophyllous leaves. We carried out comparative experiments in which periodic analyses were made on germinating seeds, in the light and in the dark, for a shorter time, the seedlings having still available some stored proteinaceous matter. In these experiments no differences of consequence were observable between the seedlings grown in the dark and those grown in the light.

¹ SCHULZE, E., and N. CASTORO. *Zeitschr. physiol. chem.*, 38, 244 (1903); 49, 72 (1906).

With mature excised leaves kept in the dark the change in amino-acid and carbohydrate content naturally becomes much more marked. Thus in table 16 are given the results of analyses of an experiment in which 40 similar leaves of about the same age and size were cut from plants. These plants had been exposed to sunlight in the greenhouse for about 9 hours. The cut leaves were placed in battery jars, with petioles in nitrogen-free nutrient solution, and kept in the dark; every 24 hours 8 leaves were removed and analyzed.

TABLE 15.—*Amino nitrogen in beans (Canada Wonder) sprouted on filter-paper.*

No. of hours.	In light.		In dark.	
	Amino nitrogen.	Dry weight.	Amino nitrogen.	Dry weight.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
0	0.252		0.252	
24			.146	41.40
48	.195	38.18	.232	39.43
96	.264	32.07	.248	31.04
120	.373	32.32	.342	30.59

The results given in table 16 show a decided and regular decrease in the carbohydrate-content of the leaves kept in the dark. Also, the amino-acids in these leaves increased with continued time in the dark, although there was no inorganic nitrogen given in the solution. It would appear, therefore, that there is a continuous formation of amino-acids, presumably from proteins, and that under these conditions the rate of protein decomposition exceeds the rate of protein synthesis, with the result that the splitting products in form of amino-acids accumulate in the leaves. The effect of this process on the rate of respiration and its relation to the carbohydrate economy of the plant can in part be gathered from the following experiments.

In table 16 were also given the results of analyses of leaves kept in the dark and showing the gradual depletion of the carbohydrate material used by the plant as material from which it derives its energy. The course which the rate of respiration follows under these circumstances has been described in figure 8. These phenomena are of fundamental importance and appear relatively simple and well known. The fate and behavior of the proteins under like circumstances are, however, quite obscure. Furthermore, it is impossible and irrational to try to follow the fate of these substances in plant respiration without simultaneously considering the carbohydrate economy. As a counterpart to the experiment sum-

marized in table 16, the results of a similar experiment are given in table 17, in which the petioles of the leaves are placed, instead of in nitrogen-free nutrient solution only, in such a solution containing also 7 per cent of d-glucose. Previous to cutting the leaves the plants had been in the sunlight in the greenhouse for about 9 hours.

TABLE 16.—Analysis of excised leaves of *Helianthus annuus* kept in the dark at 20°, with petioles in nitrogen-free nutrient solution.

No. of hours leaves were kept in dark.	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
0	13.48	0.149	2.49
24	10.76	.187	1.51
48	10.10	.268	0.90
72	13.46	.272	0.69
96	10.33	.422	0.44

From the results given in table 17 it is evident, as was shown many years ago by Boehm, that when excised leaves are placed with the petioles in a nitrogen-free nutrient solution containing 7 per cent d-glucose the sugar is taken up into the leaves and accumulates there. As will be seen later at higher temperatures, the sugar under these circumstances accumulates but very little, if at all. However,

TABLE 17.—Analysis of excised leaves of *Helianthus annuus* kept in the dark at 20°, with petioles in nitrogen-free nutrient solution plus 7 per cent d-glucose.

Number of hours leaves kept in dark.	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
0	10.73	0.164	1.86
24	13.24	.249	1.83
48	13.12	.176	2.42
72	13.29	.268	2.75
96	12.59	.183	3.91

when there is thus an abundant supply of carbohydrates, the amino-acids increase less than in the absence of sugar. The questions which arise, then, are: Do the carbohydrates in any manner influence amino-acid formation, and do the amino-acids affect the rate of carbohydrate consumption by the leaf-cells? Also, what is the influence of light and dark in the formation of amino-acids? The experiments hereinafter described may throw some light on these questions.

2. *Glycocoll*.

That amino-acids accelerate the rate of carbohydrate consumption in the leaves becomes evident from the following experiment: A number of healthy leaves of about the same age were cut from the plants in the manner already described and placed in battery jars. In one set the petioles were put in 100 c. c. nitrogen-free nutrient

TABLE 18.

Leaves in—	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Original condition.....	14.36	0.089	11.48
Nutrient solution only.....	14.31	.238	3.34
Nutrient solution plus glycocoll..	11.35	.293	2.75

solution plus 100 c. c. of water; in the other set the solution consisted of 100 c. c. of the same nutrient solution plus a solution of 0.5 gram glycocoll in 100 c. c. of water. These solutions were sterilized as usual and the experiments carried out in duplicate. After remaining in the dark for 72 hours at 20°, all the leaves were removed from the jars and analyzed. The results of the analysis are given in table 18.

TABLE 19.—Rate of CO₂ emission of 8 leaves of *Helianthus annuus* at 24°.

Petioles in nutrient solution containing 0.11 per cent of glycocoll.

No.	Time.	Hours.	Total hours.	Cubic centimeters 0.1 N HCl	Mg. CO ₂ per hour.	Mg. CO ₂ per hour per gram dry material.
0	4 ^h 30 ^m p.m. to 5 p.m.....	0.50				
1	5 p.m. to 10 ^h 15 ^m p.m.....	5.25	5.75	49.00	20.548	3.570
2	10 ^h 15 ^m p.m. to 9 a.m.....	10.75	16.50	80.10	16.412	2.852
3	9 a.m. to 3 ^h 30 ^m p.m.....	6.50	23.00	39.00	13.200	2.293
4	3 ^h 30 ^m p.m. to 8 ^h 45 ^m p.m....	5.25	28.25	26.50	11.088	1.926
5	8 ^h 45 ^m p.m. to 9 ^h 15 ^m a.m.....	12.50	40.75	55.95	9.864	1.748
6	9 ^h 15 ^m a.m. to 3 ^h 15 ^m p.m.....	6.00	46.75	26.35	9.658	1.678
7	3 ^h 15 ^m p.m. to 11 ^h 15 ^m p.m....	8.00	54.75	30.50	8.382	1.456
8	11 ^h 15 ^m p.m. to 9 a.m.....	9.75	64.50	36.30	8.184	1.422
9	9 a.m. to 4 p.m.....	7.00	71.50	28.00	8.800	1.529
10	4 p.m. to 9 ^h 15 ^m p.m.....	5.25	76.75	20.35	8.492	1.478

From the results given in table 18 it appears that the leaves placed in nutrient solution gained only in amino-acid content and that the leaves placed in a glycocoll solution showed an even greater gain in amino-acids. It is also evident that the leaves fed glycocoll consumed more carbohydrates than did the ones which had been placed in nutrient solution only.

In tables 10 and 11 and figure 8 are given the results of what was termed the normal course of respiration. This represents the rate of carbon-dioxid emission from *Helianthus* leaves in the dark at 24° in nutrient solution containing no organic material, and indicates the rate at which the carbohydrates in the leaves are consumed.

FIGURE 9.

Rates of respiration with and without glycocoll. The broken line represents the rate of respiration of 8 leaves of *Helianthus annuus* at 24°; petioles in a nutrient solution containing no organic substances. Values taken from table 10. Solid line represents the rate of respiration of 8 leaves of *Helianthus annuus* at 24°, petioles in nutrient solution containing 0.11 per cent of glycocoll. Values taken from table 19. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa the time in hours.

As a counterpart to this, an experiment was carried out in which the nutrient solution contained an amino-acid in order to determine what the influence of this is on the rate of respiration. The *Helianthus* leaves were taken from the same plant, a very large and strong one, and were treated in precisely the same manner. The results of this respiration experiment are given in table 19 and in figure 9, the graphs of the two experiments, the one with and the other without amino-acid.

The analytical data representing the original condition of the leaves and that at the end of the experiment, after having been in the dark for 76.75 hours at 24°, with the petioles of the leaves in nutrient solution containing 0.11 per cent glycocoll, are given in table 20.

In order to compare the respiratory activity of the two sets of leaves, those with and those without glycocoll in the nutrient solution, the determinations of carbon dioxide are summarized in table 21.

From the foregoing it is evident that the leaves which had been fed glycocoll respired more actively than those without the amino-acid. On the basis of fresh material, the former produced 22.43

TABLE 20.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Original condition.....	14.45	0.100	9.28
After 76.75 hours in dark...	13.12	.459	2.36

per cent more carbon dioxide than the latter, and on the basis of dry material the respective difference was 15.09 per cent in favor of the leaves given glycocoll. It is also evident that the leaves which had had glycocoll showed a much greater reduction in the amount of carbohydrates; the slight difference in the length of time the two experiments ran would have but slight effect on these results. It

TABLE 21.

	Total mg. CO ₂ formed.	Total mg. CO ₂ formed per gram fresh material.	Total mg. CO ₂ formed per gram dry material.
Without glycocoll.	549.56	16.082	129.20
With glycocoll....	862.51	19.690	149.80

might be argued that the leaves given glycocoll had an original higher content of carbohydrates and that this might account for the greater respiratory activity. However, it has been our experience in a great many cases that within certain upper and lower limits the initial carbohydrate-content does not influence the rate of respiration under controlled conditions of temperature and water-supply. Finally, it is also noticeable that the leaves given glycocoll have a higher amino-acid content at the end of the experiment.

3. d-Glucose.

From the foregoing experiments the simple facts become evident that when excised leaves are kept in the dark at 25° the amino-acid content rises, and that when the leaves are given glycocoll

this is taken up in the leaves and stimulates the rate of respiration and carbohydrate consumption. However, the excised leaves have as their only supply of carbohydrates the material stored in the leaves, which is depleted in a relatively short time. It has been shown, though, that sugars are easily taken into the leaf through the petioles from a nutrient solution. Under these circumstances the respiratory rate exhibits some interesting variations. In table 23 are given the results on an experiment in which the excised *Helianthus* leaves were fed d-glucose, so that the carbohydrate-content was practically the same at the beginning and end of the experiment. During the course of the experiment, 80 hours at 24°, the amino-acid content increased about threefold. The analytical data are given in table 22.

TABLE 22.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition.....	p. ct. 13.42	p. ct. 0.101	p. ct. 7.55
After 80 hours in dark..	12.02	.312	7.03

The determinations of the rate of respiration are given in table 23 and figure 10. These show that although the leaves were constantly receiving sugar, the rate of CO₂ emission decreased regularly during the first 30 hours. Thereafter the respiration rate increased so that after 80 hours this was slightly above the initial rate. It should be noted that at this time the carbohydrate-content was slightly below the initial condition, while the amino-acids had increased considerably.

TABLE 23.—Rate of CO₂ emission of 9 leaves of *Helianthus annuus* at 24°. Petioles in nitrogen-free nutrient solution containing 7 per cent of d-glucose.

No.	Time.	Hours.	Total hours.	Cubic centimeters, 0.1/N HCl.	Mg. CO ₂ per hour.	Mg. CO ₂ per hour per gram dry material.
0	12 ^h 15 ^m p.m. to 2 ^h 30 ^m p.m.....	2.25	2.25			
1	2 ^h 30 ^m p.m. to 9 ^h 30 ^m p.m.....	7.00	9.25	43.10	13.530	3.083
2	9 ^h 30 ^m p.m. to 9 a.m.....	11.50	20.75	64.85	12.409	2.828
3	9 a.m. to 3 p.m.....	6.00	26.75	32.05	11.748	2.677
4	3 p.m. to 9 p.m.....	6.00	32.75	31.25	11.440	2.607
5	9 p.m. to 9 ^h 15 ^m a.m.....	12.25	45.00	71.25	12.804	2.918
6	9 ^h 15 ^m a.m. to 3 ^h 45 ^m p.m.....	6.50	51.50	41.45	14.036	3.198
7	3 ^h 45 ^m p.m. to 10 ^h 15 ^m p.m.....	6.50	58.00	39.65	13.420	3.058
8	10 ^h 15 ^m p.m. to 9 ^h 15 ^m a.m.....	11.00	69.00	66.85	13.354	3.043
9	9 ^h 15 ^m a.m. to 4 ^h 45 ^m p.m.....	7.50	76.50	48.40	14.190	3.234
10	4 ^h 45 ^m p.m. to 9 p.m.....	4.25	80.75	27.00	13.970	3.183

In the following experiment the conditions were the same as in the previous one, with the exception that the initial carbohydrate-content was lower and the leaves showed a greater gain in amino acids. The petioles of the excised leaves were also placed in a nitro-

FIGURE 10.

Rate of respiration of 9 leaves of *Helianthus annuus* at 24°; petiole in nitrogen-free nutrient solution containing 7 per cent of d-glucose, from table 23. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa the time in hours.

gen-free nutrient solution containing 7 per cent of d-glucose, but the leaves also showed no gain in carbohydrate-content. There was, however, a very decided increase in the amino-acids after 94.25 hours in the dark. The analytical results are presented in table 24 and the course of respiration in table 25 and figure 11.

TABLE 24.

	Dry weight.	Amino nitrogen in dry material.	Total carbo- hydrates in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Original condition.....	14.19	0.132	1.64
After 94.25 hours in dark....	11.74	.697	1.14

The noteworthy feature of the foregoing experiment is the very marked drop in the respiration rate during the first 35 hours, and thereafter the decided increase, so that the rate was higher at the end of the experiment than at the beginning. From previous experiments, as indicated in table 17, it seems evident that when leaves are placed with the petioles in a 7 per cent glucose solution the carbohydrate-content is maintained after the first 24 hours, so that it may be assumed that these leaves, with their initial relatively low carbohydrate-content, did not suffer a decided reduction of this material. It is evident that after about 35 hours the rate of CO₂ emission increased decidedly, so that at the end of 94.25 hours, in

spite of the slightly lower carbohydrate-content, the leaves showed a higher respiration rate than at the beginning of the experiment. This indicates that after about 35 hours there are produced within the leaf conditions which favor a more active catabolism of carbohydrates. From the experiments already described and others which are to follow, it appears certain that, when leaves are kept in the dark, synchronous with the reduction of the carbohydrates

TABLE 25.—Rate of emission of CO_2 by 6 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.1169 normal, 125 c.c. of which has the equivalent of 0.3214 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per gram dry weight.
0	11 ^h 45 ^m a.m. to 1 p.m.	1.25	1.25					
1	1 p.m. to 4 p.m.	3.00	4.25	66.6	0.2522	0.0692	23.06	6.80
2	4 p.m. to 10 ^h 30 ^m p.m.	6.50	10.75	83.1	.1968	.1246	19.16	5.65
3	10 ^h 30 ^m p.m. to 5 a.m.	6.50	17.25	93.7	.1730	.1484	18.38	5.42
4	5 a.m. to 11 a.m.	6.00	23.25	69.8	.2400	.0814		
5	11 a.m. to 4 p.m.	5.00	28.25	72.6	.2298	.0916	18.32	5.40
6	4 p.m. to 10 ^h 30 ^m p.m.	6.50	34.75	74.9	.2216	.0998	15.35	4.23
7	10 ^h 30 ^m p.m. to 5 a.m.	6.50	41.25	76.9	.2154	.1060	16.30	4.81
8	5 a.m. to 10 a.m.	5.00	46.25	71.5	.2336	.0878	17.56	5.18
9	10 a.m. to 4 p.m.	6.00	52.25	76.7	.2160	.1054	17.56	5.18
10	4 p.m. to 10 ^h 30 ^m p.m.	6.50	58.75	77.3	.2140	.1074	16.52	4.87
11	10 ^h 30 ^m p.m. to 5 a.m.	6.50	65.25	82.3	.1988	.1226	18.86	5.56
12	5 a.m. to 10 a.m.	5.00	70.25	77.4	.2134	.1080	21.60	6.37
13	10 a.m. to 4 p.m.	6.00	76.25	89.8	.1812	.1402	23.36	6.89
14	4 p.m. to 10 ^h 30 ^m p.m.	6.50	82.75	99.6	.1612	.1602	24.64	7.27
15	10 ^h 30 ^m p.m. to 5 a.m.	6.50	89.25		.1340	.1874	28.83	8.50
16	5 a.m. to 10 a.m.	5.00	94.25	93.1	.1740	.1474	29.48	8.69

there takes place an increase in the amino-acids. Moreover, an increase in the amino-acids stimulates the rate of carbohydrate catabolism. Under ordinary circumstances, when the stores of carbohydrates are being depleted rapidly, the effect of the natural increase in amino-acids is but slightly noticeable, as was seen in figure 8. When, however, the leaf is fed sugar and the amino-acids increase appreciably, the result is a decided stimulation of CO_2 emission. These relations are, however, of a more complex nature than would appear at first glance. They of course involve the metabolism of the proteins as a source of the amino-acids, and the converse question arises as to what extent the formation of amino acids from proteins is affected by a decreasing supply of carbohydrates. These questions will be referred to later in this paper.

Before taking up the effect of amino-acids on respiration in more detail, there remains to be described the course of respiration under

conditions in which there is a decided increase in the carbohydrate-content of the leaves and but a slight increase in amino-acids. In the following experiment, carried out with Canada Wonder bean leaves, the experimental conditions were the same as in the two

FIGURE 11.

Rate of respiration of 6 leaves of *Helianthus annuus* at 25°; petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa the time in hours.

previous experiments, i. e., the petioles of the leaves were in a nitrogen-free nutrient solution containing 7 per cent glucose, and the CO₂ emission was determined in the dark at 25°. From the analytical

TABLE 26.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Initial condition	16.82	0.335	2.53
After 58.35 hours in dark . . .	14.70	.469	3.45

data in table 26 it is evident that there was a decided increase in the carbohydrate-content, but only a slight increase in the amino acids. The rates of respiration are given in table 27 and figure 12.

It should be noted that in the foregoing experiment, unlike the two previous ones, there is an increase in the carbohydrate-content and the increase in the amino-acids is very small. With these facts in mind, the results of the rates of respiration as plotted in figure 12 are noteworthy. The rapid drop between the fifth and tenth hours

TABLE 27.—Rate of CO_2 emission of 15 leaves Canada Wonder bean at 25° ; petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose.

CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.1169 normal, showing resistance of 53.2 ohms in cell with constant of 1.2255 at 25° .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	11 ^h 55 ^m a.m. to 1 ^h 2 ^m p.m..	1.10	1.10					
1	1 ^h 2 ^m p.m. to 5 ^h 2 ^m p.m....	4.00	5.10	68.3	0.2422	0.0792	19.8	5.13
2	5 ^h 2 ^m p.m. to 10 ^h 32 ^m p.m..	5.50	10.60	71.7	.2298	.0916	16.6	4.30
3	10 ^h 32 ^m p.m. to 3 ^h 32 ^m a.m.	5.00	15.60	73.6	.2232	.0982	19.6	5.08
4	3 ^h 32 ^m a.m. to 8 ^h 32 ^m a.m..	5.00	20.60	68.1	.2428	.0786	15.7	4.07
5	8 ^h 32 ^m a.m. to 2 ^h 32 ^m p.m..	6.00	26.60	70.9	.2328	.0886	14.7	3.81
6	2 ^h 32 ^m p.m. to 11 ^h 17 ^m p.m.	8.75	35.35	77.0	.2120	.1094	12.5	3.24
7	11 ^h 17 ^m p.m. to 4 ^h 17 ^m a.m.	5.00	40.35	64.3	.2592	.0322	12.4	3.21
8	4 ^h 17 ^m a.m. to 9 ^h 17 ^m a.m..	5.00	45.35	67.6	.2450	.0764	15.3	3.97
9	9 ^h 17 ^m a.m. to 3 ^h 17 ^m p.m..	6.00	51.35	71.4	.2310	.0904	15.1	3.91
10	3 ^h 17 ^m p.m. to 10 ^h 17 ^m p.m.	7.00	58.35	71.0	.2326	.0888	12.7	3.29

FIGURE 12.

Rate of respiration of 15 leaves of Canada Wonder bean at 24° ; petioles in nitrogen-free solution containing 7 per cent d-glucose. Values taken from table 27. The ordinate represents mg. CO_2 per hour per gram dry material, the abscissa the time in hours.

of the experiment is probably due to the fact that the sugar from the nutrient solution had not yet got into the leaf in sufficient quantity to cover the loss through respiration. Thereafter the rate of CO_2 emission shows a rapid rise, soon followed by a regular decline far below the initial rate, until the fortieth hour, when there

again occurs the characteristic rise, which, however, is not maintained and the rate again falls, so that after about 60 hours the rate of respiration is far below the initial rate, in spite of the fact that the leaves have actually increased in carbohydrate-content.

TABLE 28.

	Dry weight.*	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition	<i>p. ct.</i> 12.72	<i>p. ct.</i> 0.124	<i>p. ct.</i> 1.33
After 74 hours in dark..	12.78	.888	2.07

Undoubtedly the carbohydrate supply alone does not determine the rate of respiration, but there is necessary an accessory factor which aids the successive chemical reactions constituting this process. That amino-acids act in this stimulating manner is established by

TABLE 29.—Rate of emission of CO_2 by 6 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose and 0.11 per cent glycocoll. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.1169 normal, 125 c. c. of which has the equivalent of 0.3214 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	2 p.m. to 3 p.m.	1.0	1.0					
1	3 p.m. to 5 p.m.	2.0	3.0	53.2	0.2930	0.0284	14.20	4.45
2	5 p.m. to 11 ^h 30 ^m p.m. . . .	6.5	9.5	74.6	.2226	.0988	15.20	4.77
3	11 ^h 30 ^m p.m. to 6 a.m. . . .	6.5	16.0	72.6	.2294	.0920	14.15	4.44
4	6 a.m. to 11 a.m.	5.0	21.0	70.8	.2360	.0854	17.08	5.36
5	11 a.m. to 4 p.m.	5.0	26.0	69.3	.2412	.0802	16.04	5.03
6	4 p.m. to 10 ^h 30 ^m p.m. . . .	6.5	32.5	77.2	.2140	.1074	16.52	5.18
7	10 ^h 30 ^m p.m. to 5 a.m. . . .	6.5	39.0	80.4	.2040	.1174	18.06	5.67
8	5 a.m. to 10 a.m.	5.0	44.0	72.2	.2312	.0902	18.82	5.90
9	10 a.m. to 12 m.	2.0	46.0	60.7	.2798	.0416		
10	12 m. to 4 p.m.	4.0	50.0	68.0	.2466	.0748	18.70	5.87
11	4 p.m. to 10 ^h 30 ^m p.m. . . .	6.5	56.5	81.8	.2000	.1214	18.60	5.86
12	10 ^h 30 ^m p.m. to 5 a.m. . . .	6.5	63.0	84.6	.1932	.1282	19.70	6.19
13	5 a.m. to 10 a.m.	5.0	68.0	74.6	.2228	.0986	19.70	6.19
14	10 a.m. to 4 p.m.	6.0	74.0	81.4	.2014	.1200	20.00	6.28

the experiments here described, and the point naturally suggests itself that under normal conditions in the dark, when the carbohydrate-content decreases and the amino-acids increase, there is a similar stimulating action which would tend to maintain a relatively higher respiration rate. From such data as are available it appears that the accumulation of amino-acids is a relatively slow process, and

it seems highly probable that the rise in the respiration rate observed after 30 to 40 hours represents the time when the amino-acids have accumulated sufficiently and their influence on the rate of respiration becomes noticeable. The experiments on this point will be taken up later.

FIGURE 13.

Rate of respiration of 6 leaves of *Helianthus annuus* at 25°; petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose and 0.11 per cent glyocoll. Values taken from table 29. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa the time in hours.

Turning now to the experiments in which amino-acids were fed the leaves, it should be noted that the conditions here were precisely the same as in the foregoing experiments, with the exception that the nutrient solution contained, besides the inorganic salts and the specific sugar, a definite amount of amino-acid. In tables 23, 25,

TABLE 30.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition	<i>p. ct.</i> 14.92	<i>p. ct.</i> 0.142	<i>p. ct.</i> 1.27
After 76.25 hours in dark. . .	12.71	.653	1.07

and 27 were presented the results of respiration determinations of leaves which had been given d-glucose. In the following experiment with *Helianthus* leaves 0.11 per cent of glyocoll was added to

the nutrient solution. The analytical data show that during the course of the experiment the leaves gained both in amino-acids and in carbohydrate-content.

TABLE 31.—Rate of emission of CO_2 by 6 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose and 0.16 per cent asparagine. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.11835 normal, 125 c. c. of which has the CO_2 equivalent of 0.3254 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	11 ^h 45 ^m a.m. to 1 p.m....	1.25	1.25					
1	1 p.m. to 4 p.m.	3.0	4.25	60.7	0.2800	0.0454	15.13	4.22
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	10.75	69.0	.2428	.0826	12.7	3.54
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	17.25	71.4	.2340	.0914	14.06	3.92
4	5 a.m. to 10 a.m.	5.0	22.25	67.8	.2476	.0778	15.56	4.34
5	10 a.m. to 4 p.m.	6.0	28.25	72.2	.2312	.0942	15.70	4.38
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	34.75	74.0	.2250	.1004	15.44	4.30
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	41.25	75.3	.2202	.1052	16.18	4.51
8	5 a.m. to 10 a.m.	5.0	46.25	70.3	.2376	.0878	17.56	4.90
9	10 a.m. to 4 ^h 15 ^m p.m.	6.25	52.5	80.2	.2046	.1208	19.32	5.39
10	4 ^h 15 ^m p.m. to 10 ^h 45 ^m p.m.	6.5	59.0	80.9	.2026	.1228	18.89	5.27
11	10 ^h 45 ^m p.m. to 5 ^h 15 ^m a.m.	6.5	65.5	82.4	.1986	.1268	19.50	5.44
12	5 ^h 15 ^m a.m. to 10 a.m.	4.75	70.25	72.2	.2312	.0942	19.83	5.53
13	10 a.m. to 4 p.m.	6.0	76.25	81.5	.2010	.1244	20.73	5.78

From the determinations of the rates of CO_2 emission, given in table 29 and figure 13, it is apparent that when the leaves are given both d-glucose and glycocoll the rate of respiration rises at once and, with slight irregularities, maintains this increased rate for at least 74 hours. The stimulating action of glycocoll becomes evident when the graph of the experiment is compared with the three preceding ones. This is especially apparent during the first 35 hours, that is, before the effect of the amino-acids accumulating in the leaves becomes noticeable.

TABLE 32.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition.	<i>p. ct.</i> 15.33	<i>p. ct.</i> 0.121	<i>p. ct.</i> 1.49
After 76 hours in dark..	12.94	.569	1.03

That other amino-acids have a similar effect is shown by the following experiment with asparagine and *Helianthus* leaves. All conditions were kept precisely the same and the nutrient solution contained 0.16 per cent of asparagine. The analytical data in table 30

show that while the initial carbohydrate-content was not quite maintained at the end of the experiment, there was a decided gain in amino-acids.

The determinations of the rates of carbon-dioxid emission are given in table 31 and figure 14. A noteworthy feature of these results is the rather rapid initial drop in the respiration rate before the subsequent rise. This is probably due to the time required for the asparagine to pass through the petiole and penetrate into the cells. Glycocoll, on the other hand, has notable penetrating qualities, and the effect of this amino-acid becomes noticeable very quickly in the respiration-rate.

FIGURE 14.

Broken line indicates rate of respiration of 6 leaves of *Helianthus annuus* at 25°; petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose and 0.16 per cent asparagine; from table 31. The solid line indicates the rate of respiration of leaves of *Helianthus annuus* at 25°, petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose and 0.11 per cent of alanine; from table 33. The ordinate represents mg. CO₂ emitted per hour per gram dry material, the abscissa the time in hours.

Alanine seems to be taken up very slowly. The same conditions were maintained as in the preceding experiments and pure alanine was given as the amino-acid. The analytical data in table 32 show a relatively slight increase in amino-acids after the leaves had been

in the dark for 76 hours. The rate of respiration as shown in table 33 and figure 14 is also correspondingly low and the rise in the rate comes quite late.

TABLE 33.—Rate of emission of CO_2 by 6 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent glucose and 0.11 per cent alanine. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.11835 normal, 125 c. c. of which has the CO_2 equivalent of 0.3254 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	12 m. to 1 p.m.	1.0	1.0					
1	1 p.m. to 4 p.m.	3.0	4.0	65.5	0.2570	0.0684	22.80	6.027
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	10.5	77.8	.2122	.1132	17.41	4.602
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	17.0	77.9	.2118	.1136	17.47	4.618
4	5 a.m. to 10 a.m.	5.0	22.0	70.7	.2360	.0894	17.88	4.726
5	10 a.m. to 4 p.m.	6.0	28.0	72.5	.2300	.0954	15.90	4.203
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	34.5	73.6	.2264	.0990	15.23	4.026
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	41.0	73.9	.2252	.1002	15.41	4.073
8	5 a.m. to 10 a.m.	5.0	46.0	67.0	.2504	.0750	17.43	4.607
9	10 a.m. to 4 p.m.	6.0	52.0	78.9	.2086	.1168		
10	4 p.m. to 10 ^h 30 ^m p.m.	6.5	58.5	76.4	.2166	.1088	16.73	4.422
11	10 ^h 30 ^m p.m. to 5 a.m.	6.5	65.0	77.2	.2140	.1114	17.13	4.528
12	5 a.m. to 10 a.m.	5.0	70.0	71.6	.2332	.0922	18.44	4.874
13	10 a.m. to 4 p.m.	6.0	76.0	78.3	.2106	.1148	19.13	5.056

4. Sucrose.

Some attention was also given to the influence of sugars other than d-glucose. Thus the rate of respiration was determined of leaves which were given sucrose as well as a mixture of this sugar and an amino-acid. In these experiments the conditions as to temperature, etc., were the same as in the preceding determinations.

Table 34 gives the analytical data of the leaves in the initial condition and after having stood in the dark with the petioles in a nitrogen-free nutrient solution containing 7 per cent of sucrose.

TABLE 34.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition.	p. ct. 15.00	p. ct. 0.126	p. ct. 2.14
After 76.5 hours in dark.	14.00	.335	2.28

The rates of respiration of this experiment are given in table 35 and figure 15. It will be noted that although there is a slight increase in both the amino-acid and carbohydrate-content, the leaves do

not maintain the initial rate of CO_2 emission and the general course of the rate is down. The rise after 35 hours is very noticeable; this, however, is not maintained and the rate gradually drops.

TABLE 35.—Rate of emission of CO_2 by 6 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent sucrose. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.11835 normal, 125 c. c. of which has the CO_2 equivalent of 0.3254 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	11 ^h 30 ^m a.m. to 12 ^h 30 ^m p.m.	1.0	1.0					
1	12 ^h 30 ^m p.m. to 4 p.m.	3.5	4.5	64.6	0.2608	0.0646	18.45	5.42
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	11.0	75.9	.2184	.1070	16.46	4.84
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	17.5	76.1	.2172	.1082	16.64	4.89
4	5 a.m. to 10 a.m.	5.0	22.5	69.6	.2402	.0852	17.04	5.00
5	10 a.m. to 4 p.m.	6.0	28.5	73.6	.2264	.0990	16.50	4.84
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	35.0	75.5	.2198	.1056	16.24	4.77
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	41.5	78.9	.2088	.1166	17.93	5.26
8	5 a.m. to 10 a.m.	5.0	46.5	70.7	.2360	.0894	17.88	5.25
9	10 a.m. to 4 p.m.	6.0	52.5	75.4	.2198	.1056	17.60	5.17
10	4 p.m. to 10 ^h 30 ^m p.m.	6.5	59.0	75.7	.2188	.1066	16.40	4.81
11	10 ^h 30 ^m p.m. to 9 a.m.	10.5	69.5	102.7	.1554	.1700	16.19	4.75
12	9 a.m. to 10 ^h 30 ^m a.m.	1.5	71.0	56.7	.3020	.0234	15.60	4.58
13	10 ^h 30 ^m a.m. to 4 p.m.	5.5	76.5	72.6	.2298	.0956	17.38	5.10

When, on the other hand, the leaves are given, besides sucrose, a small amount of glycocoll, the course of the respiration rates is a gradual rise. The changes in material are given in the analytical data in table 36.

TABLE 36.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition	<i>p. ct.</i> 15.49	<i>p. ct.</i> 0.086	<i>p. ct.</i> 2.11
After 76 hours in dark. . .	14.11	.310	2.70

The rates of respiration are given in table 37 and figure 15. From these it is apparent that after an initial drop in the rate of CO_2 emission, which probably represents the time required for the material in the nutrient solution to migrate into the leaves, there is an irregular rise which becomes pronounced after about 35 hours. The principal difference between the leaves which have been given glycocoll and those without is that in the former there is a marked rise in the rate immediately after the initial drop. This is probably

attributable to the influence of the glycocoll from the nutrient solution, while the rise after 35 hours, which is noticeable in both experiments, represents the effect of the natural accumulation of amino-acids in the leaves, which becomes noticeable even in those leaves which have been given no sugar in the nutrient solution.

FIGURE 15.

Rates of respiration with sucrose. The broken line indicates the rate of respiration of 6 leaves of *Helianthus annuus* at 25°; petioles in nitrogen-free nutrient solution containing 7 per cent sucrose. Values taken from table 35. The solid line indicates the rate of respiration of 6 leaves of the same plant at 25°; petioles in nitrogen-free nutrient solution containing 7 per cent sucrose and 0.11 per cent glycocoll. Values taken from table 37. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa time in hours.

5. d-levulose.

As was pointed out in the introductory discussion, the purely chemical experiments of Nef, as well as the physiological studies of Lusk, point to the conclusion that d-levulose is more easily oxidized than any of the other hexose sugars. Among plants, particularly the lower ones, there is a great diversity in the capacity for using different sugars. There exists little information on the relative value of the various sugars in respect to respiratory activity of higher plants. Palladin¹ attempted to determine the influence of various sugars on the rate of respiration. He found that d-levulose produced a greater carbon-dioxid emission than either d-glucose, sucrose, maltose, raffinose, glycerine, or mannit. However, Palladin worked with etiolated bean shoots. These were placed in solutions of the various sugars for 2 to 4 days and then the rate of respiration

¹ PALLADIN, W. *Rev. Gén. de Bot.*, 13, 19, 93, 127 (1901).

was determined during a few hours. The results from these experiments can, of course, not be taken as giving definite results as to the use in respiration of the various sugars employed. First of all, no account is taken of the possible conversion of the sugars in the leaf during the time the sprouts were in the sugar solution. Also, the length of time during which respiration determinations were made was entirely too short to gain any conclusive idea as to the true rate of this process, and it is very questionable whether it is justifiable to draw conclusions as to the normal behavior of plants from a study of etiolated shoots. Finally, Palladin carried out his respiration determinations with levulose in diffuse light, which is another disturbing factor.

Such evidence as is now available seems to point to the conclusion that in the plant, unlike the animal, d-levulose is not the most easily oxidized sugar. Brown and Morris¹ made comparative analyses of the sugars in *Tropæolum majus*. Two sets of excised leaves were used; the one was analyzed immediately, the other after having remained in the dark for 24 hours with the petioles standing in water. Thus they found:

TABLE 37.—Rate of emission of CO₂ by 6 leaves of *Helianthus annuus* at 25°.

Petioles in nitrogen-free nutrient solution containing 7 per cent sucrose and 0.11 per cent glycocholl. CO₂ absorbed in Ba(OH)₂ solution 0.11835 normal, 125 c. c. of which has the equivalent of 0.3254 gram CO₂.

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO ₂ equivalent of 125 c. c. Ba(OH) ₂ solution.	Gram CO ₂ absorbed.	Mg. CO ₂ per hour.	Mg. CO ₂ per hour per gram dry weight.
0	12 m. to 1 p.m.....	1.0	1.0					
1	1 p.m. to 4 p.m.....	3.0	4.0	59.6	0.2856	0.0398	13.26	3.84
2	4 p.m. to 10 ^h 30 ^m p.m....	6.5	10.5	67.1	.2500	.0754	11.60	3.36
3	10 ^h 30 ^m p.m. to 5 a.m....	6.5	17.0	69.8	.2400	.0854	13.13	3.80
4	5 a.m. to 10 a.m.....	5.0	22.0	66.3	.2534	.0720	14.40	4.17
5	10 a.m. to 4 p.m.....	6.0	28.0	70.1	.2384	.0870	14.50	4.20
6	4 p.m. to 10 ^h 30 ^m p.m....	6.5	34.5	70.6	.2366	.0888	13.66	3.95
7	10 ^h 30 ^m p.m. to 5 a.m....	6.5	41.0	71.5	.2334	.0920	14.15	4.10
8	5 a.m. to 10 a.m.....	5.0	46.0	68.3	.2456	.0798	15.96	4.62
9	10 a.m. to 4 p.m.....	6.0	52.0	73.8	.2256	.0998	16.63	4.81
10	4 p.m. to 10 ^h 30 ^m p.m....	6.5	58.5	73.8	.2256	.0998	15.35	4.44
11	10 ^h 30 ^m p.m. to 5 a.m....	6.5	65.0	74.7	.2224	.1030	15.84	4.58
12	5 a.m. to 10 a.m.....	5.0	70.0	69.5	.2406	.1848	16.96	4.91
13	10 a.m. to 4 p.m.....	6.0	76.0	73.9	.2250	.1004	16.73	4.84

Evidently there is a considerable loss of carbohydrates due to respiration, as well as a decided change in the relative amounts of the various sugars. In all probability the starch was converted into maltose, which yields dextrose, and the cane sugar was inverted to

¹ BROWN, H. T., and G. H. MORRIS. *Jour. Chem. Soc. London*, 63, 671 (1893).

equal quantities of dextrose and levulose. There was thus undoubtedly a preponderance of dextrose. Nevertheless, there was a much greater increase of levulose than of dextrose during the 24

TABLE 38.

Experiment No..	Leaves analyzed at once.		Leaves kept in dark 24 hours.	
	III.	IV.	III.	IV.
Starch.....	3.693	5.425	2.980	0.906
Cane sugar.....	9.98	7.33	3.49	3.35
Dextrose.....	0.00	0.00	0.53	1.34
Levulose.....	1.41	2.11	3.46	3.76
Maltose.....	2.25	2.71	1.86	1.28

hours the leaves were in the dark. Brown and Morris calculate from these results that far more dextrose than levulose is used in the respiratory process, as is shown in table 39.

TABLE 39.—*Sugars used up in respiration expressed as percentages of the dry-leaf material.*

	Expt. III.	Expt. IV.
Dextrose.....	2.66	0.65
Levulose.....	1.19	0.34
Maltose.....	1.10	5.94

The work of Parkin¹ substantiates the conclusions of Brown and Morris. Working with *Galanthus nivalis*, the leaves of which do not store starch, Parkin found that levulose, as a rule, is in excess of glucose, irrespective of the time of day the leaves are taken for analysis.

"Out of 52 duplicate leaf analyses made, 47 had the fructose in excess of the glucose, and only 7 the reverse. Representing fructose as unity, in the former cases the ratio varied from 1: 0.4 to 1: 0.76, and in the latter from 1:1.01 to 1:1.06. The calculation of the separate amounts of glucose and fructose depends chiefly upon one observation, viz, the optical angle of rotation. A slight error in the reading of this will affect the results considerably; consequently it might hardly be expected that any further conclusion beyond the bare fact of the excess of one hexose sugar over the other could be reached."

A later investigation of the carbohydrates of foliage leaves by Gast² yielded very similar results. *Tropæolum majus* and *Vitis vinifera* during 24 hours of respiration in the dark showed a rela-

¹ PARKIN, J. *Biochem. Jour.*, 6, 1-47 (1911).

² GAST, W. *Zeit. physiol. chem.*, 99, 1-53 (1917).

tively greater decrease of glucose than of levulose. While *Musa ensente* Gruel, which forms very little starch, showed a decrease in cane sugar and maltose during the period of darkness, glucose increased and levulose remained the same. The results with *Cucurbita ficifolia* Beté and with *Canna viridiflora* R. and Pav. are not as definite,

TABLE 40.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Initial condition	16.21	0.144	1.88
After 76.75 hours in dark . . .	14.95	.552	1.60

but seem also to indicate that in the course of respiration more of the glucose is utilized. The expression of analytical data in terms of percentages of the dry-leaf material makes uncertain the calculation of the amounts of the various sugars used up.

TABLE 41.—Rate of emission of CO₂ by 9 leaves of *Helianthus annuus* at 25°.

Petioles in nitrogen-free nutrient solution containing 7 per cent levulose. CO₂ absorbed in Ba(OH)₂ solution, 0.12153 normal, 125 c. c. of which has CO₂ equivalent of 0.3343 gram CO₂.

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO ₂ equivalent of 125 c. c. Ba(OH) ₂ solution.	Gram CO ₂ absorbed.	Mg. CO ₂ per hour.	Mg. CO ₂ per hour per gram dry weight.
0	11 ^h 15 ^m a.m. to 12 ^h 15 ^m p.m.	1.0	1.0					
1	12 ^h 15 ^m p.m. to 4 p.m.	3.75	4.75	65.3	0.2548	0.0795	21.20	5.500
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	11.25	74.1	.2214	.1129	17.36	4.503
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	17.75	76.9	.2122	.1221	18.78	4.871
4	5 a.m. to 10 a.m.	5.0	22.75	68.2	.2426	.0917	18.35	4.760
5	10 a.m. to 4 p.m.	6.0	28.75	73.2	.2246	.1097	18.30	4.747
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	35.25	74.0	.2218	.1125	17.30	4.487
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	41.75	76.3	.2140	.1203	18.50	4.799
8	5 a.m. to 10 a.m.	5.0	46.75	69.0	.2396	.0947	18.94	4.913
9	10 a.m. to 4 p.m.	6.0	52.75	75.0	.2184	.1159	19.31	5.009
10	4 p.m. to 10 ^h 30 ^m p.m.	6.5	59.25	75.6	.2164	.1179	18.13	4.703
11	10 ^h 30 ^m p.m. to 5 a.m.	6.5	65.75	75.8	.2160	.1183	18.20	4.721
12	5 a.m. to 10 a.m.	5.0	70.75	68.3	.2422	.0921	18.42	4.778
13	10 a.m. to 4 p.m.	6.0	76.75	74.4	.2238	.1105	18.41	4.775

While the evidence is as yet by no means conclusive, it appears that levulose in leaves is more stable than glucose. The question resolves itself into whether d-glucose is actually more easily consumed in the leaf or whether d-levulose is, under certain conditions, converted into d-glucose. The problem of the glucose-fructose relation is a very fundamental one in the carbohydrate economy of

plants. Its solution is associated with the extraordinary difficulty of quantitatively determining the various sugars in leaves which have been subjected to a series of experimental conditions.

TABLE 42.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Initial condition	<i>p. ct.</i> 15.91	<i>p. ct.</i> 0.190	<i>p. ct.</i> 1.969
After 76.25 hours in dark . . .	13.85	.615	1.563

Unfortunately the results of experiments which we have carried out with this problem in view had to be discarded on account of some uncertainties which developed later in our analytical methods. However, the investigation of this phase of the carbohydrate economy problem is to be continued.

TABLE 43.—Rate of emission of CO_2 by 7 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent d-levulose and 0.11 per cent glycocoll. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution, 0.11335 normal, 125 c. c. of which has the CO_2 equivalent of 0.3254 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 ab- sorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	11 ^h 45 ^m a.m. to 1 p.m. . . .	1.25	1.25					
1	1 p.m. to 4 p.m.	3.0	4.25	64.4	0.2620	0.0634	21.13	6.00
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	10.75	74.3	.2238	.1016	15.63	4.44
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	17.25	76.6	.2160	.1094	16.83	4.78
4	5 a.m. to 10 a.m.	5.0	22.25	68.7	.2438	.0816	16.32	4.64
5	10 a.m. to 4 p.m.	6.0	28.25	71.7	.2332	.0922	15.36	4.36
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	34.75	72.8	.2290	.0964	14.83	4.21
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	41.25	74.0	.2246	.1008	15.50	4.40
8	5 a.m. to 10 a.m.	5.0	46.25	69.0	.2426	.0828	16.56	4.71
9	10 a.m. to 4 p.m.	6.0	52.25	73.9	.2250	.1004	16.73	4.75
10	4 p.m. to 10 ^h 30 ^m p.m.	6.5	58.75	73.6	.2264	.0990	15.23	4.33
11	10 ^h 30 ^m p.m. to 5 a.m.	6.5	65.25	74.3	.2238	.1016	15.63	4.44
12	5 a.m. to 10 a.m.	5.0	70.25	69.0	.2426	.0828	16.56	4.71
13	10 a.m. to 4 p.m.	6.0	76.25	74.2	.2242	.1012	16.86	4.79

In the three following experiments d-levulose was used as the sugar in the nutrient solution. The first of these was carried out with d-levulose as the only organic substance in the nutrient solution. The analytical data of the changes of materials in the leaves are given in table 40.

These leaves also showed the usual gain in amino-acids after remaining 76.75 hours in the dark. There was, however, a decrease

in the carbohydrate-content, although d-leulose is notably a good nutrient for starch-forming plants. The rates of respiration are given in table 41 and figure 16.

TABLE 44.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
Original condition.....	<i>p. ct.</i> 18.31	<i>p. ct.</i> 0.157	<i>p. ct.</i> 2.16
After 77 hours in dark..	14.79	.480	1.59

The preceding experiment was repeated, all conditions remaining the same, except that the nutrient solution contained, besides the d-leulose, 0.11 per cent of glyocoll. The analyses of the leaves are compiled in table 42.

TABLE 45.—Rate of emission of CO₂ by 7 leaves of *Helianthus annuus* at 25°.

Petioles in nitrogen-free nutrient solution containing 7 per cent d-leulose and 0.138 per cent asparagine. CO₂ absorbed in Ba(OH)₂ solution. Nos. 1 to 8, inclusive, in solution 0.11835 normal, 125 c. c. of which has the CO₂ equivalent of 0.3254 gram CO₂. Nos. 9 to 13 in solution 0.12153 normal, 125 c. c. of which has the CO₂ equivalent of 0.3343 gram, CO₂.

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO ₂ equivalent of 125 c. c. Ba(OH) ₂ solution.	Gram CO ₂ ab- sorbed.	Mg. CO ₂ per hour.	Mg. CO ₂ per hour per gram dry weight.
0	10 ^h 30 ^m a.m. to 12 m.....	1.5	1.5					
1	12 m. to 4 p.m.....	4.0	5.5	73.0	0.2282	0.0972	24.30	5.521
2	4 p.m. to 10 ^h 30 ^m p.m.....	6.5	12.0	79.9	.2056	.1198	18.43	4.187
3	10 ^h 30 ^m p.m. to 5 a.m.....	6.5	18.5	81.5	.2010	.1244	19.13	4.346
4	5 a.m. to 10 a.m.....	5.0	23.5	70.5	.2368	.0886	17.72	4.026
5	10 a.m. to 4 p.m.....	6.0	29.5	74.5	.2232	.1022	17.03	3.869
6	4 p.m. to 10 ^h 30 ^m p.m.....	6.5	36.0	74.6	.2226	.1028	15.81	3.592
7	10 ^h 30 ^m p.m. to 5 a.m.....	6.5	42.5	73.9	.2250	.1004	15.44	3.508
8	5 a.m. to 10 a.m.....	5.0	47.0	68.7	.2438	.0816	16.32	3.708
9	10 a.m. to 4 p.m.....	6.0	53.0	70.5	.2366	.0977	16.28	3.700
10	4 p.m. to 10 ^h 30 ^m p.m.....	6.5	59.5	71.2	.2346	.0997	15.33	3.483
11	10 ^h 30 ^m p.m. to 5 a.m.....	6.5	66.0	73.1	.2280	.1063	16.35	3.715
12	5 a.m. to 10 a.m.....	5.0	71.0	69.3	.2414	.0929	18.58	4.222
13	10 a.m. to 4 p.m.....	6.0	77.0	76.0	.2182	.1161	19.35	4.396

The rates of respiration for this experiment are given in table 43 and in the graphs in figure 16.

The experiment was again repeated, the glyocoll being replaced in this instance by asparagine. The analytical data are presented in table 44.

The rates of respiration are shown in table 45 and in the graphs in figure 16.

A comparison of the results of the foregoing three experiments as presented in figure 16 shows at once that apparently d-levulose does not produce an increased respiratory activity in *Helianthus* leaves. Also, the graphs of the rates of respiration differ considerably from the experiments in which d-glucose and sucrose were fed the leaves. Of these sugars, d-levulose seems to produce the least active respiratory activity and d-glucose the most active, while sucrose lies midway between the two. In none of the experiments

FIGURE 16.

Rates of respiration with d-levulose. The dotted line indicates the rate of respiration of leaves of *Helianthus annuus* at 25°; petioles in a nitrogen-free nutrient solution containing 7 per cent d-levulose; values from table 41. The broken line indicates the rate of respiration at 25° with a nitrogen-free solution containing 7 per cent d-levulose and 0.11 per cent glycocoll, as per table 43. The solid line indicates the rate of respiration at 25° with a nitrogen-free nutrient solution containing 7 per cent d-levulose and 0.138 per cent asparagine. The ordinates represent mg. CO₂ per hour per gram dry material, and the abscissa the time in hours.

with *Helianthus* leaves in which these sugars alone were given was the initial sugar-content maintained. However, the reduction in all these cases was about the same, as was also the approximate increase in relative water-content. Nevertheless, these sugars show decided variation in their effect on respiratory activity. Moreover, the influence of adding amino-acids to the nutrient solutions containing either d-glucose, sucrose, or d-levulose is very different. With d-glucose the effect is marked, particularly before the fortieth

hour of the experiment with the more easily penetrating glycocoll and asparagine. With d-levulose no stimulating effect can be noticed in our experiments; in fact the respiration rates in the experiments with glycocoll or asparagine and d-levulose are relatively below those with this sugar alone during the first half of the time.

6. d-mannose.

A sugar which is closely related in structure to both d-glucose and d-levulose is d-mannose. These three sugars have a common enol and are easily converted into each other.¹ Aside from its rather common occurrence as an anhydride, mannan, in seed coats and similar organs, the physiological behavior of d-mannose in plants has been investigated very little. Knudson² found that d-mannose has a decidedly toxic action on the root tips of wheat and peas grown in sterile agar cultures.

We carried out but two complete experiments with d-mannose, one without and the other with glycocoll in the nutrient solution. The results were quite surprising in that there was no toxic action apparent and that the rates of respiration developed a very great increase over the initial rate.

In table 46 the analytical data are given representing the changes in material in leaves which have been given d-mannose.

TABLE 46.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Initial condition.....	14.68	0.153	1.24
After 77.5 hours in dark....	13.64	0.788	0.95

The results of the determination of respiration-rates for this experiment are given in table 47 and figure 17.

The foregoing experiment was repeated with the addition of 0.11 per cent of glycocoll to the nutrient solution. The analytical data are given in table 48.

The results of the determination of the respiration rates are compiled in table 49 and figure 17.

The experiments with d-mannose are noteworthy on account of the great increase in amino-acids in both cases. Although the petioles of the leaves were standing in a 7 per cent solution of d-mannose, there was a very appreciable decrease in the total sugar-

¹ SPOEHR, H. A. The carbohydrate economy of cacti. Carnegie Inst. Wash. Pub. No. 287 (1919).

² KNUDSON, L. *Amer. Jour. of Bot.*, 4, 430-437 (1917).

content of the leaves. Nevertheless, the respiration rates at the end of the experiment show a remarkable increase over the initial rates, although the latter were relatively not low. There is here the same phenomenon, exhibited by d-glucose in figure 11, of an

TABLE 47.—Rate of emission of CO_2 by 8 leaves of *Helianthus annuus* at 25° .

Petioles in nitrogen-free nutrient solution containing 7 per cent mannose. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.12153 normal, 125 c. c. of which has the CO_2 equivalent of 0.3343 g. CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	10 ^h 30 ^m a.m. to 12 m.	1.5	1.5					
1	12 m. to 4 p.m.	4.0	5.5	64.1	0.2598	0.0745	18.62	5.540
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	12.0	72.7	.2262	.1081	16.63	4.947
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	18.5	76.4	.2140	.1203	18.50	5.504
4	5 a.m. to 10 a.m.	5.0	23.5	68.1	.2428	.0915	18.30	5.444
5	10 a.m. to 4 p.m.	6.0	29.5	71.9	.2294	.1049	17.48	5.200
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	36.0	75.1	.2180	.1163	17.89	5.322
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	42.5	76.1	.2148	.1195	18.38	5.468
8	5 a.m. to 10 a.m.	5.0	47.5	71.0	.2326	.1017	20.32	6.045
9	10 a.m. to 4 p.m.	6.0	53.5	79.6	.2040	.1303	21.71	6.459
10	4 p.m. to 10 ^h 30 ^m p.m.	6.5	60.0	83.2	.1944	.1399	21.52	6.402
11	10 ^h 30 ^m p.m. to 5 a.m.	6.5	66.5	87.0	.1854	.1489	22.90	6.813
12	5 a.m. to 10 a.m.	5.0	71.5	79.3	.2048	.1295	25.90	7.706
13	10 a.m. to 4 p.m.	6.0	77.5	99.0	.1622	.1721	28.68	8.533

increased respiration rate with decreased total sugar-content and increased amino-acids. The sugar-content alone is no index of the rate at which the leaves utilize this material. Moreover, under the conditions of the experiment, where sugar is being constantly sup-

TABLE 48.

	Dry weight.	Amino nitrogen in dry material.	Total sugars in dry material.
	<i>p. ct.</i>	<i>p. ct.</i>	<i>p. ct.</i>
Initial condition.	15.00	0.252	1.18
After 76.75 hours in dark. . .	13.29	0.741	0.72

plied, it is quite conceivable that this is oxidized as rapidly as it is taken up. This state of affairs must, of course, also frequently exist under natural conditions. But the increased rate of respiration at the end of the experiment can not be due to a greater supply of sugar, but to the cooperation of some other factor which increases its activity with time. The increase in amino-acids under the circumstances is more than a coincidence and plays an important

role in the respiratory activity. In the experiments with d-mannose the glycocoll which was given in the nutrient solution had apparently very little effect, as the normal accumulation of amino-acids in the leaf in this case was so very great. There must, of course, also be limits beyond which there is little increase in amino-acids and above which the rate of respiration does not rise, so that further addition of an amino-acid would be of little consequence.

TABLE 49.—Rate of emission of 8 leaves of *Helianthus annuus* at 25°.

Petioles in nitrogen-free nutrient solution containing 7 per cent d-mannose + 0.11 per cent glycocoll. CO₂ absorbed in Ba(OH)₂ solution 0.12153 normal, 125 c.c. of which has the CO₂ equivalent of 0.3343 gram CO₂.

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO ₂ equivalent of 125 c. c. Ba(OH) ₂ solution.	Gram CO ₂ absorbed.	Mg. CO ₂ per hour.	Mg. CO ₂ per hour per gram dry weight.
0	11 ^h 15 ^m a.m. to 12 ^h 15 ^m p.m.	1.0	1.0					
1	12 ^h 15 ^m p.m. to 4 p.m.	3.75	4.75	61.3	0.2730	0.0613	16.34	4.932
2	4 p.m. to 10 ^h 30 ^m p.m.	6.5	11.25	67.1	.2468	.0875	13.46	4.036
3	10 ^h 30 ^m p.m. to 5 a.m.	6.5	17.75	71.1	.2320	.1023	15.73	4.748
4	5 a.m. to 10 a.m.	5.0	22.75	66.3	.2493	.0850	17.00	5.131
5	10 a.m. to 4 p.m.	6.0	28.75	68.0	.2432	.0911	15.18	4.582
6	4 p.m. to 10 ^h 30 ^m p.m.	6.5	35.25	70.0	.2358	.0985	15.15	4.573
7	10 ^h 30 ^m p.m. to 5 a.m.	6.5	41.75	69.7	.2370	.0972	15.00	4.528
8	5 a.m. to 10 a.m.	5.0	46.75	65.2	.2552	.0791	15.82	4.775
9	10 a.m. to 4 p.m.	6.0	52.75	69.4	.2380	.0963	16.05	4.845
10	4 p.m. to 10 ^h 30 ^m p.m.	6.5	59.25	72.0	.2288	.1055	16.23	4.899
11	10 ^h 30 ^m p.m. to 5 a.m.	6.5	65.75	76.4	.2138	.1205	18.53	5.593
12	5 a.m. to 10 a.m.	5.0	70.75	73.0	.2254	.1089	21.78	6.574
13	10 a.m. to 4 p.m.	6.0	76.75	90.0	.1786	.1557	25.95	7.833

7. Effect of the Natural Increase in Amino-Acids. Influence of Light on Amino-Acids and Effect on Respiration.

In the experiments which have been described attention has repeatedly been called to the rise in the rates of respiration after about 35 or 40 hours. An examination of the graphs shows this rise in practically all of the experiments, and particularly is it noticeable in the cases in which no amino-acid was contained in the solution. It is perhaps unnecessary to point out that these graphs do not represent the true course of respiratory activity, for the points in the curves represent the rates of carbon-dioxid emission during a certain period, usually 6 hours. Naturally such an experimental procedure makes the variations appear much more abrupt than in all probability they are, so that the rise in respiration rate between the thirty-fifth and fortieth hour must be regarded with the foregoing in view.

When excised *Helianthus* leaves are kept in the dark, with the petioles in nitrogen-free nutrient solution (table 16), there is a gradual

rise in the amino-acid content of the leaves. Our experiments indicate that amino-acids have a stimulating effect on the respiratory activity of leaves containing the natural sugars, or when they are fed d-glucose or sucrose. It seems permissible to assume that the amino acids which accumulate in the leaves when kept in the dark would exert a similar stimulating effect if the leaves contained sufficient

FIGURE 17.

Rates of respiration with d-mannose. The broken line indicates the rate of respiration of 8 leaves of *Helianthus* at 25°; petioles in a nitrogen-free nutrient solution containing 7 per cent d-mannose, values taken from table 47. The solid line indicates the rate of respiration of 8 leaves of *Helianthus* at 25° with petioles in a nitrogen-free nutrient solution containing 7 per cent d-mannose and 0.11 per cent glycocoll, values taken from table 49. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa the time in hours.

sugar. Normally, of course, when excised leaves are kept in the dark, besides an accumulation of amino-acids there is a rapid reduction of the sugar-content, so that any stimulating effect of the accumulating amino-acids could not be exerted on account of the reduced fuel material.

If, however, after the leaves have remained in the dark for a time and the amino-acids have accumulated, glucose is then fed, the

effect of this increased amino-acid content becomes noticeable at once. Thus excised leaves which had been kept in the dark for about 40 hours and then given d-glucose exhibited an immediate increase in the rate of carbon-dioxid emission. This is not the case when leaves are given d-glucose immediately after cutting from the plant; there is then a primary drop in the CO_2 curve and a rise after about 40 hours (see figs. 10 and 11). The combination, then, of the accumulated amino-acids after 40 hours of darkness and an abundant sugar supply results in an accelerated rate of respiration.

TABLE 50.—Rate of CO_2 emission, at 24° , of 8 leaves of *Helianthus annuus*.

The excised leaves were placed in a nutrient solution free from organic material and nitrogen and kept in the dark for 43.5 hours. They were then transferred to a nitrogen-free nutrient solution containing 7 per cent d-glucose and the rates of respiration determined.

No.	Time.	Hours.	Total hours.	Cubic centimeters 0.1/N HCl.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry material.
1	11 ^h 30 ^m a.m. to 12 ^h 12 ^m p.m..	0.75	0.75			
2	12 ^h 15 ^m p.m. to 5 p.m.	4.75	5.50	27.65	12.80	2.387
3	5 p.m. to 8 ^h 30 ^m p.m.	3.50	9.00	22.25	13.99	2.608
4	8 ^h 30 ^m p.m. to 9 a.m.	12.50	21.50	84.50	14.85	2.768
5	9 a.m. to 3 p.m.	6.00	27.50	51.00	18.70	3.486
6	3 p.m. to 9 ^h 45 ^m p.m.	6.75	34.25	56.20	18.26	3.404
7	9 ^h 45 ^m p.m. to 9 ^h 30 ^m a.m.	11.75	46.00	85.80	16.19	3.018
8	9 ^h 30 ^m a.m. to 4 ^h 15 ^m p.m.	6.75	52.75	54.65	17.60	3.283
9	4 ^h 15 ^m p.m. to 9 ^h 45 ^m p.m.	5.50	58.25	43.20	17.18	3.203
10	9 ^h 45 ^m p.m. to 9 ^h 15 ^m a.m.	11.50	69.75	87.85	17.01	3.171
11	9 ^h 15 ^m a.m. to 3 ^h 15 ^m p.m.	6.00	75.75	56.45	20.68	3.855

This fact becomes evident from the following experiment, in which 8 *Helianthus* leaves were cut from the plant, placed immediately in nitrogen-free mineral nutrient solution, and kept in the dark. After 43.5 hours the leaves were put in the respiration chamber with fresh nutrient solution containing 7 per cent of d-glucose. From the results of the determinations of the rates of carbon-dioxid emission in table 50 and figure 18 it is apparent that the rate of respiration rises immediately and, with some irregularities, then attains its maximum.

It is, moreover, a noteworthy fact that when the foregoing experiment is repeated in such a manner that the nutrient solution contains, besides d-glucose, 0.11 per cent of glycocoll, there is practically no difference in the rate of carbon-dioxid emission. In other words, it appears that the natural accumulation of amino-acids is just as effective in stimulating respiratory activity as when amino-acids are fed to the leaves. It was shown in the previous experiments that the influence of amino-acids when fed to the leaves was especially noticeable in the first 40 hours of the experiment, that is, before the natural accumulation of amino-acids in the dark becomes effective.

It might be argued that this rapid increase in the rate of respiration of leaves, which had been previously left in the dark and then given d-glucose, was due simply to the greater available supply of

TABLE 51.—Rate of CO_2 emission, at 24° , of 8 leaves of *Helianthus annuus*.

The excised leaves were placed in a nutrient solution free from organic material and nitrogen and kept in the dark for 42.75 hours. They were then transferred to a nutrient solution containing 7 per cent d-glucose and 0.11 per cent glycocoll and the rates of respiration determined.

No.	Time.	Hours.	Total hours.	Cubic centimeters 0.1/N NCl.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry material.
1	11 ^h 45 ^m a.m. to 12 ^h 30 ^m p.m....	0.75	0.75			
2	12 ^h 30 ^m p.m. to 5 p.m.	4.50	5.25	27.60	13.49	2.389
3	5 p.m. to 9 ^h 45 ^m p.m.	4.75	10.00	34.10	15.80	2.798
4	9 ^h 45 ^m p.m. to 11 a.m.	13.25	23.25	96.00	15.97	2.829
5	11 a.m. to 9 ^h 30 ^m p.m.	10.50	33.75	92.30	19.36	3.429
6	9 ^h 30 ^m p.m. to 9 a.m.	11.50	45.25	87.40	17.63	3.087
7	9 a.m. to 3 ^h 30 ^m p.m.	6.50	51.75	58.40	19.76	3.500
8	3 ^h 30 ^m p.m. to 11 ^h 30 ^m p.m.	8.00	59.75	68.45	18.81	3.332
9	11 ^h 30 ^m p.m. to 9 ^h 15 ^m a.m.	9.75	69.50	85.55	19.34	3.408
10	9 ^h 15 ^m a.m. to 3 ^h 15 ^m p.m.	6.00	75.50	61.25	22.44	3.975

sugar. In the experiments already described it has been pointed out that the rate of respiration bears no direct relation to the carbohydrate-supply. There are numerous instances in which an increase of carbon-dioxid emission was obtained with a decreased total sugar-content. In these cases the amino-acids had consistently

FIGURE 18.

The solid line indicates the rate of respiration at 25° of 8 leaves of *Helianthus* which were kept in the dark for 43.5 hours previous to putting in a nitrogen-free nutrient solution containing 7 per cent d-glucose, as per table 49. The broken line indicates the rate of respiration at 25° of 8 similar leaves which were kept in the dark for 42.75 hours previous to putting in a nitrogen-free nutrient solution containing 7 per cent glucose and 0.11 per cent glycocoll, as per table 50. The ordinate represents mg. CO_2 per hour per gram dry material and the abscissa the time in hours.

increased. In table 27 and figure 12 is also given a case of decreased respiration rate accompanied by increased sugar-content and decreased amino-acids. However, in table 12 were given the results of the carbohydrate and amino-acid determinations of leaves from plants which were kept in the dark, showing the gradual decrease in carbohydrates and increase in amino-acids. When these plants were again placed in the light the reverse process occurred; there was an increase in sugars and a decrease in amino-acids.

FIGURE 19.

Rate of respiration subsequent to periods of illumination. Six leaves of *Helianthus* at 25°; petioles in a nitrogen-free nutrient solution containing 7 per cent d-glucose. The solid lines indicate the CO₂ emission in the dark, the dotted lines indicate the periods during which the leaves are illuminated. The ordinate represents mg. CO₂ per hour per gram dry material, the abscissa the time in hours.

There remains, therefore, to show the effect on the rate of respiration of temporarily decreasing the amino-acids while the carbohydrate-content remains high. It has been shown that the effect of light is to decrease the amino-acids. The following experiment was carried out so that the leaves which from the beginning of the experiment were fed d-glucose were illuminated for 6 to 7 hours after having been in the dark for about 25 hours. Thus during the course of the experiment there was a period of illumination of 7 hours after the twenty-fifth hour, another period of illumination of 6 hours after the fiftieth hour, and a third period of illumination after the seventy-fourth hour. As a source of illumination there was used a 750-watt tungsten filament lamp at 40 cm. distance. The glass cover of the respiration chamber was below the level of the water in the thermostat. The results of this experiment are given in table 52 and figure 19.

During the periods of illumination the amount of carbon dioxide absorbed was very low. This would indicate that a large part of

the carbon dioxide of respiration was fixed through photosynthesis. The carbohydrate-content of the leaves, at the end of the periods of illumination, was therefore, in all probability, higher than at the beginning of illumination. It must also be borne in mind that the petioles of the leaves were in a 7 per cent d-glucose solution during the entire experiment.

TABLE 52.—Rate of emission of CO_2 by 6 leaves of *Helianthus annuus* at 25° ; petioles in nitrogen-free nutrient solution containing 7 per cent d-glucose.

The leaves were illuminated during periods Nos. 5, 10, and 15. CO_2 absorbed in $\text{Ba}(\text{OH})_2$ solution 0.12170 normal, 125 c. c. of which has the equivalent of 0.3346 gram CO_2 .

No.	Time.	Hrs.	Total hours.	Observed resistance in ohms.	Gram CO_2 equivalent of 125 c. c. $\text{Ba}(\text{OH})_2$ solution.	Gram CO_2 absorbed.	Mg. CO_2 per hour.	Mg. CO_2 per hour per gram dry weight.
0	2 ^h 30 ^m p.m. to 3 ^h 30 ^m p.m.	1	1.0					
1	3 ^h 30 ^m p.m. to 4 ^h 30 ^m p.m.	6	7.0	71.3	0.2436	0.0910	15.16	7.38
2	4 ^h 30 ^m p.m. to 5 ^h 30 ^m a.m.	6	13.0	72.9	.2376	.0970	16.16	7.87
3	5 ^h 30 ^m a.m. to 6 ^h 30 ^m a.m.	6	19.0	73.2	.2366	.0980	16.33	7.95
4	6 ^h 30 ^m a.m. to 7 ^h 30 ^m p.m.	6	25.0	70.7	.2456	.0890	14.83	7.22
5	7 ^h 30 ^m p.m. to 8 ^h 30 ^m p.m.	7	32.0	60.4	.2920	.0426	6.08	2.96
6	8 ^h 30 ^m p.m. to 9 ^h 30 ^m a.m.	3	35.0	59.5	.2978	.0368	12.26	5.97
7	9 ^h 30 ^m a.m. to 10 ^h 30 ^m a.m.	6	41.0	67.9	.2570	.0776	12.93	6.29
8	10 ^h 30 ^m a.m. to 11 ^h 30 ^m a.m.	3	44.0	60.0	.2948	.0398	13.23	6.44
9	11 ^h 30 ^m a.m. to 12 ^h 30 ^m p.m.	6	50.0	69.1	.2524	.0822	13.70	6.67
10	12 ^h 30 ^m p.m. to 1 ^h 30 ^m p.m.	6	56.0	60.3	.2930	.0416	6.93	3.37
11	1 ^h 30 ^m p.m. to 2 ^h 30 ^m a.m.	3	59.0	60.1	.2938	.0408	13.60	6.62
12	2 ^h 30 ^m a.m. to 3 ^h 30 ^m a.m.	6	65.0	68.5	.2550	.0796	13.26	6.45
13	3 ^h 30 ^m a.m. to 4 ^h 30 ^m a.m.	3	68.0	60.4	.2920	.0426	14.20	6.91
14	4 ^h 30 ^m a.m. to 5 ^h 30 ^m p.m.	6	74.0	70.5	.2466	.0880	14.66	7.14
15	5 ^h 30 ^m p.m. to 6 ^h 30 ^m p.m.	6	80.0	59.5	.2978	.0368	6.13	2.98
16	6 ^h 30 ^m p.m. to 7 ^h 30 ^m a.m.	3	83.0	59.8	.2960	.0386	12.86	6.26
17	7 ^h 30 ^m a.m. to 8 ^h 30 ^m a.m.	6	89.0	68.0	.2570	.0776	12.93	6.29
18	8 ^h 30 ^m a.m. to 9 ^h 30 ^m p.m.	6	95.0	68.0	.2570	.0776	12.93	6.29

The experiment shows clearly that following a period of illumination there is a decrease in the rate of respiration; with continued darkness the rate rises slowly again until the next period of illumination. It must be remembered that under the conditions of the experiment the leaves had a large supply of available sugar in the form of d-glucose. From the experiments which have been described it becomes evident that there are at least two factors affecting the rate of the respiratory process. One of these is the supply of carbohydrates and the other is the amount of amino-acids. A change in either of these factors alters the rate of respiration. Under most circumstances light seems to affect these two factors in an opposite manner: the carbohydrates are increased by the photosynthetic activity of light, while under these conditions the amino-acids decrease. It is conceivable that in this manner the rate of respiration tends to be equalized under varying external conditions.

The increased rate of growth in the dark, which has been very commonly observed, can also in a measure be explained on the basis of the foregoing experiments. In the dark there is an accumulation of amino-acids in most plant parts. Given an adequate supply of carbohydrates, stored in the stems and other parts of the plant, an increase in amino-acids resulting from the absence of light would cause an increased respiratory rate. The behavior of an entire plant has been described in the first experiment, under table 8 and figure 7. It is therefore suggested that the augmented respiratory activity is at least one important factor contributing to the increased rate of growth during periods of darkness.

There are many points regarding the relation of carbohydrates and amino-acids to light which require further study. These questions seem to us to be of great importance to the problems of photosynthesis and are now being subjected to investigation.

It would, of course, have been highly desirable if the proteins as well as the amino-acids could have been determined in the leaf material. This, however, was impossible, largely because of the relatively small amount of material remaining from the respiration determinations and from the analyses for amino-acids and sugars. In view of the fact that the leaves were kept in a nutrient solution containing no nitrogen, it seems highly probable that the amino-acid increase came from the breakdown of the proteins. That the leaves contained ample proteinaceous material to supply the increase in amino-acids becomes evident from the following analyses. A typical dry-leaf material of *Helianthus* contained 5.50 per cent of nitrogen as NH_2 , determined by the Folin micro-Kjeldahl method. The water extract yielded 0.119 per cent nitrogen as NH_2 by the Van Slyke method, representing free amino-acids, and 0.463 per cent nitrogen as NH_2 by the Folin micro-Kjeldahl method. Evidently some nitrogenous material other than amino-acids is extracted by means of water. This, however, does not affect the Van Slyke results. Some idea of the amount of protein in the dry-leaf material can be gained from the results of the hydrolysis with 20 per cent hydrochloric acid. This hydrolyzed material contains total nitrogen as NH_2 of 5.03 per cent by the Folin micro-Kjeldahl method. The discrepancy between this figure and the 5.50 per cent representing the total nitrogen of the material which had not been previously treated with 20 per cent acid must represent the humin formation resulting from the reaction of the amino-acids with the carbohydrate. The hydrolyzed material yielded amino nitrogen by the Van Slyke method of 2.88 per cent. Although the latter value is undoubtedly a little low on account of the humin formation during hydrolysis, it represents a considerable reserve of protein when compared with the original amino nitrogen in the water extract of 0.119 per cent. Thus the hydrolyzed material contains about twenty times more

amino-acids than the water extract. This gives an indication of the amount of protein in the leaf as compared with the amino-acids. A considerable number of analyses such as the one just described were carried out, all of which represented about the same proportions, but as the results have not a real quantitative value they are omitted here.

Special tests were also made to determine whether there was an accumulation of nitrates in the leaves which had been kept in the dark. However, no indication could be found that this was the case. In fact, no tests for nitrates could be obtained in the water extracts. Similarly, the amount of ammonia was so small that it can be considered as insignificant. The ammonia was determined by treating the leaf powder, suspended in water, with magnesium oxide and distilling in a stream of air at 35° to 40° under reduced pressure, 15 mm. The distillate was taken up in standard acid solution.

It is well known that with an ample supply of carbohydrates, protein synthesis can take place in the leaf in the dark from inorganic nitrates.¹ The observation that light acts favorably on protein synthesis—more than three times the amount which is formed in the dark—has been made by a number of workers.² Since it had been shown that protein synthesis from inorganic nitrogen compounds occurs only in the presence of an abundance of carbohydrates, the beneficial influence of light was attributed to photosynthetic elaboration of carbohydrates by means of light. That this was not the only interpretation to be given to experiments showing the favorable action of light on protein syntheses was made evident by the experiments of Godlewski.³ He showed that protein synthesis from nitrates was very much greater in the light, even when photosynthesis was largely excluded by keeping the plants in a carbon-dioxid-free atmosphere, and that his wheat seedling grown under these conditions contained not only as much protein as was in the original seeds, but had formed a certain amount over and above this. Such was not the case with the seedlings grown in the dark. Evidently, then, light exercises a very direct influence on protein synthesis. These observations have led to much experimentation and extensive speculation in an endeavor to ascertain the mode of synthesis of the complex proteins.

Emmerling⁴ and others consider that amino-acids are the first products of nitrogen assimilation in the leaves. However, this part of the problem is still largely in the speculative stage and little direct experimental evidence is available. That leaves take up amino-acids and, in the presence of sugar, increase in protein-content was demonstrated in a qualitative manner by Hansteen.⁵

¹ ZALESKI, W. *Ber. deutsch. bot. Ges.*, 15, 536-542 (1897). PRIANISCHNIKOW, D. *Ibid.*, 17, 151 (1899).

² LAURENT, E., and M. MARCHAL. *Bull. de l'Acad. Roy. de Belgique*, 32, 55 (1903).

³ GODLEWSKI, E. *Bull. Acad. Science de Cracovie*, 313 (1903).

⁴ EMMERLING, E. *Landw. Versuch. Stat.*, 34, 113 (1880).

⁵ HANSTEEN, B. *Jahrb. wiss. Bot.*, 33, 417 (1899).

From the experiments of Saposchnikow¹ it appears that excised leaves rapidly take up asparagine and in the light show an increase in protein-content.

The increase of amino-acids in leaves kept in the dark offers the key to the interpretation of some observations of long standing which have never been adequately explained. In an excellent paper on the chemistry and physiology of foliage leaves, published by Brown and Morris² in 1893, determinations are reported of the increase of diastatic activity of leaves kept in the dark. Thus, for example, in experiments with *Hydrocharis morsus-ranæ* they found:

TABLE 53.

	Diastatic activity.	Increase in diastase.
	<i>p. ct.</i>	<i>p. ct.</i>
After full insolation.....	0.267	
In darkness 47 hours.....	0.476	78.2
In darkness 96 hours.....	0.676	153.1

The explanation given for this phenomenon by Brown and Morris is, in brief, that the protoplasm elaborates diastase according to the requirements of the leaf.

"As long as conditions are favorable for assimilation, the leaf-cells are supplied with an abundance of newly assimilated materials and so plentifully that the supply exceeds their powers of metabolism and translocation. The excess of nutritive material is in part at least deposited as starch. At this period there is little or no elaboration of diastase of the protoplasm, probably none at all in those cells in which starch deposition is in active progress. When the light fails, and assimilation falls off, the living cells speedily use up or translocate the excess of the soluble assimilative products, e. g., cane sugar, and begin to draw their supplies from the reserve of starch. To enable them to do this effectually, the somewhat starved protoplasm now commences to elaborate the needed diastase more rapidly, and this secretion becomes still more marked as the starvation point of the cell is neared."

It seems to us that this argument ascribes to protoplasm final causes beyond the justification of the experiments, in a manner already briefly considered in the introductory discussion of this paper. The fact that amino-acids increase in the leaves kept in darkness has been established repeatedly. The accelerating influence of amino-acids on the diastatic activity as determined by Sherman and others offers a more direct explanation of the periodic variations of diastase in leaves than the one originally advanced by Brown and Morris. Thus an increase in diastatic activity of leaves which had been kept in the dark would simply mean that the amino-acids in the leaves had increased and thereby produced conditions which are favorable to diastatic activity.

¹ SAPOSCHNIKOW, W. *Bol. Zentrbl.*, 63, 246 (1895).

² BROWN, H. T., and G. H. MORRIS. *Jour. Chem. Soc. London*, 63, 644 (1893).

8. *The Action of Amino-Acids on Sugars.*

In searching for a possible chemical explanation of the stimulating effect of amino-acids on the respiration of leaves, the action of amino acids on sugars deserves some consideration. In this regard the work of Maillard¹ has received considerable notice as offering the key to the amino-acid-carbohydrate and protein relation in living organisms. By the action of amino-acids on glycerine, Maillard obtained substances to which he ascribed the properties of polypeptides. Subsequently he studied the action of amino-acids on a variety of sugars and obtained the well-known reactions which lead to the formation of humin compounds. It is very questionable whether these reactions can find application to living organisms such as leaves. Maillard used very concentrated solutions, e. g., 4 grams of glucose in 3 to 4 c. c. of water and 1 gram of glycocoll. Moreover, the reaction proceeds only very slowly at ordinary temperature, so that his conditions can not be considered as having direct biological significance.

A solution of d-glucose, 5.6853 grams, in 100 c. c. water in a 2-dm. tube, gave a rotation $\alpha = +5.50$ at 10° . To 50 c. c. of this solution 0.2401 gram of glycocoll was added; the rotation of this solution was $\alpha = +5.47^\circ$. After 24 hours both solutions gave the same rotation. The d-glucose solution containing the glycocoll was heated for 4 hours at 90° with a reflux condenser. After making up carefully to volume, the rotation was practically unchanged, $\alpha = +5.52^\circ$.

The experiment was repeated with d-levulose. To a solution of 8.9675 grams d-levulose in 100 c. c. water was added 0.5002 gram glycocoll. This gave a rotation in a 2-dm. tube of $\alpha = -16.50^\circ$. After heating on a boiling water-bath for 5 hours the solution was very slightly yellow and gave a corresponding rotation of $\alpha = -16.40^\circ$.

It is therefore very doubtful whether the amino-acids in this dilution have any influence toward the mutual transformation of the hexoses such as is exerted by the weak alkalies. Nor does there seem to be, under the conditions of dilution and temperature employed in these experiments, any other profound action on the sugars.

The foregoing experiment with solutions of glycocoll and d-levulose were repeated, using instead of water the nitrogen-free mineral nutrient solution employed in the respiration. Also under these conditions the rotation remained unchanged in the solution kept at ordinary temperature as well as in one heated on the boiling water-bath for 2 hours. Similarly, only negative results were obtained in experiments with solutions of d-glucose and glycocoll to which was added the juice obtained by thoroughly grinding *Helianthus* leaves with quartz sand.

¹ MAILLARD, L. C. *Ann. de Chimie*, (9 série.), 5, 258-317 (1918); 2, 210-268 (1914). IVANOFF, N. N. *Biochem. Zeitschr.*, 120, 1-80 (1921).

II. THE INTERNAL FACTOR IN PHOTOSYNTHESIS.

INTRODUCTORY DISCUSSION.

It has been realized for some time that in the photosynthetic process taking place in chlorophyllous leaves there is an essential internal factor the nature of which has thus far not been discovered. That such a factor exists is concluded not only from the failure of all attempts which have been made to reproduce photosynthesis outside of the living cell, but recently also from direct experiments with living leaves. The existence of such a factor is especially noticeable under circumstances where the rate of the photosynthetic activity varies quite independently of external conditions. The nature of this factor and the seat of its activity have been given a large variety of purely hypothetical explanations.

In the course of investigations on certain phases of the problem of photosynthesis which have been in progress for a number of years, it was recognized that a better understanding of the internal factors affecting respiration was a prerequisite to a rational interpretation of photosynthesis. Considerable information on the nature of carbohydrate consumption in leaves has been gained and has aided materially in the experimental work on photosynthesis. It now seems highly probable that a solution of the problem of the internal factor in photosynthesis can be found in the intimate interrelation between photosynthesis and respiration.

Pantaneli¹ maintained that in photosynthesis the major role is to be ascribed to the protoplasmic function of the colorless components of the chloroplasts. Willstaetter and Stoll,² in their splendid and thorough investigation of the relation of the chlorophyll components to photosynthesis, attempt to determine whether the differences in the photosynthetic activity of a leaf can be explained by ascribing a dual function to a single chemical component of the chlorophyll. That this factor is not to be sought in the chlorophyll components or in such physical conditions as the degree of dispersion of the chlorophyll pigments follows from the great and irregular disproportionality which has been found to exist between the chlorophyll-content and photosynthetic activity. Willstaetter and Stoll are of the opinion that besides the chlorophyll there is another chemical agent essential to the photosynthetic process. The function of this agent they believe can be ascribed either to the general behavior of the plant protoplasm or, in attempting to enter more deeply into its chemical nature, it must be ascribed to a definite

¹ PANTANELI, E. *Jahrb. f. wiss. Bot.*, 39, 165 (1903).

² WILLSTAETTER, R., and A. STOLL. *Untersuchungen ueber die Assimilation der Kohlensaure*, 41-166 (1919).

component of the protoplasm. This component they assume to be a specific enzyme contained in the stroma.

So far as these conclusions go they are apparently in undeniable agreement with observational facts, for it is well known that even mild disturbances of the structure of the chlorophyllous cell result in inhibition of photosynthesis. Of great interest also is their observation that the internal factor is affected by temperature in much the same manner as most enzyme reactions.

In Willstaetter and Stoll's experiments on the effects of variations in temperature and light intensity with leaves rich and poor in chlorophyll, the disproportionality between chlorophyll-content and photosynthetic activity becomes clearly evident. Moreover, leaves poor in chlorophyll are more dependent upon variations in light intensity, while those rich in chlorophyll show greater variations with temperature. The two components of the photosynthetic apparatus thus operate in such a manner that the pigment reacts to variations in light intensity more in the case of the leaf with low chlorophyll-content than the one with high chlorophyll-content, while the internal factor reacts more directly to temperature and manifests itself to a larger degree in the leaves of high chlorophyll-content. This fact can be expressed in terms of limiting factors in such a way that in leaves poor in chlorophyll this component is the limiting factor, while in leaves rich in chlorophyll the internal factor is the limiting one, and complete utilization can not be made of the higher chlorophyll-content on account of the relatively low activity of the internal factor. } - ill

The most valuable experimental data on this subject are those pertaining to the temperature coefficients. Unfortunately, however, while there have been made a number of careful determinations of this nature, these represent a variety of rather isolated observations on different plant material, with varying methods, and the nutritional conditions of the plant have not been taken into consideration sufficiently. We are at present engaged in a more exhaustive study of the subject with mature leaves.

Warburg¹ found that the temperature coefficient with high light intensity and high carbon-dioxid concentration decreases with increasing temperature, so that at 5° it is 4.3 and at 32° it is 1.6. With low light intensity the temperature coefficient is about unity. These observations can be explained on the basis of Blackman's theory of limiting factors; but on careful consideration the question is also raised whether this theory is not due for a revision or extension, not as to its observational basis, but rather regarding the interpretation of the dynamics of the factors involved. Temperature relations

¹ WARBURG, O. *Biochem. Zeitschr.*, 100, 230-370 (1919).

such as were found by Warburg are not at all uncommon for physiological processes.¹ Becking² has recently published a theoretical discussion of the physical factors involved in the temperature coefficients of vital phenomena. Osterhaut and Haas,³ on the basis of determinations of the temperature coefficient of photosynthesis of *Ulva rigida*, conclude that this process involves two reactions—one a light reaction with a low temperature coefficient and an ordinary chemical reaction with a high Q_{10} .

A detailed discussion of temperature coefficients can not be entered upon here. Suffice it to say, however, that in a process such as photosynthesis there enters such a large number of factors that the question naturally arises whether the very small number of really concordant results is not due to chance or more probably to the choice of limited conditions to the exclusion of the broader aspect of the question. The many factors and steps which contribute to the complete process naturally produce a complicated situation, and determinations of temperature coefficients must represent the mean of a number of reactions. Thus, while the rate of photosynthesis is reduced with a decrease in temperature, the absorption of carbon dioxide at 25° is about half that at 5°.⁴

Recent investigations in the field of photochemistry emphasize the complex nature of these reactions, and it will require an enormous amount of experimental data before the kinetics of the photosynthetic process are made clear. Stoklasa's⁵ attempt to explain the internal factor in photosynthesis on the basis of the radioactivity of potassium, which is found in higher concentration near the chloroplasts, still requires much experimental evidence before it is beyond the realm of the purely hypothetical.

In the consideration of the relation between respiration and photosynthesis it is primarily the mature leaf which must be studied. The principal interest in photosynthesis centers about the leaf which is producing carbohydrate material above its immediate needs. For this reason also the behavior of germinating seeds or seedlings which have available reserve food material is of rather secondary importance in the study of photosynthesis.

The problem of photosynthesis is essentially one of energy transfer. Unfortunately, however, our knowledge of the energy relations in photosynthesis, as well as of plant respiration, is most rudimentary. The former is practically confined to the observations of Brown

¹ FAWCETT, H. S. University of California Publications in Agricultural Sciences, 4, No. 8, 217 (1921).

² BECKING, L. B. Dissertation, University of Utrecht (1921).

³ OSTERHAUT, W. J. V., and A. R. C. HAAS. *Jour. Gen. Physiol.*, 1, 295-298 (1919).

⁴ WILLSTAETTER, R., and A. STOLL. *l. c.*, p. 181.

⁵ STOKLASA, J. *Biochem. Zeitschr.*, 108, 159-184 (1920).

and Escombe¹ and of Puriewitsch;² thus far no measurements have been made which determine *directly* the amount of radiant energy used in photosynthesis. Such data as we now possess have been obtained from calculations based either on the amount of carbon dioxid absorbed (Brown and Escombe) or on the heat of combustion of the photosynthate (Puriewitsch). Fundamentally the measurements of Brown and Escombe and of Puriewitsch are based upon the same principle, i. e., taking the heat of combustion of the photosynthate or of glucose, or its equivalent in CO₂, as a measure of radiant energy utilized. The notable facts in Brown and Escombe's studies are that only a small proportion of the energy absorbed by the leaf is used in photosynthesis and that the amount actually used is a variable quantity.

Puriewitsch's figures exhibit similar variations in the percentage of radiant energy used in photosynthesis, ranging from 0.6 per cent to 7.7 per cent. In these experiments the intensity of illumination was high and normal air was used, so that the carbon dioxid was probably the limiting factor. Under such conditions it is to be expected that the percentage of radiant energy used in photosynthesis would vary inversely with the intensity of illumination. This, however, is not the case. He finds that the longer the period of illumination the lower is the percentage of radiant energy utilized in photosynthesis. Thus for *Polygonum sacchalinese* the time and percentage of energy used are as follows: 1 hr. 20 min. 7.7 per cent; 2 hrs. 20 min. 3.7 per cent; 5 hrs. 1.1 per cent and 2.5 per cent. Since the "time factor" in photosynthesis does not become evident under 25°, these variations can probably not be ascribed to it, and we must assume that as the plant accumulates carbohydrates less radiant energy is utilized. It can not be assumed, as Puriewitsch does, that in his experiments this decrease is due to the accumulation of the photosynthate, which would mean the interference by the well-known Saposchnikoff effect—in brief, an inhibition of the photosynthetic activity, probably due to the accumulation of carbohydrates.³ This effect seems to become apparent in land plants only after more prolonged exposure than in the experiments of Puriewitsch. Unfortunately, Puriewitsch gives no data as to the rate of respiration; but it is safe to assume, from the experiments of Matthaei⁴ and our own on the relation of carbohydrate-content

¹ BROWN, H. T., and F. ESCOMBE. *Proc. Roy. Soc. London*, B. 76, 29-111 (1905).

² PURIEWITSCH, K. *Jahrb. f. wiss. Bot.*, 53, 210-254 (1914).

³ SAPOSCHNIKOFF, W. *Ber. d. deut. bot. Ges.*, 11, 391-393 (1893).

EWART, A. J. *Jour. Linnean Soc.*, 30, 439-443 (1896); 31, 573 (1897); *Ann. of Bot.*, 11, 439-480 (1897).

⁴ MATTHAEI, G. *Phil. Trans. Roy. Soc. London*, B. 197, 50 (1904).

BORODIN, J. *Mem. de l'acad. imp. des Sciences de St. Petersburg* (Serie 7), 28, 4 (1881).

to respiration, that as photosynthesis proceeds the rate of respiration increases. It would appear, then, that the amount of radiant energy utilized in the photosynthetic process decreases as photosynthesis proceeds, and the plant's internal-energy release presumably increases, due to higher respiratory activity from the greater available carbohydrate-supply.

That a general relationship exists in leaves between the rate of carbohydrate synthesis and consumption has been recognized for a long time.¹ Boysen-Jensen² summarizes his studies as follows:

"In *Sinapis* the intensity of the CO₂ assimilation is very great, rising to at least 6 mg. CO₂ per 50 cm.² per hour at 20°. Also respiration in the leaves is great, about 8 mg. CO₂ per 50 cm.² per hour at 20°. The point of equilibrium between CO₂ assimilation and respiration lies at a light intensity of 1.0 (Bunsen units ×100). The development of a *Sinapis* plant is very weak. In 44 weeks the dry-matter content rises from 0.5 gram to 38 grams per 100 of plant. In favorable conditions the daily percentage production of dry matter can be estimated as about 15.

"In *Oxalis* the maximal intensity of CO₂ assimilation is very small, about 0.8 mg. CO₂ per cm.² per hour at 20°. Also respiration of the leaves is very small, about 0.1 to 0.2 mg. CO₂ per 50 cm.² per hour at 20°. The point of equilibrium between CO₂ assimilation and respiration lies at a light intensity of 0.2. The daily production of dry matter is at 2.1."

If an actual chemical or energetic relationship exists between the photosynthetic and respiratory activities of the leaf it should be expected that any disturbance in the respiratory activity would be reflected in photosynthesis. The observations on the effect of reduced oxygen pressure and of narcotics on photosynthesis are of special interest in this connection. Boussingault³ and Pringsheim⁴ investigated the effect of lack of oxygen on the rate of photosynthesis and concluded that it is inhibited thereby. Willstaetter and Stoll,⁵ from their extensive experiments decide that oxygen is absolutely necessary for the photosynthetic reaction, but that very small amounts of oxygen suffice. This small amount of oxygen, they claim, need not be present as free oxygen, but as loosely bound, "dissoziabel gebundener." Although their experiments were apparently carried out with great care, it is a question whether they succeeded in removing all the oxygen which is occluded in the leaves. This fact, however, stands out clearly in their experiments, that the more perfectly the oxygen has been removed the greater is the inhibition of the photosynthetic activity. Also, leaves which had been previously kept in the dark and their carbohydrate-content thus reduced showed no photosynthesis in an atmosphere freed from oxygen. From these experiments it appears that leaves with low

¹ ECKERSON, S. *Bot. Gaz.*, 48, 224-228 (1909).

² BOYSEN-JENSEN, P. *Botanisk Tidsskrift*, 36, 219-259 (1918).

³ BOUSSINGAULT, J. B. *Compt. rend.*, 61, 608 (1865).

⁴ PRINGSHEIM, N. *Sitzber. der Preuss. Akad. der Wiss.*, 763 (1887).

⁵ WILLSTAETTER, R., and A. STOLL. *l. c.*, 344-370.

carbohydrate-content, i. e., in which the respiratory energy release is very low, can stand the absence of oxygen less easily than can leaves with higher carbohydrate-content. Also, an anaerobic form of respiration, in which the course of the reaction is different and the energy of release quite low, does not suffice.

In harmony with the conception of the dependence of photosynthesis on respiration are the observations of the effects of anesthetics or narcotics on photosynthesis. Claude Bernard discovered the inhibiting effect on photosynthesis of chloroform, and this fact has been repeatedly confirmed.¹ In fact, photosynthesis is far more sensitive to the action of chloroform and ether than is respiration, so that amounts of these anesthetics, which hardly affect the rate of CO₂ emission, exert a decidedly inhibiting effect on photosynthesis, and with higher concentrations the capability for photosynthetic work is entirely lost. Willstaetter and Stoll² have shown that in leaves which have lost their photosynthetic power the four chlorophyll components are unchanged. Unfortunately, we have not been able to gather sufficient data on the nature of carbohydrate metabolism in leaves during narcosis to show definitely how the energy release is affected. It would be using very unreliable evidence to draw conclusions as to this point from the tropic and general movement phenomena or other responses of plants under the influence of narcotics.

The very interesting experiments of Pelster³ bear directly on the relation of photosynthesis and respiration. He found that while photosynthesis is much lower in the varieties of the same species containing little chlorophyll, there is no direct ratio between photosynthetic activity and chlorophyll-content. Furthermore, the light-green or *aurea* varieties, with low chlorophyll-content, also have a low respiratory activity as compared with the normal varieties. However, here also there is no direct relation between respiration and chlorophyll-content. But Pelster's results seem to show a relation between respiratory and photosynthetic activity. The quotient of the respiration and photosynthesis values of the light-green types are: *Ptelea* 84.1/47.5=1.77, *Catalpa* 58.8/34.2=1.72, *Mirabilis*=2.0, *Ulmus*=2.0, *Populus*=2.1, while *Atiflex* showed the very low quotient 1.3.

The findings of Pelster can be explained, of course, in a large measure also, on the ground that due to the low photosynthetic

¹ BERNARD, CL. Leçons sur les phénomènes de la vie, 278 (1878).

IRVING, A. A. *Ann. of Bot.*, 25, 1077-1099 (1911).

KEGEL, W. Inaug. Diss., Goettingen (1905).

EWART, A. J. *Jour. Linnean Soc.*, 31, 439 (1895).

BONNIER, G., and L. MAGIN. *Ann. d. Sci. Nat. Bot.*, (7), 3, 14 (1886).

² WILLSTAETTER, R., and A. STOLL. *l. c.*, 39.

³ PELSTER, W. *Beiträge zur Biologie der Pflanzen*, 11, 249-304 (1912).

activity the leaves have a relatively low supply of oxidizable material, resulting in correspondingly low respiration rates. Therefore, the experimental conditions in the investigations of Willstaetter and Stoll were destined to give more definite results.

In just what manner respiration can participate in the photosynthetic process it is as yet impossible to say with any degree of certainty. That the heat liberated in the process of plant respiration is to be regarded largely as an energetic waste product now seems highly probable.

If respiration is to be considered as a producer of energy for the maintenance of the so-called life processes, the potential energy of the food materials can not be converted entirely into heat, for if that were the case the heat of respiration could be substituted by heat applied from without. This, of course, is not the case. There must be produced in the course of respiration other forms of energy which are used in the metabolic processes of the plant. That such is the case was indicated in the old experiments of Bonnier,¹ who found that considerably less heat was evolved than could be expected from the respiratory coefficient. Further calorimetric studies of this kind would be highly desirable, carried out on the basis of knowledge obtainable regarding the transformation of the carbohydrates and other food material and with the application of modern temperature-measuring devices of high accuracy. While such calorimetric investigations would serve as excellent guides and checks, it is evident that they can tell us little of the more intricate details of the energy release in respiration.

Besides heat, then, it would appear that the plant has available and uses a considerable amount of energy derived from the breaking down and combustion of carbohydrates and other food material. Plant physiologists have widely accepted the dictum that this energy is converted into work by the plant. The nature of the work which is thus performed by the combustion of organic substances has been described in only vague and indefinite terms, although it is the basic problem of plant life. In the case of animals, where the factors of locomotion, balance, and other purely mechanical movements are involved, the work done is of a far more evident nature.² In the plant, however, it requires rather more searching perception to determine in what manner this release of energy in respiration takes form, for it is a strictly chemical phenomenon. The discussion here is limited to the energy liberated by the breakdown and oxidation of food material. The tensions and pressures related to turgor or the possible mechanical work involved in the activity of the so-called

¹ BONNIER, G. *Ann. Sci. Nat. Bot.*, (7), 18, 1 (1893).

SPOEHR, H. A. *Carnegie Inst. Wash. Pub.* No. 287, 21 (1919).

² OPPENHEIMER, C. *Der Mensch als Kraftmaschine. Die Naturwissenschaften*, 23, 64-72 (1920).

osmotic energy are, of course, of enormous importance in the life of the plant. Similarly, the phenomena dependent upon inhibition of water by the colloidal material and surface-tension energy are of great significance to the economy of the plant and are capable of many external manifestations. Nevertheless, their relation to the energy transformations of food material can be considered at most to be only an indirect one.

In general, photosynthesis and respiration bear an intimate association, not only on the basis of direct observation, but because oxidation and reduction actions in the living organism are intimately connected and apparently dependent upon the same or very closely allied agents. The two processes of photosynthesis and respiration, proceeding in opposite directions, may be related either on the basis that the energy released in respiration actually aids or is essential for one of the steps of the reduction process, or the relation may be based upon the action of an enzyme which functions in both reactions. Under no circumstances, of course, can all the energy for the reduction of the carbon-dioxid come from the oxidation of the carbohydrates. An extraneous source of energy is essential.

Thermodynamically, the contribution which respiration could make in the photosynthetic process would naturally be relatively small, so that this amount of energy could at best serve only as a partial source.

For a molecular relationship recourse must be taken to our modern conceptions of the nature of carbohydrate breakdown or glycolysis. According to this view, as has been elaborated in an earlier publication,¹ precursory to oxidation there must take place a cleavage or dissociation of the molecule. This action has as its result the formation of a very large number of enormously reactive substances. These pieces, the products of dissociation, either rearrange, react with each other, or react with some other substance present in the medium. Now, these pieces, on account of their enormous reactivity, can not be isolated. It is, in fact, only from the products formed by their reaction or rearrangement that they can be known. They are, nevertheless, of foremost importance in the chemical reactions involved in carbohydrate breakdown. A molecular relation of photosynthesis and respiration would depend upon the activity of these intermediary products of sugar catabolism. Just as these products serve as the building-blocks from which other compounds of higher potential energy may be formed in the cell, it is conceivable that they also may react with carbon dioxid or with some of the primary products of the photochemical breakdown of carbon dioxid.²

¹ SPOEHR, H. A. Carnegie Inst. Wash. Pub. No. 287, 5-24 (1919).

² SIEGFRIED, M., and S. HOWWJANZ. Zeit. f. Physiol. Chem, 59, 376 (1909).

One of the most remarkable and puzzling features of the carbohydrate synthesis in the plant is the fact that the sugars found in nature are confined to a small number of those which are theoretically possible on the basis of stereoisomeric constitution. A conception of the manner in which the plant-cell is able to synthesize carbohydrates from compounds which are already extant in the cell has been suggested by E. Fischer. This is based upon the fact that in the artificial synthesis of sugars from compounds containing a smaller number of carbon atoms, by means of the cyanhydrine reaction, the influence of existing stereoasymmetry of these carbon compounds is exerted on the final product. In other words, an asymmetric carbon compound yields an asymmetric product. In the same way, the reactive products of the primary carbohydrate dissociation would also be asymmetric substances, and these, by reaction roughly analogous to the cyanhydrine reaction, have the power of uniting with the products of photochemical decomposition of carbon dioxid and thereby yield an asymmetric product in the form of d-glucose, d-fructose, or its condensation product, sucrose. As these substances in turn are used for the formation of the large variety of other compounds found in plants, they would serve as the basis of the asymmetry of these.

The most noteworthy result of the energy release of respiration in plants is the formation of new compounds. These compounds may be of higher energy content, although they may not again serve the plant as food material, but enter only into the structural or plasmic elements of the organism. Or respiratory glycolysis may serve the plant in such a way that the primary products thereof are substances on to which carbon dioxid or its primary splitting product can be added to form a further supply of hexose sugars. Given, then, a properly functioning respiratory system, together with the necessary simpler materials and an apparatus capable of utilizing radiant energy, the plant is able to manufacture not only the large variety of substances found in its structural elements, but also to add organic material derived from the carbon dioxid of the air.

METHODS AND APPARATUS.

1. *The Experimental Material.*

The experiments on the photosynthetic activity were carried out with single excised leaves. For this work leaves of the Canada Wonder bean and of the sunflower were used. The methods of culture were the same as those employed for the material used in the respiration experiments and the same precautions were observed in regard to using only perfect leaves and in the method of cutting and handling the leaves. These always remained in perfectly

healthy condition during the course of the experiment. A considerable number of experiments was carried out; but as these in each case yielded consistent results, only one, representative of the altered conditions, is described here.

2. *The Apparatus.*

The method employed for determining the rate of photosynthetic activity was that based upon the differential determination of carbon dioxide. A stream of air containing a known concentration of carbon dioxide was passed over the leaf. The rate of carbon-dioxide emission from the leaf when this was in the dark was first determined; this gave the value for the rate of respiratory activity. The leaf was then illuminated and the amount of carbon dioxide in the gas-stream after it had passed over the leaf was determined.

FIGURE 20.

Arrangement of apparatus for study of the rates of photosynthesis. The entire apparatus is in a constant-temperature room. The leaf-container is in a water-thermostat, which is electrically heated, controlled, and stirred.

Thus the total amount of carbon dioxide fixed by the leaf could be calculated. The rate of the gas-stream was maintained absolutely constant throughout the course of the experiment. The entire apparatus was in a constant-temperature room which was thermostatically controlled. The leaf was in a container which was placed in an electrically controlled water-thermostat, usually at 24° ; the room was kept at 20° .

For these experiments a single excised sunflower or bean leaf was used. The same advantages and disadvantages of working with excised leaves for determining rates of respiration which have already

been discussed apply to the work on photosynthesis. With a single leaf the illumination in relation to the surface of the leaf can be more accurately controlled than when there is a larger number of leaves. In this way the leaf can at all times be kept at right angles to the source of light, and the shading by other leaves, or changing of the angle of incidence of the light, can be absolutely avoided.

The same precautions of culture, cutting, and handling of the leaves which were observed in the work on respiration, already described, apply to the material used for this work. The experience and knowledge gained from a study of the rates of respiration and the changes of material under varying conditions were naturally of great value in a study of the photosynthetic activity.

FIGURE 21.

Container for leaf used in photosynthesis experiments. *E* shows the elevation and a leaf through the glass plates with tube *N*, containing the nutrient solution. *S* is the cross section of the container. *T* is the transverse section showing the glass plates *G* and the mercury seal.

A diagram of the apparatus used in these experiments is shown in figure 20. As has been stated, the experiments were carried out in a dark-room which was thermostatically controlled and kept at 20°. The air was taken from a large gasometer. This was first partially filled with carbon dioxide in amounts as required. The carbon dioxide was prepared from white marble and hydrochloric acid and was washed through a solution of sodium carbonate and

one of potassium permanganate. The gasometer was then filled with air drawn from out of doors. Special experiments were carried out to insure that there was no stratification of carbon dioxide in the gasometer and that this gas was of the same concentration at the beginning and end of the experiment. The gasometer was sealed by means of heavy mineral oil.

By means of glass tubing, the gasometer was connected to a spiral of metal tubing which stood in the water of the thermostat, so that the air attained the temperature of the bath before entering the leaf-container. The connections made by means of heavy wall rubber tubing were wired and covered with several coats of Bakelite paint.

For these experiments a single leaf was placed in a chamber or cell of special construction. This consisted, essentially, of a metal frame, 15 by 25 cm., two sides of which were glass plates, 5 mm. apart. This leaf-cell is shown in figure 21, in elevation (*E*) in section (*S*) and in transverse section (*T*). The upper part of the metal frame carries a metal trough, similar to that of the respiration chamber, already described. This trough was also electroplated with copper and nickel and covered with lacquer, so that it could hold mercury. Into this trough fits a metal cover (*C* in fig. 21). Distilled water was poured over the surface of the mercury, and when the cover was placed in the trough a completely air-tight seal was made. Through the cover passes a metal tube by means of which the air-stream enters into the leaf-container. A narrow-bore tube through the lower part of one edge of the container provides the means for carrying off the air-stream. At the bottom of the leaf-container is a glass tube with a flared opening. Into this was placed the petiole of the leaf, which was supplied with nutrient solution through the tube *N*. This tube was filled with nutrient solution and sealed at the upper end with a piece of rubber tubing and a screw-clamp. During the course of an experiment, the level of the solution in which the petiole stood went down; this was adjusted from time to time by opening the screw-clamp at the upper end of the tube.

To the exit-tube of the leaf-container is attached a water-trap containing phosphorus pentoxide. The experiments were carried out in a saturated atmosphere. This was assured by filling the gasometer with moist air; moreover, the water over the mercury seal and the nutrition solution provided ample water-vapor. The tubes through which the air-stream passes after leaving the leaf-container are all of small bore, 2 mm. It was therefore essential to avoid the formation of water-drops by condensation in these tubes. This was accomplished by means of the water-trap filled with phosphorus pentoxide.

From the leaf-container the air-stream passed to the control-valve. This is shown in figure 22, and consists of a very fine needle-

valve which pierces just through a piece of lead *L*. By means of this valve the rate at which the air-stream passes through the apparatus could be very accurately regulated. The valve is attached to a small vessel containing a little phosphorus pentoxide.

From the control-valve the air-stream passes through narrow-bore glass tubes to the distributing tube. This consists of glass stopcocks by means of which the air-stream can be directed to the absorption tubes. Each stopcock is connected to a narrow glass tube slightly bent up at the end. Over these tubes are fitted the absorption tubes by means of a small rubber stopper. The air-stream can thus be directed from one absorption tube to another without interrupting the rate of flow.

The absorption tubes consist of 10-bulb glass tubes, the lower end of which is slightly bent. They are filled by means of a pipette, carefully graduated. For these experiments 68.12 c. c. of barium hydroxide was used for each determination. It was found that the bulb-tubes break up the air-bubbles more thoroughly and thus assure more complete absorption than the straight-walled Pettenkoffer tubes.

FIG. 22.—Needle control valve by means of which the rate of the air-stream passing through the apparatus is regulated. The valve is shown in section; a fine needle pierces the sheet of soft lead *L*, and is raised and lowered by means of a packed screw.

From the absorption tubes connection is made to a bottle which served to determine the rate of the gas-stream. This bottle is partly filled with water, over which is a layer of mineral oil. The glass tube from the absorption tubes extend to the bottom of this bottle. The rate of the gas-stream is determined by counting the gas-bubbles passing through the water in the bottle during a certain period. The time measurements were made with a stop-watch, and by means of the control-valve the rate of flow could be very accurately adjusted. A rate of 70 bubbles per minute represented a flow of 310 c.c. per hour. Care was taken to maintain constant the pressure in the aspirator. This was indicated by a manometer filled with aniline oil. Blank experiments showed that with the constant temperature of the room and in the water thermostat a very regular flow of air, and consequently of carbon dioxide, is obtainable and obviates the use of cumbersome and inaccurate gasometers.

The aspirator consists of an inverted galvanized-iron cylinder which is drawn out of a vessel of water. The cylinder is attached to a weight by means of a light wire cable. This passes over the rim

of a bicycle wheel which is suspended from the ceiling of the room. The wheel has excellent ball-bearings and is fastened, so that there is no play and very little friction. As the weight descends it lifts the cylinder out of the water, which causes the air-stream to be drawn through the entire apparatus. It is, however, necessary to make an adjustment for the increasing weight of the cylinder, as a larger portion of this comes out of the water. This is attained by increasing the pull on the other end of the cable through a supplementary weight placed on the wheel. When the cylinder is completely down this weight is at the top of the wheel, exerting no pull. As the cylinder is drawn out of the water, the wheel turns and the pull of the supplementary weight increases as the horizontal distance between it and the center of the wheel increases. Thus the increasing weight of the cylinder is adjusted; and while the two phenomena do not mathematically balance each other, the adjustment can be made sufficiently accurate so that no differences in the rate of the air-stream are noticeable.

The length of time for each observation was usually two hours. The amount of carbon dioxide fixed by a single leaf in this length of time is very small. It was therefore essential that the accuracy of the method of determining carbon dioxide be correspondingly high. For these experiments the same principle of carbon-dioxide determination was employed as in the respiration experiments. On account of the shorter periods and smaller amounts of carbon dioxide to be absorbed, a smaller range in the specific resistance curve of barium-hydroxide solution was used. Greater accuracy was attained by using an electrolytic cell of higher resistance. The original barium-hydroxide solution was 0.1001 normal, and in the absorption of the carbon dioxide this was reduced to about 0.086 normal. For each determination 68.12 c. c. of solution was introduced by means of a pipette

FIG. 23.—Electrolytic cell for determining the conductivity of the barium hydroxide solutions used to absorb the CO_2 in the photosynthesis experiments. The solution is protected from the air by a soda-lime tube.

into the absorbing tube, and the method of calculating the carbon dioxid for each period was essentially the same as in the respiration experiments. A curve of the specific resistance for concentrations of 0.1001 to 0.0860 normal, similar to the one used for the respiration work, was determined by means of the high-resistance cell, and was used for establishing the amount of carbon dioxid absorbed.

The barium-hydroxide solution, after absorption of the carbon dioxid, was transferred rapidly to narrow bottles; these were then stoppered and sealed with paraffine. After all the barium carbonate had settled out, the conductivity of the clear supernatant liquid was determined by means of the electrolytic cell shown in figure 23. This is essentially a pipette form of cell with a glass stopcock, and the solution is protected from the air by means of a soda-lime tube. The cell had a resistance of 1,950 ohms when filled with 0.1 normal potassium-chloride solution. For making the conductivity determinations, the cell was submerged slightly above the upper electrode in a water thermostat kept at 25°. A difference of 1 ohm in observed resistance represented about 0.00014 gram of carbon dioxid when using 68.12 c. c. of the barium-hydroxide solution. For the determination of the carbon dioxid in the different periods of a single experiment, differences of at least several ohms were observed, which gives an indication of the degree of accuracy of the method.

As a source of light there was used a 500 or 750 watt tungsten Mazda lamp. The distance of the filament from the leaf was 35 cm., and the light traversed about 8 cm. of water in the thermostat. The electric lamp was so placed that the light fell on the leaf at right angles. Immediately above and fitting over the top of the glass bulb of the lamp was a metal hood in the form of an inverted funnel. This hood was connected by means of a 5-cm. pipe to an electric suction fan. In this way the hot air surrounding the electric lamp was drawn off.

After each experiment the leaf was removed from the leaf-container and placed in a photographic printing-frame with blue-print paper. From the print thus produced the area of the leaf was determined by means of a planimeter.

EXPERIMENTAL RESULTS.

The problems of photosynthesis must be met in succession. It is questionable whether the ultimate or final causes can be discovered before a clearer understanding is gained of the workings of the immediate causes and relationships. The introduction by Blackman of the conception of limiting factors into the experimentation on this phenomenon, while it has contributed little to the ultimate causes involved, has aided very materially in the experimental investigation.

On the other hand, the many claims of simple and complete explanation of the process, based usually on very limited and inadequate experimentation, have done more to befog the issues than to clarify them. The factors of carbon-dioxid supply, light intensity, the quantity of chlorophyll, temperature, and water-supply have all received more or less exhaustive treatment. Practically all of these investigations have revealed the fact that there are other internal factors operative. The nature of these internal factors is unquestionably complex, though there is no reason for supposing that they are not amenable to physical and chemical investigation.

TABLE 54.—*Rates of respiration and synthesis of an excised bean leaf (Canada Wonder) kept in the dark 64 hours previous.*

In atmospheric air at the constant rate of the air-stream equal to 0.64 mg. CO₂ per 2-hour period. Observations made at 23.8°. Area of leaf₁ = 111.84 sq. cm. Light intensity 7,140 lux.

No.	Time.	Leaf exposed to—	Hrs.	Total hours.	Mg. CO ₂ absorbed.	Respiration, mg. CO ₂ emitted.	Photo-synthesis, mg. CO ₂ fixed.	Respiration per sq. cm. per hour in mg. CO ₂ .	Photo-synthesis per sq. cm. per hour in mg. CO ₂ .
Dec. 13:									
1	3 ^h 15 ^m p.m. to 5 ^h 15 ^m p.m.	Dark	2	66.00	1.34	0.70		0.0063	
2	5 ^h 30 ^m p.m. to 7 ^h 30 ^m p.m.	Do.	2	68.25	1.22	0.58		0.0051	
3	7 ^h 45 ^m p.m. to 9 ^h 45 ^m p.m.	Do.	2	70.50	1.12	0.48		0.0042	
4	10 p.m. to 12 n.	Do.	2	72.75	0.98	0.34		0.0033	
Dec. 14:									
5	12 ^h 15 ^m a.m. to 2 ^h 15 ^m a.m.	Do.	2	75.00	0.89	0.25		0.0022	
6	2 ^h 30 ^m a.m. to 4 ^h 30 ^m a.m.	Light	2	77.25	0.27		0.62		0.0054
7	4 ^h 45 ^m a.m. to 6 ^h 45 ^m a.m.	Do.	2	79.50	0.10		0.79		0.0072
8	7 a.m. to 9 a.m.	Do.	2	81.75	0.04		0.85		0.0076
9	9 ^h 20 ^m a.m. to 11 ^h 20 ^m a.m.	Do.	2	84.08	0.05		0.84		0.0076
10	11 ^h 40 ^m a.m. to 1 ^h 40 ^m p.m.	Dark	2	86.41	1.01	0.37		0.0034	
11	1 ^h 55 ^m p.m. to 3 ^h 55 ^m p.m.	Light	2	88.66	0.17		0.84		0.0076
12	4 ^h 05 ^m p.m. to 6 ^h 05 ^m p.m.	Do.	2	90.82	0.10		0.91		0.0080
13	6 ^h 20 ^m p.m. to 8 ^h 20 ^m p.m.	Do.	2	93.07	0.09		0.92		0.0082
14	8 ^h 35 ^m p.m. to 10 ^h 35 ^m p.m.	Do.	2	95.32	0.23		0.78		0.0072
15	10 ^h 45 ^m p.m. to 12 ^h 45 ^m a.m.	Dark	2	95.48	1.38	0.74		0.0066	

The course of carbon-dioxid emission by leaves as the supply of available carbohydrates diminishes has been described in the first section of this publication. The decrease in the rate of carbon-dioxid emission is a well-known feature of these conditions. However, the course of the photosynthetic process under these circumstances has received little attention.

1. In table 53 the results are given of the rates of respiration and subsequent photosynthesis of a bean leaf which had previously been kept in the dark for 64 hours. The carbohydrate supply had thus been very appreciably diminished and the decreasing rate of carbon-dioxid emission is evident. For the experiment atmospheric air was employed and throughout the experiment the temperature of the thermostat was kept at 23.8°.

After the leaf had been in the dark for 75 hours, the rate of respiration was 0.0022 mg. CO₂ per square centimeter per hour. During the subsequent 2 hours of photosynthesis the leaf fixed 0.0054 mg. CO₂ per square centimeter per hour. Thereafter the rate of photosynthesis rose, and after 86.41 hours the rate of respiration had also

FIGURE 24.

Rates of respiration and photosynthesis of an excised leaf of Canada Wonder bean kept in the dark 64 hours previously, as per data given in table 53. The values above the zero line indicate rates of photosynthesis; those below, the rates of respiration. The ordinates represent mg. CO₂ fixed and emitted per sq. cm. per hour × 10; the numbers of each square correspond to the periods as given in table 54.

risen to 0.0034 mg. CO₂ per square centimeter per hour. Apparently the carbohydrate-supply had been increased through the photosynthetic activity. During the next periods of exposure to light the photosynthetic rate continued to rise, and the final period of respiration shows a considerably higher rate. The photosynthetic rates are in each case calculated by subtraction from the last preceding respiration period. This undoubtedly accounts for the relatively low values of the last photosynthetic period (Nos. 9 and 14), for the leaf was at this time emitting carbon dioxide at a higher rate than during the last respiration period. If the last photosynthetic rates were calculated from the first succeeding respiration period, the rate of the former would result considerably higher. This fact

stands out and has been observed repeatedly, that under the conditions of the experiment, the rate of photosynthesis of a leaf, the store of carbohydrates of which has been greatly reduced, is initially low and rises with continued exposure to light. Thereby the respi-

TABLE 55.—*Rates of respiration and photosynthesis of leaves of Helianthus annuus at 24°.*

Taken after plant had been exposed to sunlight and after increasing periods of darkness. In atmospheric air, light intensity 7,140 lux.

[First leaf, 106.55 sq. cm.]

No.	Time.	Leaf exposed to—	Hrs.	Total hours.	Mg. CO ₂ absorbed.	Respiration, mg. CO ₂ emitted.	Photosynthesis mg. CO ₂ fixed.	Respiration per sq. cm. per hour in mg. CO ₂ .	Photosynthesis per sq. cm. per hour in mg. CO ₂ .
March 5.									
1	5 ^h 15 ^m p.m. to 7 ^h 15 ^m p.m.	Dark	2	2.00	1.82	1.180		0.0111	
2	7 ^h 27 ^m p.m. to 9 ^h 27 ^m p.m.	Do.	2	4.20	1.41	0.770		0.0073	
3	9 ^h 40 ^m p.m. to 11 ^h 40 ^m p.m.	Do.	2	6.40	1.39	0.750		0.0071	
4	11 ^h 55 ^m p.m. to 1 ^h 55 ^m a.m.	Light	2	8.65	0.26		1.130		0.0107
5	2 ^h 05 ^m a.m. to 4 ^h 05 ^m a.m.	Do.	2	11.15	0.24		1.150		0.0109
6	4 ^h 15 ^m a.m. to 6 ^h 15 ^m a.m.	Dark	2	13.31	1.27	0.63		0.0059	
7	6 ^h 30 ^m a.m. to 8 ^h 30 ^m a.m.	Do.	2	15.56	1.22	0.58		0.0054	

[Second leaf, 93.97 sq. cm.]

March 6.									
8	7 ^h 30 ^m p.m. to 9 ^h 30 ^m p.m.	Dark	2	28.56	1.02	0.38		0.0040	
9	9 ^h 45 ^m p.m. to 11 ^h 45 ^m p.m.	Do.	2	30.81	1.03	0.39		0.0041	
10	11 ^h 55 ^m p.m. to 1 ^h 55 ^m a.m.	Light	2	32.97	0.27		0.76		0.0080
11	2 ^h 05 ^m a.m. to 4 ^h 05 ^m a.m.	Do.	2	35.13	0.23		0.80		0.0085
12	4 ^h 15 ^m a.m. to 6 ^h 15 ^m a.m.	Dark	2	37.29	0.92	0.28		0.0029	
13	6 ^h 25 ^m a.m. to 8 ^h 25 ^m a.m.	Do.	2	39.45	0.90	0.26		0.0028	

[Third leaf, 98.29 sq. cm.]

March 10.									
14	4 ^h 05 ^m p.m. to 6 ^h 05 ^m p.m.	Dark	2	119.11	1.07	0.43		0.0043	
15	6 ^h 15 ^m p.m. to 8 ^h 15 ^m p.m.	Do.	2	121.27	1.06	0.43		0.0042	
16	8 ^h 25 ^m p.m. to 10 ^h 25 ^m p.m.	Light	2	123.43	0.35		0.71		0.0071
17	10 ^h 35 ^m p.m. to 12 ^h 35 ^m a.m.	Do.	2	125.59	0.43		0.63		0.0064

[Fourth leaf, 91.91 sq. cm.]

March 12.									
18	8 ^h 10 ^m a.m. to 10 ^h 10 ^m a.m.	Dark	2	179.17	0.82	0.18		0.0019	
19	10 ^h 25 ^m a.m. to 12 ^h 25 ^m p.m.	Light	2	181.42	0.52		0.30		0.0032
20	12 ^h 35 ^m p.m. to 2 ^h 35 ^m p.m.	Do.	2	183.58	0.79		0.03		0.0003
21	2 ^h 45 ^m p.m. to 4 ^h 45 ^m p.m.	Dark	2	185.74	1.77	1.13		0.0159	

ration rates also show an increased rate and subsequent periods of photosynthesis exhibit correspondingly higher rates. The results of this experiment are shown graphically in figure 24.

2. The following experiment was carried out in a different manner. A mature leaf was cut from a large, healthy sunflower plant which

had been exposed to bright sunlight in the greenhouse during the two previous days. The leaf was taken in the afternoon and placed immediately in the apparatus for determination of the respiration and photosynthesis rates. The entire plant was covered with a large hood of black paper, so as to exclude all light without altering other conditions. On succeeding days leaves were cut from this

FIGURE 25.

Rates of respiration and photosynthesis of leaves of *Helianthus annuus* at 24°, after increasing periods of darkness. The values above the zero line indicate rates of photosynthesis, those below, rates of respiration, expressed on the ordinates in mg. CO₂ fixed, or emitted, per sq. cm. per hour × 10. Data taken from table 55.

plant and their respiration and photosynthesis rates determined. In this manner leaves were used which had been attached to the plant during the period of starvation and their store of carbohydrates was not depleted as rapidly as when they were cut from the plant. Moreover, the temperature of the greenhouse in which the covered plant stood did not rise above 22°, while the determinations were carried out at 24°. For these reasons some of the leaves at first

show a slightly higher initial rate of respiration than the ones which had been cut and placed in the apparatus in the preceding period. Thus the first leaf was taken after an entire day of insolation, and the succeeding ones after 26.56, 117.11, and 177.17 hours of darkness. The results are given in table 55.

From the graph (fig. 25) in which these determinations are plotted, it is evident that the leaves which had been kept in the dark show a continual decrease in respiratory activity. Moreover, the amount of carbon dioxid fixed also shows a decline with decreasing respiration. In the last two periods of the fourth leaf there are signs of internal

TABLE 56.—*Rates of respiration and photosynthesis on three successive days of an excised leaf of Helianthus annuus kept in the dark.*

CO₂ concentration was nine times that of atmospheric air; temperature 24°; light 7,140 lux; area of leaf, 86.43 sq. cm. The air-stream equals 5.30 mg. CO₂ per hour.

No.	Leaf exposed to—	Time	Hrs.	Total hours.	Mg. CO ₂ absorbed.	Respiration, mg. CO ₂ emitted per hour.	Photosynthesis, mg. CO ₂ fixed per hour.	Respiration per sq. cm. per hour in mg. CO ₂ .	Photosynthesis per sq. cm. per hour in mg. CO ₂ .
March 17.									
1	Dark	1 ^h 45 ^m p.m. to 3 ^h 45 ^m p.m..	2	2.000	20.76	5.08		0.0587	
2	Light	3 ^h 50 ^m p.m. to 5 ^h 50 ^m p.m..	2	4.082	11.16		4.80		0.0554
3	Dark	5 ^h 55 ^m p.m. to 7 ^h 55 ^m p.m..	2	6.166	20.61	5.00		0.0578	
March 18.									
4	Dark	9 ^h 05 ^m a.m. to 11 ^h 05 ^m a.m..	2	21.533	19.18	4.60		0.0531	
5	Light	11 ^h 10 ^m a.m. to 1 ^h 10 ^m p.m..	2	23.616	13.31		3.25		0.0376
6	Dark	1 ^h 15 ^m p.m. to 3 ^h 15 ^m p.m..	2	25.700	20.21	4.81		0.0556	
March 19.									
7	Dark	3 ^h 15 ^m p.m. to 5 ^h 15 ^m p.m..	2	49.700	16.61	3.01		0.0348	
8	Light	5 ^h 20 ^m p.m. to 7 ^h 20 ^m p.m..	2	51.783	15.86		0.38		0.0044
9	Dark	7 ^h 25 ^m p.m. to 9 ^h 25 ^m p.m..	2	53.866	16.26	2.83		0.0327	

disturbance. The leaf had a slightly mottled appearance and was curled in on the edges, so that the very high rate of carbon-dioxid emission in the final period, No. 21, was probably due to protoplasmic disturbances. In two subsequent 2-hour periods the rate of carbon-dioxid emission gave even slightly higher values and the leaf showed unmistakable signs of injury, so that these results are not included. In the experiment described under 1, the longer periods of illumination are followed by an increase in respiratory activity. It is apparent that in the present experiment, however, there apparently has not been sufficient carbohydrate synthesis during the periods of illumination to produce an increase in respiration when the leaf is again put in the dark.

3. In the two preceding experiments the concentration of carbon dioxid was relatively low, that of atmospheric air. In the following experiment this was increased ninefold. The leaf was cut from the

plant after the latter had been exposed to the sunlight in the greenhouse for 7 hours. The respiration and photosynthesis rates were then determined. The leaf remained in the frame in the dark with a constant air-stream passing over it. After about 24 hours the respiration and photosynthesis rates were again determined, and likewise after about 48 hours. The results of this experiment are given in table 56 and in figure 26.

FIGURE 26.

Rates of photosynthesis and respiration, on three successive days, of a leaf of *Helianthus annuus* kept in the dark. The values above the zero line indicate rates of photosynthesis, those below, rates of respiration, expressed on the ordinates in mg. CO₂ fixed, or emitted, per sq. cm. per hour. Data and conditions as per table 56.

In this experiment, in which the carbon-dioxid content of the air was greatly increased, the rate of photosynthesis shows, as in the previous experiments, a course which in general parallels that of the respiratory activity of the leaf. In all of these experiments the same source and intensity of illumination was employed.

4. The carbon-dioxid content of the air-stream was increased further, so that it was 15.5 times that of atmospheric air. In this experiment a sunflower leaf was also used. It is doubtful whether with the intensity of light employed the carbon dioxid was the limiting factor, because the photosynthesis per square centimeter per hour in this experiment is slightly less than in the previous one, in which the carbon-dioxid concentration was almost half of that used in the present experiment. In table 57 and figure 27 the results of this experiment are given. The general decline of the rate of photosynthesis, with decreasing respiratory activity, also holds for these conditions. The photosynthetic rates are calculated from the last

preceding respiration rate. For this reason the last photosynthesis period, No. 7, appears low. Calculated on the basis of the next following respiration rate, which represents more nearly the true condition, the photosynthesis rate would be 0.0523 instead of 0.0413 mg. CO₂ per square centimeter per hour.

TABLE 57.—*Rates of respiration and photosynthesis of a leaf of Helianthus annuus.*

The CO₂ concentration was 15.5 times that of atmospheric air, temperature 24°; light 1,740 lux; area of leaf, 120.81 sq. cm. The air-stream equals 9.98 mg. CO₂ per hour.

No.	Leaf exposed to—	Time.	Hrs.	Total hours.	Mg. CO ₂ absorbed.	Mg. CO ₂ absorbed per hour.	Respiration, mg. CO ₂ emitted.	Photosynthesis, mg. CO ₂ fixed.	Respiration per sq. cm. per hour in mg. CO ₂ .	Photosynthesis per sq. cm. per hour in mg. CO ₂ .
March 30.										
1	Dark	4 p.m. to 5 ^h 30 ^m p.m.....	1.5	1.50	18.66	12.44	2.46		0.0204	
2	Light	5 ^h 45 ^m p.m. to 7 ^h 15 ^m p.m..	1.5	3.00	8.81	5.87		6.57		0.0543
3	Dark	7 ^h 30 ^m p.m. to 9 p.m.....	1.5	4.50	18.66	12.44	2.46		0.0204	
March 31.										
4	Dark	10 ^h 40 ^m a.m. to 12 ^h 10 ^m p.m.	1.5	19.66	16.91	11.27	1.29		0.0106	
5	Light	12 ^h 25 ^m p.m. to 1 ^h 55 ^m p.m.	1.5	21.16	9.56	6.57		4.90		0.0405
6	Light	1 ^h 55 ^m p.m. to 3 ^h 25 ^m p.m..	1.5	22.66	9.21	6.14		5.13		0.0424
7	Light	3 ^h 25 ^m p.m. to 5 ^h 25 ^m p.m..	2.0	24.66	12.56	6.28		4.99		0.0413
8	Dark	5 ^h 35 ^m p.m. to 7 ^h 35 ^m p.m..	2.0	26.66	22.81	11.40	1.32		0.0109	

In the experiments described under 2, 3, and 4, the decrease in photosynthetic activity occurs after the leaves had remained in the dark for more or less prolonged periods. The possibility naturally suggests itself that the reduction might be due to a decrease in the chlorophyll of the leaves, caused by keeping the plants in the dark. On the basis of the investigations of Willstaetter and Stoll,¹ it is highly improbable that this is the case. They kept leaves in the dark at 34° to 37° for 48 hours, and although, toward the end of this period, there were evidences of protoplasmic disturbances (abnormally high CO₂ evolution), the determinations of the chlorophyll components at the end of the period gave the same results as normal leaves.

It is doubtful whether there exists between the respiratory and photosynthetic activities anything like a fixed or quantitative ratio. The quotients determined by Pelster, after all, represent rather confined conditions. Similarly, we found that with atmospheric air the quotients are about of the same magnitude as those found by Pelster. With increasing carbon-dioxid content, however, the quantitative relation of these values changes, although the relative magnitude seems to indicate an interrelationship. Moreover, this interrelationship appears to be of a more intricate nature than if it were

¹ WILLSTAETTER, R., and A. STOLL. Untersuchungen ueber die Assimilation der Kohlensaure, p. 35.

simply a case of high photosynthesis supplying carbohydrates in such large amounts that the leaf is at all times well supplied and thus burns this sugar at a correspondingly high rate.

The experiments on the relation of respiration and photosynthesis are being extended, especially with a view of determining the temperature coefficients under a variety of conditions. There is considerable evidence for believing that the photosynthetic process is of a dual

FIGURE 27.

Rates of photosynthesis and respiration, on successive days, of a leaf of *Helianthus annuus*. The values above the zero line indicate rates of photosynthesis, those below, rates of respiration, expressed on the ordinates in mg. CO₂ fixed, or emitted, per sq. cm. per hour. Data and conditions as per table 57.

nature or even more complex, involving the general principle of coupled reactions. That one of these, or one group of these, reactions is photochemical there seems to be little doubt. The nature of the other reaction (Willstaetter's enzymatic reaction), or that associated with the respiratory activity, is still very obscure. In what manner these two sets of reactions complement each other or how they are coupled is one of the most vital points of the photosynthesis problem. Some speculations hereon were given in the introductory discussion of this section. However, at this stage of development the only method which promises any real advance is the experimental one. In view of the importance and the insight which can be gained from the interpretation of temperature coefficients on the basis of recent physical-chemical investigations, an extension of these determinations seems highly desirable and offers another means of analyzing the phenomenon of photosynthesis.