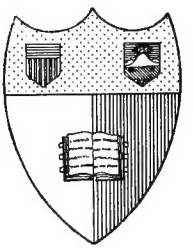


CARBON ASSIMILATION

A REVIEW OF RECENT WORK ON
THE PIGMENTS OF THE GREEN LEAF AND
THE PROCESSES CONNECTED WITH THEM.

BY

INGVAR JÖRGENSEN,

AND

WALTER STILES.

NEW PHYTOLOGIST REPRINT, No. 10.

PRICE - FOUR SHILLINGS (NET). POSTAGE - 4D.

LONDON:
WILLIAM WESLEY & SON,
28, ESSEX STREET, STRAND.
1917.

CARBON ASSIMILATION

A REVIEW OF RECENT WORK ON THE PIGMENTS OF THE GREEN LEAF AND THE PROCESSES CONNECTED WITH THEM.

BY

INGVAR JÖRGENSEN

AND

WALTER STILES.

NEW PHYTOLOGIST REPRINT No. 10.

LONDON:
WILLIAM WESLEY & SON,
28, ESSEX STREET, STRAND.

"There is not a fact or a law in the whole of science which is in its finished form. Everywhere is growth and change."

W. H. Bragg.

PREFACE.

IN these pages we attempt to give a brief account of our present knowledge of carbon assimilation. For this purpose we deal mainly with work done during the last twenty years, and we do not aim at any complete historical account of the development of the various aspects of the subject. Rather, as far as possible we select representative pieces of work and attempt to arrange the facts brought out in these investigations so as to give a clear conception of the present position of the various problems.

It may be that this review will appear to the reader to be very critical. In this case we hope it will not be concluded that the writers fail to appreciate the efforts of the many contributors to the subject, both along its main lines and its side channels. Our desire is to emphasize those facts which will help towards the realisation of the idea that the work done on carbon assimilation justifies the position of plant physiology among the pure sciences. It is to be hoped that this realisation will stimulate plant physiologists to attempt a similar development of our knowledge of other phases of the life of the plant.

A recent botanical writer has said that the function of pure science is to pursue *useful* knowledge; the duty of the leaders of science to direct the pursuit along what appear to them the most promising lines.

This proposition we regard as thoroughly unsound. It is impossible, for instance, in plant physiology for anyone to foretell which particular plant process is the most promising one to investigate in order to obtain results of utilitarian value. It is true indeed that the economic importance of a pure science depends upon the utilisation of the principles evolved by it. Thus the ever-growing importance of plant physiology is due to the fact that the principles brought out by it are utilised in plant cultivation. But the growth and development of a pure science cannot be controlled by the applied science dependent upon it.

Although the principles of carbon assimilation exposed in this review may still appear somewhat vague, yet they have sufficient definiteness to indicate that their application in agriculture will open up new possibilities of development in that field. For this reason we hope that the following pages will be of interest to those concerned in the development of scientific agriculture, as well as to those interested in plant physiology for its own sake.

I.J.
W.S

LONDON,
April, 1917.

ERRATA.

p. 6, lines 32, 36, for "benzol" read "benzene"

p. 11, line 8 of footnote, for "already referred to" read "referred to overleaf"

p. 16, scheme, for "phæophytin" read "phæophytin a"

p. 20, line 5, delete "in petrol ether"

p. 49, line 16, for "1902" read "1899"

p. 70, legend of Fig. 7, for "Krogh" read "Ege and Krogh"

p. 113, lines 12, 13, delete "glucose resulting from the hydrolysis of"

CONTENTS.

CARBON ASSIMILATION.

A Review of Recent Work on the Pigments of the Green Leaf and the Processes connected with them.

Chapter I.

Introduction.

THE elements contained in the plant are such as are always present in ample quantity in air or soil, but in the plant they exist in compounds possessing greater energy than the simpler substances in the surroundings.

The fundamental characteristic of the green plant is that during its life it produces these compounds of greater energy, the energy required for this being obtained from the radiant energy of the sun.

One of the chief elements thus built up into the plant is carbon, which is provided by the carbon dioxide of the air. It is in this sense, and this only, that we use the term carbon assimilation.

We consider the use of this rather non-committal expression preferable to that of such terms as photosynthesis, photolysis of carbon dioxide, etc., which suggest definite theories of the nature of the processes.

The main feature of carbon assimilation is the photochemical reaction or reactions in which, by means of the green pigment of the leaf, radiant energy is transformed into chemical energy.

A discussion of photochemical reactions is, however, outside the scope of this review because, unfortunately, no information is available from plant physiological investigations which throws any light on the particular photochemical reaction which is the essential feature of carbon assimilation.

The study of photochemical reactions forms an important branch of physical chemistry at present undergoing rapid development, and one which no doubt deserves more attention from plant physiologists than it has so far received, not only in connection with carbon assimilation but also in regard to many other processes,

e.g., those concerned in growth and irritability. However, the subject is likely to prove an extremely difficult one in respect to plant physiological problems, more especially because it is so difficult in a complex series of reactions to distinguish between purely photochemical reactions and the chemical actions coupled with them.

Consequently the usual characteristic of a photochemical reaction of a temperature coefficient close to unity is one of which cautious use must be made in drawing conclusions as to reactions in the living organism where a complex of physical and chemical processes may between them produce any possible temperature coefficient. Yet the fairly high temperature coefficient, between 2 and 3, obtained by F. F Blackman for carbon assimilation is fairly clear evidence of the complexity of the processes of carbon assimilation, as it indicates that the photochemical process must be coupled with actions of another kind.

Similar difficulties present themselves when we attempt to analyse the energy relations of the green leaf. The radiant energy absorbed by the leaf is partly transformed into heat energy which results in raising the temperature of the leaf, and partly into chemical energy, but it is extremely difficult to distinguish between the amount of energy transformed into heat and that transformed into chemical energy.

Yet this transference of radiant energy into chemical energy is of immense importance in the world, for upon it all life depends. Without it of course the organic world as we know it would be non-existent. Also our present well-developed industrial civilisation can be directly traced to this transformation of radiant energy into chemical energy ages ago. It is of course owing to the sun's energy stored in coal, the result of carbon assimilation in past periods, that industrial development occupies its present position. It has been well shown by A. H. Gibson (1913) how comparatively inadequate are other natural sources of energy to replace that of coal when the world's coal supply becomes exhausted, and it becomes evident that some means of utilising the sun's energy such as the plant is able to do, will have to be developed if our civilisation is to continue. Whether this can be secured by growing plants which produce material of high calorific value, or whether the energy of sunlight can be better stored directly by some photochemical reaction, cannot even be guessed at present.

Although it has been recognised for a long time that this essential transformation of radiant energy into chemical energy was accomplished by means of a pigment, yet even the recent brilliant researches on the chemistry of the leaf pigments has not so far helped in the elucidation of the problem. Nevertheless it is clear that a good knowledge of the pigments concerned is absolutely necessary in order that an attack may be made on the problems of carbon assimilation.

Further scope for organic chemistry occurs in regard to the products of the assimilatory processes; those substances at some stage in the formation of which the transference of radiant energy to chemical energy takes place. This seems likely to be a more difficult problem even than the determination of the chemical nature of the pigments. Apart from the inherent difficulties attending all work on a group of substances of similar properties such as the carbohydrates, the problem is further complicated by the possibility of intermediate substances between the crude materials and the final product, and also of degradation products of this latter preparatory to translocation away from the leaf. Again there is the possibility of "multiple carbon-assimilation," *i.e.* the formation of not one but several final products in the assimilatory processes.

Room there is undoubtedly for an enormous amount of organic chemical work on these problems, but it must never be forgotten that we cannot expect, nor perhaps should we even aim at getting the processes of the plant pictured by means of simple reactions. For this reason we think that the most promising aspects of carbon assimilation are not those made evident in the contributions of organic chemistry, necessary and valuable as they are, but in those investigations which constitute a struggle towards a clear cut conception of the main problems involved; no easy matter when these are obscured by a mass of secondary issues due to the complexity of the setting provided by organism and environment.

The investigations to which we refer are those in which an analysis of the various factors concerned in carbon assimilation has been attempted. It was indeed recognised long ago that there were various environmental factors which influenced carbon assimilation, but it was due to F. F. Blackman that the effect of each of these factors and the manner of their interaction was systematically investigated by a careful control of the possible external factors. This same line of investigation has been con-

tinued by Willstätter in regard to the internal factors with results which are full of suggestion for future investigators.

By analysis of this kind it seems most likely that we shall attain correct ideas of what has been called the mechanism of carbon assimilation, rather than by the more favourite and much easier method of conducting chemical experiments in support of various theories for which evidence derived from the facts of assimilation gives insufficient support.

Chapter II.

The Pigments of the Leaf.

A. General Remarks.

It is necessary to review the present knowledge of pigments in the leaf before discussing the actions taking place there, more particularly as this knowledge has been considerably increased during recent years by the researches of Willstätter and his co-workers, and as the results of these researches do not appear as yet to have penetrated very deeply into the botanical world.

Willstätter's researches have extended over a period of more than ten years, during which time he has been assisted by many expert chemists, working under conditions which have enabled them to conduct experiments on a properly large scale. The result is that the chemistry of chlorophyll has been made at least as clear as that of any other plant substance, and there is every reason to hope that in applying the experience of Willstätter and his colleagues to experiments in plant physiology, great progress will be made.

Besides working out the chemistry of the leaf pigments and isolating them, Willstätter has made a large number of analyses of pigments from various species of different families, from plants growing under different ecological conditions, and from plants collected at different seasons and at different times of day.

The main facts derived from Willstätter's researches are that the chloroplasts contain four pigments, two green and two yellow. These are:—

1. Chlorophyll component a, $C_{55}H_{72}O_5N_4$ Mg, blue black in the solid state, green blue in solution.
2. Chlorophyll component b, $C_{55}H_{70}O_6N_4Mg$, green black in the solid state, pure green in solution.
3. Carotin, forming orange red crystals of the composition $C_{40}H_{56}$.
4. Xanthophyll, forming yellow crystals of the composition $C_{40}H_{56}O_2$.

It was found that these pigments were identical in all plants examined. The chlorophyll always contained 2·7% of magnesium, which is the only metal present in its ash. Neither iron nor phosphorus is present.

In fresh leaves these four pigments were found in about the following quantities :—

Chlorophyll a	...	...	2 parts per 1000
„ b	...	...	$\frac{3}{4}$ „ „ 1000
Carotin ...	...	...	$\frac{1}{6}$ „ „ 1000
Xanthophyll	...	...	$\frac{1}{3}$ „ „ 1000

In the chloroplasts these pigments are also mixed with various colourless substances; fats, waxes and salts of fatty acids. Thus in an alcoholic extract of dried leaves chlorophyll is accompanied by about six times its weight of other substances.

Willstätter has worked out methods for freeing chlorophyll extracts from these accompanying colourless substances, and also methods for isolating each of the four pigments. In all that follows, when we speak of chlorophyll we refer to the green pigments freed from the yellow ones.

In thus being able to obtain pure pigments a very great advance is made. All the earlier experiments on the reactions taking place in the green leaf were made with extracts, such as alcoholic extracts of leaves, which contained many substances besides the pigments.

Yet a further complication becomes obvious from Willstätter's investigations, and this is also a fact which has not yet received its due attention in physiological researches. Moreover it is a fact not only important in this branch of plant physiology but in all cases where plant substances are extracted and purified. This is that by the methods of extraction the state of matter in which the substance generally exists may be altered. For this reason it may become difficult to draw conclusions from the behaviour of the extracted substance as to the function of the substance in its natural condition in the plant.

From Willstätter's researches it is clear that solvents which dissolve the pure extracted substance do not extract the substance from the dried leaf. For instance, the pure pigment is readily soluble in acetone, ether and benzol. If the dried powder of nettle leaves is placed in pure acetone it can remain there for half an hour without the acetone becoming at all coloured. But if a little water is added the colour immediately becomes intensely green. Neither ether nor benzol becomes coloured quickly when powdered nettle leaf is added. Yet both are immediately coloured strongly green when a few drops of water are added. This behaviour of chlorophyll suggests that chlorophyll in the leaf is in a different state of matter from extracted chlorophyll.

The extracted pigment is soluble in petrol-ether as long as it

is mixed with accompanying oils, waxes, etc., but petrol-ether does not extract at all from dried leaves, so it is feasible to suppose that the chlorophyll in the chloroplast is in the colloidal condition, that water added to the pure organic solvents dissolves the mineral substances in the leaf, and the salt solution so formed alters the colloidal condition of chlorophyll in the chloroplast and makes it easily soluble.

In this connection it should be pointed out that the pure organic solvents can extract the chlorophyll from fresh leaves, as there is of course, abundant water present in them.

Support for this assumption is given by the fact that colloidal solutions of chlorophyll in water made up from the pure extracted pigment behave in a similar way to the dried leaf powder. Thus if a colloidal solution of chlorophyll is mixed with ether, the ether remains colourless, but if a little salt solution, for instance, a solution of calcium chloride or calcium nitrate, is added, on shaking the ethereal layer becomes coloured green. The salt solution has precipitated some of the chlorophyll from its colloidal condition and it is now easily soluble in ether.

In regard to the actual state of chlorophyll in the leaf there has been some difference of opinion. Arnaud (1885) supposed that capillary forces kept the chlorophyll back in the leaf, and Willstätter himself at one time assumed that chlorophyll in the leaf was present in the form of adsorption compounds with colloids. Similarly Tswett (1901) held that the pigment was bound to the skeleton of the chloroplast by molecular adsorption. Recently Palladin (1910a, 1910b) suggested that the chlorophyll is present in the leaf in a state of chemical combination, particularly with the so-called lipoid substances, and he shows that the use of solvents for extraction could be explained by their dissociating power in regard to the adsorption compound.

Willstätter's present opinion is that the chlorophyll in the chloroplast is present in a colloidal mixture, and there appears to be a good deal of experimental evidence in support of this view, even if it may have to be slightly modified when we have more experimental knowledge of the kinetics of the physiological processes involved.

We may mention briefly some other reasons for the assumption that chlorophyll is present in the colloidal condition. There is first evidence derived from spectroscopic examination. According to Tswett (1910) and other writers the absorption bands in the spectrum of the living leaf are displaced towards the red end of the spectrum as compared with the bands in the spectrum of extracted chloro-

phyll. Herlitzka (1912) has shown that the spectrum of living leaves agrees with that of colloidal chlorophyll solutions while both differ in the same way from the spectrum of true chlorophyll solutions. Willstätter's own experiments confirm the observations of Herlitzka. He made measurements of the bands in the spectrum of leaves of different plants and found them to occupy the same positions as the absorption bands of the spectrum of colloidal solutions of pure chlorophyll a.

Again the condition of the chlorophyll in fresh leaves is altered if the leaves are plunged in boiling water. After such treatment the chlorophyll is much more easily extracted. Microscopic examination shows that the chloroplasts are deformed as a result of such treatment, they are displaced from the normal position in the cell and diffusion out from them of chlorophyll follows almost immediately. Externally the leaves change in colour to a deep green. Spectroscopically this change in colour is shown to be accompanied by a displacement of the absorption bands towards the violet end of the spectrum so that they occupy practically the same position as those in the spectrum of a chlorophyll extract. This is explained at once on the view that the chlorophyll has changed from a colloidal to a true solution and is now dissolved in waxy substances which have become liquid as a result of the alteration of temperature. As would be expected, pure acetone and ether easily extract the pigment from a powder made from leaves previously steeped in boiling water.

It is worth mentioning that if fresh nettle leaves are treated with acetone or other solvents, and are then examined spectroscopically when they have become deep green but before any pigment has diffused out of the tissues, the same bands in the spectrum are observed as with the spectrum of the extract. It is thus possible to obtain within the leaf tissue a solution of the same kind as that obtained by extraction.

The various chlorophyll samples obtained by Willstätter by different methods of extraction are identical, whether obtained from fresh leaves, or from leaves put in boiling water, or from dried leaves. They showed no difference in chemical composition, solubility or optical properties.

While in the higher land plants examined the same four pigment are always present, and the ratio of the quantities in which the four are present does not vary very much, Willstätter found a somewhat marked variation in the green algæ, and a very different state of affairs in the brown algæ.

The green alga examined was *Ulva lactuca*. Here were found

the same four pigments as are present in higher plants, but the alga is comparatively richer in chlorophyll b and also contains, relatively to the chlorophyll, more of the yellow pigments than is present in the green leaves of land plants. Willstätter gives the following table for the pigments of Ulva, the numbers representing parts per thousand of fresh thallus:—

Chlorophyll a...	0·16
„ b...	0·12
Carotin	0·02
Xanthophyll	0·06

The brown algæ stand of course in great contrast to the green algæ and higher plants as far as their external appearance goes in the matter of colour, and many views have been held in regard to the presence of pigments causing this colour. Thus Cohn (1865, 1867) supposed the cells of the Phæophyceæ contained a brown pigment called phæophyll nearly allied to chlorophyll. Molisch (1905) supported this view. In these brown forms he supposed the only pigment present to be a brown chlorophyll derivative which changes easily into ordinary chlorophyll when the thallus is immersed in warm air or water or is treated with organic solvents. Potassium hydroxide reacts with chlorophyll to produce a brown derivative which easily gives rise to green compounds, and with this brown derivative he compares phæophyll.

The theory generally taught in this country, which is the one held by Tswett (1906, 1910) and Czapek (1911), is that chlorophyll is present in the plastids of the brown algæ but that its presence is masked by yellow pigments. The well-known class experiment of putting the thallus of a brown alga in boiling water which results in an immediate change of brown to green, is usually explained by supposing the brown covering pigment to be extracted by the water. Tswett suggests as an alternative explanation the alteration of the yellow pigment.

The completion of the proof that chlorophyll is actually present in the brown algæ has been made by Willstätter and Page (1914). These workers in dealing with the phæophyll theory of Cohn and Molisch show that if the algæ contained a pigment similar to that produced by the action of potassium hydrate on chlorophyll it would give different derivatives when subjected to different treatments, but this is not the case.

A second argument against Molisch's view is to be found in spectroscopic examination of the pigments. The brown chlorophyll derivatives give a spectrum quite different from that of chlorophyll, there is no absorption in the red, but strong absorption in the green

and violet. The spectrum of the brown algal thallus on the other hand is not at all like this, but is not much different from that of the green leaf. On putting into boiling water a change in the spectrum is observed similar to that observed in the case of the green leaf and this can be explained in a similar way by supposing a change in the chlorophyll from the colloidal condition to a solution in fats and waxes.

A microscopic examination of the thallus before and after treatment with hot water confirmed other lines of evidence.

As regards the composition of the pigments in the brown algæ some workers, including Tswett, have supposed a third chlorophyll to be present; Tswett, for example, speaks of chlorophyll γ. Willstätter, however, could find no sign of such a substance when he treated fresh brown algæ with cold solvents. But he has obtained this third chlorophyll derivative from stale or dried thallus. It is clear that the chlorophyll of the brown algæ changes on standing, and the pigment is unstable in the dried plant.

The result of Willstätter's researches on the pigments of the brown algæ is to show that the same pigments are present in their plastids as in the chloroplasts of the green leaf, and that a third yellow pigment, fucoxanthin is present in addition, a pigment with the formula $C_{40}H_{56}O_6$. The green pigment is nearly all chlorophyll a; only traces of chlorophyll b were found. Willstätter gives the result of analyses of Fucus, Dictyota and Laminaria. The yellow pigments are much more abundant than in green plants. Instead of being present in the molecular ratio of 3 to 5 of green to 1 part of yellow pigment as in higher plants, the ratio is here more nearly 1 to 1.

Willstätter gives the following figures as the result of his analysis of Fucus pigments:—

Chlorophyll	... 0·503
(nearly all a, not more than 5% b).	
Carotin	... 0·089
Xanthophyll	... 0·087
Fucoxanthin	... 0·169

These numbers represent parts per 1,000 of the fresh thallus The molecular ratios are:—

Chlorophyll	... 3·64
Carotin	... 1·08
Xanthophyll	... 1·00
Fucoxanthin	... 1·75

In the following sections of this chapter we propose to deal more in detail with the chemistry of the pigments, their method of extraction and variations in their amounts. It must, of course, be understood that anyone wishing to obtain first-hand knowledge of these aspects of the subject must consult Willstätter's book (1913) or his original papers referred to therein.[1] Most of the information given in the succeeding sections of this chapter is due to Willstätter and his co-workers. It is impossible to offer any criticism of the methods or the results of Willstätter, and in many cases in the following we have simply had to quote him without comment.

In view of the information given by Willstätter's researches it becomes unnecessary and would only be confusing, to enter into any discussion of the different results obtained by other continental investigators of this subject such as Hoppe-Seyler (1879, 1880, 1881), Gautier (1879) and Stoklasa (1907, 1909, 1913). It is sufficient to indicate that their results now appear due to their imperfect methods of extraction.

The difficulties of isolating chlorophyll, partly because it changes so easily to other substances, and is so soluble in many solvents, has had the result of producing a very voluminous literature, but not even the most elementary questions had been solved before Willstätter's researches. Thus it was not known whether there was one chlorophyll substance or more than one and as recently as 1906, Étard claimed to have found in one plant a whole series of different chlorophyll pigments, and an unlimited number of chlorophylls from different plants. Also the elementary questions of analysis had not been solved. It was not even known which elements were contained in the chlorophyll molecule.

[1] How little notice has been taken of Willstätter's work by plant physiologists can be made clear by reference to recent work involving the use of extracted chlorophyll. It is perhaps understandable that Wager in a paper published in 1914 should be unaware of Willstätter's results, for not much more than half a year had elapsed since the publication of Willstätter's book; yet even so, accounts of many of the researches summarised in that book have been easily accessible in original papers for some years. Again, in the paper by Ewart (1915) already referred to, in which Wager's work is criticised, there is no evidence of close acquaintance with Willstätter's work, for the author gives as the source of his information, the account of Willstätter's researches given by Haas and Hill (1913). This, however, may be explained by the fact that Ewart is working in Australia and some time is required for German publications to reach so far. But it is difficult to understand how Chodat, working in a country so near Germany as Switzerland, in a paper (1915) published three months after Ewart's, should make no reference to Willstätter's, but should recommend the methods of chlorophyll extraction worked out by Hoppe-Seyler which date back to the years 1879 to 1881.

Now, owing to Willstätter's work, which is undoubtedly one of the most brilliant achievements of organic chemistry, our knowledge of the chemistry of chlorophyll is as complete as, or more complete than, that of any other plant substance. His researches have therefore cleared the way for a vast amount of plant physiological work of the greatest importance. It seems impossible that this unique work of Willstätter and his co-workers should not influence and stimulate work in plant physiology, and it is surprising how little this work has influenced plant physiological research so far. Thus, in some recent work (Ewart, 1915) where it is contended that Willstätter's methods of extraction have been followed, it would have been more convincing if the author of the paper had stated what chemical tests he applied to test the purity of his extracted pigment.

B. The Chemistry of Chlorophyll.

Chlorophyll is a neutral substance which on treatment with alkalis yields salts of acids which are known as chlorophyllins. These salts of the chlorophyllins are soluble in water and are also green in colour like chlorophyll. In the production of chlorophyllins, a group which was bound to an acid radicle, has been split off from the chlorophyll molecule, that is, the chlorophyll has undergone saponification like an ester.

The chlorophyllins which are formed by alkaline hydrolysis from alcoholic extracts of leaves are easily decomposed. They were, however, isolated by Willstätter, and were found on analysis to contain magnesium, which was bound to the nitrogen in a complex way. The magnesium cannot be electrolytically dissociated as in a magnesium salt. The magnesium containing group is very easily affected by acids, but is stable in presence of alkalis.

On heating chlorophyllins with concentrated alcoholic alkalis, a series of decomposition products, phyllins, are obtained by removal of carboxyl groups, until in the final phyllin only one remains. The phyllins are also acids containing magnesium. On removal of the last carboxyl group a substance devoid of oxygen, ætiophyllin, is produced, having the composition $C_{31}H_{34}N_4Mg$, in which also the magnesium is bound to the nitrogen.

If mineral acids and acetic acid are allowed to act on the phyllins, these lose their magnesium. The series thus obtained

from the phyllins by the action of acids are called porphyrins. Thus ætiophyllin gives ætioporphyrin, $C_{31}H_{36}N_4$.

While the action of alkalis on chlorophyll produces no change in the optical properties of the chlorophyll derivatives, with acids the colour becomes olive-green and the fluorescence becomes less. It is another group of the chlorophyll that is attacked, but the resulting substance is incapable of forming salts: no saponification has taken place.

For instance, the action of oxalic acid or dilute alcoholic hydrochloric acid on an alcoholic extract of leaves is to produce a wax-like chlorophyll derivative called phæophytin. It contains no magnesium, and the replacement of that metal by hydrogen is the only change which takes place. The substance is not easily soluble in alcohol and so is precipitated easily. Its solution differs from that of chlorophyll in colour, but if a metal is introduced into the molecule again, it regains the chlorophyll colour. This may easily be effected with copper and zinc by adding their acetates to phæophytin. Magnesium is not so easily replaced, but Willstätter has succeeded in doing this by treating phæophytin with magnesium methyl iodide.

If phæophytin is saponified with alkali, nitrogen-containing acids are produced and a nitrogen-free alcohol called phytol of the formula $C_{20}H_{39}OH$. Willstätter has also shown that a $-COOCH_3$ group is broken up by this hydrolysis.

From the results of the treatment of chlorophyll with alkalis and acids Willstätter has thus been able to write the formula of chlorophyll a as $(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_{20}H_{39})$, that of chlorophyll b as $(C_{32}H_{28}O_2N_4Mg)(COOCH_3)(COOC_{20}H_{39})$.

When a mixture of chlorophyll a and b is saponified with alkali the green colour changes first to a deep brown (chlorophyll a changes to yellow, chlorophyll b to red). After a few minutes the colour changes back to the original green. Willstätter explains this as possibly due to the presence of a lactam ring CO—NH which is opened when the brown phase is produced.
| |
The reappearance of the green colour is supposed to be due to the formation of another lactam ring which is more alkali-stable.

During the production of the brown phase the complex combination of the magnesium is affected. On the reproduction of the green colour the carboxyl group might combine with the same nitrogen group or with a different nitrogen group, or the nitrogen

might combine with another carboxyl group.[1]

From the formulæ of chlorophyll given above it will be observed, that the phytol component amounts to one-third of the weight of the chlorophyll.

Now analysis of chlorophyll from different plants gave very various numbers for the phytol content, and plants yielding chlorophyll containing very little phytol were found to be excellent material from which to isolate chlorophyll in a crystalline form. According to Willstätter and Stoll the chlorophyll in plants is accompanied by an enzyme chlorophyllase, active in alcoholic media, which brings about the replacement of the phytol of the chlorophyll molecule by alcohol, so that one gets alcoholysis of the chlorophyll. The substances so produced were known formerly as crystalline chlorophyll. They constitute a group called chlorophyllides. Ethyl chlorophyllide (crystalline chlorophyll) is produced according to the equation

$$(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_{20}H_{39}) + C_2H_5OH =$$
$$C_{20}H_{39}OH + (C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_2H_5).$$

Phytol. Ethyl chlorophyllide.

Similar chlorophyllides are produced with other alcohols.

Of much interest is the manner in which the presence of two chlorophylls in leaves was discovered. It has been mentioned already that the treatment of an alcoholic extract of leaves with dilute acid yields a wax-like substance called phæophytin. It was observed by Willstätter that the decomposition of phæophytin results in a considerable number of products, but that these consist of two distinct groups, one called the phytochlorins which are olive-green in solution, and another group, comprising those which give solutions of a beautiful red colour, called phytorhodins. These compounds were so numerous that they were simply differentiated by letters so that they were designated phytochlorin a, phytochlorin b, etc.

The method by which they were separated by Willstätter and Mieg (1906) is based on the different distributions of these substances between ether and hydrochloric acid. The concentration of the hydrochloric acid determines how much of the substance is extracted by it from ether. Thus only traces of phæophytin a are extracted

[1] In alcoholic solution chlorophyll undergoes a change which Willstätter calls allomerisation. He supposes the lactam ring is opened and another lactam ring formed. Such allomerised chlorophyll does not give the brown phase. This change does not take place in ether or chloroform solutions. It is accelerated in alkaline solutions but inhibited by small quantities of acid. Therefore, in the separation of the two chlorophylls, a small quantity of oxalic acid is added. See section D (6), p. 23.

from an ether solution by an equal volume of 25% hydrochloric acid, while with 32% it is almost entirely extracted: again only traces of phytochlorin e are extracted by 0·5% acid, but it is almost entirely extracted with 4 to 5% acid. The "hydrochloric acid number" is the percentage content of that acid which by shaking removes approximately two-thirds of the dissolved substance from an equal volume of an ethereal solution.

It was thus possible to separate the decomposition products of phæophytin by fractionating the mixture of them in ether with hydrochloric acid of different concentration.

The formation of the large number of decomposition products of phæophytin is due to the instability of chlorophyll in alcoholic solution. By uniform treatment of material, however, Willstätter has been able to ensure obtaining only two, but never fewer, decomposition products from phæophytin: phytochlorin e, $C_{32}H_{32}ON_4(COOH)_2$, and phytorhodin g, $C_{32}H_{30}O_2N_4(COOH)_2$.

As the molecular weight of phæophytin is of the same order of magnitude as that of phytochlorin e and phytorhodin g, and as these cannot be converted into one another, and as moreover they are formed in definite proportions by weight, it follows that they are formed from two different phæophytins and ultimately from two different chlorophylls.[1]

The summary on the next page may serve to make clear the action of different reagents on the chlorophyll molecule. In each case we have represented the actions with chlorophyll a. Similar reactions take place with chlorophyll b.

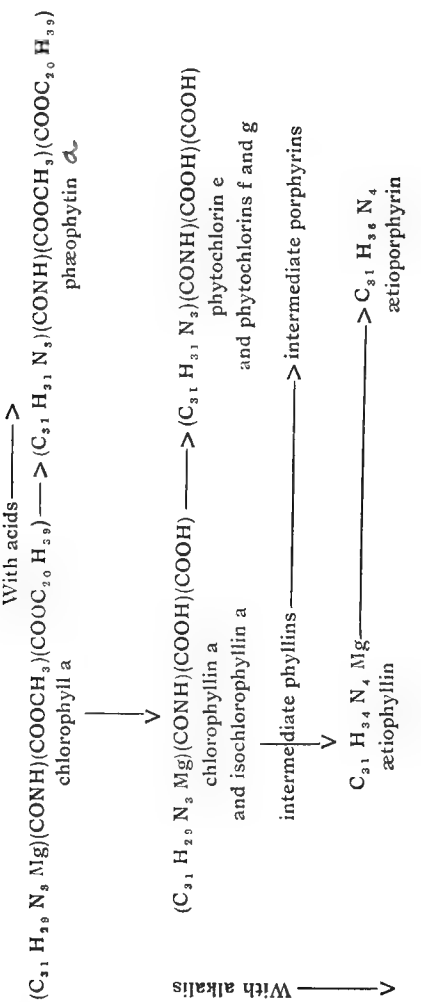
Description of Chlorophyll. Chlorophylls a and b as precipitated out by petrol ether are microcrystalline. Chlorophyll a forms a blue black powder which makes a green mark. Chlorophyll b forms a green to green black powder.

Chlorophyll a is easily soluble in ethyl alcohol, acetone, chloroform, ether and carbon-disulphide, pyridin and benzene, moderately soluble in methyl alcohol and soluble with difficulty in 80% ethyl alcohol, 90% methyl alcohol (even warm) and petrol ether (even warm). It is practically insoluble in 80% methyl alcohol.

Chlorophyll b has much the same solubility properties as chlorophyll a, except that the solubility is generally slightly less. It is completely insoluble in petrol ether and practically insoluble in 90% methyl alcohol.

[1] For further information on the chemistry of chlorophyll see Willstätter and Stoll (1913). A short account of the chemistry of chlorophyll has also recently been published by Willstätter in English; see Willstätter (1915a).

The ethyl alcoholic solution of chlorophyll a is blue green with deep red fluorescence, the colloidal solution in water is pure green and does not fluoresce. The alcoholic solution of chlorophyll b is greener, compared with a it is tinted a little yellow and the fluorescence is red with a tinge of brown.



The different behaviour of the two chlorophylls on saponification has been mentioned previously. Chlorophyll a gives a pure yellow and b a red colour both of which change back subsequently to green.

C. The Yellow Pigments.

Although the yellow pigments may have physiological importance in carbon-assimilation there is not much to be said in regard to their chemistry. They both give non-fluorescent yellow solutions stable in alkaline but very easily dissociated in acid media.

Carotin is identical with the yellow pigment of carrots. It is an unsaturated hydrocarbon of the formula $C_{40}H_{56}$ crystallising in rhombohedra with a lustrous blue surface, but appearing red in transmitted light. It is easily soluble in chloroform, carbon-disulphide and benzene, soluble with difficulty in petrol ether and ether, and in even boiling methyl and ethyl alcohol; in the cold it is almost insoluble.

Characteristic of it is its distribution between petrol ether and methyl alcohol. If to a solution in petrol ether is added methyl alcohol containing a little water, the alcohol layer remains colourless.

It undergoes auto-oxidation. If it stands in air it becomes bleached and increases in weight by 35% in dry air and by 41% in moist air. With the halogens it forms addition compounds.

It gives a red solution in carbon-disulphide and a deep blue solution in concentrated sulphuric acid.

Xanthophyll has the formula $C_{40}H_{56}O_2$. The crystals are pleochromatic often with a steel blue lustre. In transmitted light they are yellow and only red where two or more cross one another and in this way are easily distinguishable from those of carotin although the colour of the two pigments in solution is very similar. The behaviour of xanthophyll with sulphuric acid and halogens is the same as that of carotin. It is insoluble in petrol ether, the solvent is not even coloured; in methyl alcohol it is soluble with difficulty but more easily than carotin. It is also soluble with difficulty in carbon-disulphide. In ether it is more soluble, and is easily soluble in chloroform. Like carotin it undergoes auto-oxidation and a solution of xanthophyll bleaches in presence of air very quickly, much quicker than carotin.

If a xanthophyll solution in methyl alcohol is mixed with petrol ether and a little water added, the greatest part of the pigment remains in the methyl alcohol layer.

Although carotin and xanthophyll give very similar solutions it is difficult to compare the colour intensities of the two because the colour varies with the solvent and the concentration. The carotin is always stronger and in dilute solutions they are not comparable because the shade varies.

The alcohol solution of carotin or xanthophyll has a spectrum with one band in the blue and another in the indigo blue and the end absorption commences in the violet. The bands in the case of xanthophyll are displaced a little towards the violet as compared with carotin.

In carbon-disulphide solutions the difference between the two spectra is greater, and the absorption bands are displaced towards the red end as compared with those in alcoholic solution.

D. The Extraction and Preparation of the Pure Pigments from the Leaf.

(1) *The Choice of a species.* In obtaining the pigments of the leaf in the free state it is obvious that the first question to arise is the choice of material from which the pigment should be extracted. This question immediately resolves itself into two: firstly, the choice of a species on which to work, and secondly, the preparation of the leaves for treatment with the solvent.

In regard to the choice of a species, Willstätter divides plants into two groups. (i) Those rich in the enzyme chlorophyllase, which on extraction of the pigment give the substance known as "crystalline chlorophyll" (chlorophyllides). In this group are hogweed (*Heracleum sphondylium*), hempnettle (*Galeopsis Tetrahit*) and the hedge woundwort (*Stachys sylvatica*). (ii) Those poor in chlorophyllase, which on extraction of the pigment give true chlorophyll. Of the plants in this group Willstätter recommends for use the nettle (*Urtica* sp.) which is very abundant, is rich in chlorophyll, and poor in enzymes. Nettles are easily dried and when dried they keep well. They have the disadvantage that in the process of extraction the chlorophyll is easily altered, but this disadvantage can be obviated by quick preparation.

It is interesting, as Willstätter points out, that as long ago as 1852, G. G. Stokes proposed the use of nettles as a source of chlorophyll.

(2) *The Preparation of the Leaves.* The earlier preparations of chlorophyll were nearly all made by boiling fresh leaves in alcohol, and for this purpose, on account of its abundance, grass was very commonly used. Sometimes the fresh leaves were first boiled with water, after which the pigment could be extracted with warm alcohol. Hoppe-Seyler first treated the leaves with ether in order to extract the waxes, before extracting the pigment with boiling alcohol.

Willstätter first dried his leaves and powdered them before extracting the pigment. The advantages and disadvantages of using fresh and dried leaves may be summarised as follows. Preparations from fresh leaves are important (i) for analytical purposes when small quantities only are necessary; (ii) when it is necessary to find the true proportions of the various pigments; (iii) when the action of chlorophyllase on chlorophyll is utilised for the preparation of crystalline chlorophyll. On the other hand fresh leaves have the disadvantages that (i) they are more difficult to divide finely; (ii) it is more difficult to prevent the alteration of the chlorophyll in fresh leaves than in the dry powder. However, this difficulty may be overcome by treating with a watery solution of methyl or ethyl alcohol of such concentration that no chlorophyll is extracted while at the same time the enzymes are destroyed.

Willstätter himself used the dried powder of leaves for all ordinary extractions. The use of the dried powder has these advantages. (i) To obtain the same quantity of pigment a much smaller quantity of material is required than if fresh material is used. This allows of the use of smaller vessels for the extraction operations, a very important advantage when the small quantity of chlorophyll present in the crude material is considered. (ii) A saving in the solvents is effected. These are not diluted by the water content of the leaves which constitutes about 75% of the fresh leaves. (iii) As a leaf can be chosen the dried powder of which keeps well, the preparation of the pigments can be made independent of the season and growing place of the plants.

The disadvantages of using the dried material are as follows: (i) Loss of chlorophyll owing to drying. If the drying is done properly this loss is very small. Thus Willstätter found in alcoholic extracts of dried nettle and of *Galeopsis* 95 to 96% of the chlorophyll in fresh leaves. (ii) Again, if the leaves are not properly dried, alteration of the pigments may take place. Some dried leaves are spoilt by being kept (e.g., Grass), others (e.g., Elder and Conifer leaves) are even spoilt by drying. But even in such cases the chlorophyll may be preserved unchanged if the leaves are dried in a vacuum desiccator over sulphuric acid.

It should be mentioned that Willstätter has compared the pigments extracted from fresh leaves and dried leaf powder and has found them identical.

(3) *The Solvents.* For the various reasons given above Willstätter used the dried powder of nettle leaves for all ordinary

extractions of chlorophyll. Now it has already been pointed out in an earlier section of this chapter that the efficiency of a solvent for extraction is not necessarily conditioned by the solubility of extracted chlorophyll in it. Thus pure extracted chlorophyll is easily soluble in benzene, ~~in petrol ether~~ and in water-free acetone, as well as in alcohol, ether, and carbon-disulphide, yet chlorophyll is only very slowly extracted from dried leaf powder by pure alcohol, ether and acetone, and not at all by benzene, petrol ether and carbon-disulphide. On the other hand it is immediately extracted by methyl alcohol.

The foundation then of Willstätter's method of extraction is the use of solvents containing a moderate content of water. This latter forms a salt solution with some of the cell contents, and this salt solution effects an alteration in the condition of the chlorophyll which thus becomes easily soluble in the organic solvent. If the solvent contains the correct water-content the pigment is almost entirely extracted. The pure solvents are effective in the order, methyl alcohol, acetone, ethyl alcohol, ether. When 1% water is added, acetone, ethyl alcohol and methyl alcohol are all equally effective as solvents. With a higher water-content acetone is better than any of the others. The best solvent is acetone containing 15% (by volume) of water. Willstätter however, uses 80% acetone, because with this somewhat higher water-content, a quantity of accompanying substances are not extracted and the separation of the pigments becomes easier. If alcohol is used for chlorophyll extraction the most satisfactory solvent is one containing 10% (by volume) of water.

The earlier extractions of chlorophyll were always made with hot or at least warm solutions. All Willstätter's extractions have been made in the cold, i.e., at ordinary laboratory temperature, thus preventing any alteration in the pigments which might take place with rise of temperature.

(4) *The Method of Extraction of the Pigments.* The nettle leaves having been collected, their stalks are removed and the leaves dried at air temperature. They are then powdered as finely as possible, and the resulting powder then dried at a temperature of 30°C. to 40°C. A quantity of this powder, say 500 grams,[1] is then put on a filter paper in a Buchner funnel 24 cms. in diameter and sucked to it by means of a strong water pump, or better, by a vacuum pump. Half a litre of solvent is now allowed to permeate the powder on the filter paper for five minutes without the use of

[1] The quantities of material and reagents and the dimensions of apparatus quoted in this and succeeding sections are those given by Willstätter and Stoll (1913).

the pump. Then 250 c.c. of solvent is added and slowly sucked through with the pump. After five minutes another 250 c.c. of solvent is added and sucked by the pump for ten minutes. This operation is repeated with two further additions of 250 c.c. of solvent, and finally the pump is allowed to work as strongly as possible and the powder is sucked dry. The 1,500 c.c. of solvent used gives 800 to 900 c.c. of extract. It will be noticed that the solvent only passes once through the powder, and that the extraction is rather rapid.

In order to obtain good results with small quantities of solvents in so short a time, a good deal of care is necessary. It is essential to have the powder dry and to get it sucked into a coherent mass on the Buchner funnel before commencing the extraction. The layer of powder on the funnel must not be too high: not more than 5 cms.

The amount of solvent required and the time necessary for extraction depend on the chlorophyll content and on the fineness of the powder, but at the end of the extraction the powder should remain colourless or coloured only slightly yellow.

(5) *The Separation and Purification of Chlorophyll.* Various methods for the separation of the green pigments have been worked out by Willstätter and his co-workers. The most successful of these, of which we give a résumé below, is that of Willstätter and Stoll.

The essentials of this method are, firstly, the transference of the pigment from acetone to petrol ether and the removal from the petrol ether solution of accompanying substances by washing with watery acetone. The xanthophyll is then removed by means of methyl alcohol. By washing the petrol ether solution with water, the last traces of acetone and methyl alcohol are removed. As chlorophyll is insoluble in pure petrol ether, it is precipitated, and so is filtered from the carotin which remains in solution.

The details of the method are as follows. The extract from 2 kilos of nettle powder is obtained as indicated in the preceding section. Four litres of petrol ether (S.G. ·64 to ·66) are put in a 7-litre separating funnel and the extract added to this in two successive portions. With each of these additions is also added ½-litre of water, and the funnel is gently rotated at the same time. The liquid separates into an upper deep green layer and a lower weak yellow green layer. The latter is run off. The remaining petrol ether layer is mixed with two successive litres of 80% acetone which removes impurities but very little chlorophyll. The acetone

is then removed by adding 4 successive ½-litres of water, with gentle rotation of the liquid and running off the lower layer each time. (The first time 0·6 litre of acetone is removed and in successive removals 0·5, 0·4 and 0·2 litre). In the acetone thus removed are many of the impurities accompanying chlorophyll in the crude extract.

From the solution remaining, the xanthophyll is first separated by shaking the solution with 3 successive additions of 2 litres of 80% methyl alcohol. After each addition and shaking, the methyl alcoholic layer is removed, and if the last extract is still considerably yellow, one or two further additions of methyl alcohol are made. From these methyl alcohol extracts xanthophyll is prepared.

From the petrol ether solution, which should now have a volume of 3·6 litres, the last traces of acetone and methyl alcohol are removed by washing with water four times, each time using 2 litres of water. With the disappearance of the last parts of the acetone and methyl alcohol, the chlorophyll is precipitated as a suspension from the petrol ether, which thus loses its fluorescence.

This suspension in petrol ether is shaken with some fused sodium sulphate and about 150 gms. of talc, and then filtered through a layer of talc on a Buchner funnel. From the filtrate carotin can be isolated as described later.

The talc and chlorophyll on the Buchner funnel are washed with ordinary petrol ether until this runs off yellow in colour, and then the washing is completed with 300 c.c. petrol ether of B.P. 30° to 50°C. The talc is then sucked completely dry with the pump, and the chlorophyll in it dissolved in pure ether. The ether solution of chlorophyll so obtained is filtered through fused sodium sulphate, concentrated to 100 c.c., filtered twice more, and evaporated to 25 c.c.

From this solution the chlorophyll is precipitated by the slow addition of 800 c.c. of low B.P. petrol ether. The precipitate so obtained may be a blue black powder easily filtered, or it may be so fine that it has to be filtered on talc.

The precipitate is again dissolved in ether, and the solution concentrated to 20 c.c. and dried in a dish in a desiccator.

The pure chlorophyll so obtained consists of about 13 grams (i.e., 6·5 grams per kilo of dried leaves of mixed chlorophyll a and chlorophyll b) forming a thin shining steel blue crust. The yield is about 75% of the total chlorophyll content of the leaves.

(6) *The Separation of the two Chlorophyll Components from one another.* Although no doubt for many plant physiological purposes it will be sufficient to extract a mixture of the pure chlorophyll pigments, yet in other cases it will doubtless be of the first importance to obtain the two chlorophyll components isolated from one another. In order to make this review as complete as possible we have therefore thought it worth while to give Willstätter's method of separation of chlorophyll a and chlorophyll b in spite of its laboriousness.

The principle involved in the separation is that of the distribution of the two components in petrol ether and methyl alcohol. In a mixture of these two solvents the *a* component goes to the petrol ether, the *b* to the methyl alcohol.

Eight grams[1] of chlorophyll isolated according to the method described in section 5 are dissolved in 150 to 200 c.c. of ether, and filtered into a 7-litre separating funnel containing 4 litres of petrol ether (S.G. ·64 to ·66). The chlorophyll begins to precipitate out, and 50 to 100 c.c. of methyl alcohol are added to clear it again.

Before separating the components by fractionation the ether is first removed by washing with 2 litres of 80% methyl alcohol once or twice.

The chlorophyll b is now separated by repeated extractions (14 of them) with 2 litres of 85% or 90% methyl alcohol. The component *a* remains in the petrol ether. The methyl alcohol must first be saturated with petrol ether (5·5% and 10% respectively is required for this) and immediately before use it must be acidified with ·01 gram oxalic acid per litre.

(7) *Purification of Chlorophyll b.* The first methyl alcohol extract is brought to a concentration of about 90% by the addition of a litre of methyl alcohol. It is washed with a litre of petrol ether, separated from it, added to 2 litres of ether and mixed with much water, by which means the chlorophyll b is brought into ethereal solution.

The second methyl alcohol extract is similarly mixed with a litre of methyl alcohol. It is shaken with the washed petrol ether of the first extract to which has been added another ½-litre of petrol ether. The solution containing component *b* is separated from the petrol ether and added to the ether solution of the first extract to which another ½-litre of ether is added.

Each of the petrol ether portions used in washing is freed by

[1] See note on page 20.

means of water from its methyl alcohol, whereon the pigment in it is precipitated.

The third and fourth and fifth alcohol extracts are similarly treated. The content of component *b* is now considerably reduced.

The sixth methyl alcohol extract is treated with 900 c.c. methyl alcohol, and each successive extract with 100 c.c. less, so that to the 14th extract only 100 c.c. methyl alcohol is added.

These extracts are cleaned in pairs with 1 litre of petrol ether, for the second of each pair a further ½-litre of petrol ether is added.

All extracts thus cleaned are added to the same ether solution which is increased by continual additions of ether, beginning with 1 litre, and decreasing in amount to about ½-litre with the 10th extract.[1]

A 15th and 16th extraction with methyl alcohol is made in order to free chlorophyll a from the last traces of chlorophyll b.

The chlorophyll b solution is now freed from methyl alcohol by washing with water, it is dried with sodium sulphate and evaporated to 500 c.c., and then to 30 or 40 c.c. *in vacuo*.

The chlorophyll b is then precipitated by the addition of 300 c.c. petrol ether of B.P. 30° to 50°C. and filtered on talc. The filtrate contains much chlorophyll a. It is purified by solution in ether and precipitation with petrol ether, which is repeated several times. It is finally filtered and dried in a vacuum desiccator.

(8) *Purification of Chlorophyll a.* The petrol ether solution, from which the last traces of chlorophyll b have been removed as indicated in the preceding section, is further purified by shaking it three times with 2 litres of 90% methyl alcohol.

The methyl alcohol is removed and the petrol ether solution of chlorophyll a is washed with water until the chlorophyll is precipitated in quantity. Talc is added to the extent of from 30 to 100 grams, and the whole filtered on a layer of talc on a Buchner funnel. The petrol ether should then run off colourless.

The talc is washed with petrol ether of low B.P. and sucked dry with the pump till all petrol ether smell has disappeared. It is then transferred to a bottle and shaken with as little ether as possible. On filtration on a small Buchner funnel the beautiful deep blue ether solution of chlorophyll a runs through. The chlorophyll and talc are completely freed from one another by further filtration.

[1] The large quantities of ether are necessary because the watery methyl alcohol dissolves much ether and the petrol ether which separates out on dilution makes it difficult to carry the chlorophyll over from methyl alcohol to ether.

Finally the ether solution is concentrated by evaporation and the concentrated solution put in a dish in a vacuum desiccator. On complete removal of the ether, the chlorophyll a is left as a blue black mass.

(9) *Purification of Xanthophyll.* To the extract containing xanthophyll obtained as described in section 5, 4 to 5 litres of ether are added and a quantity of water. Any chlorophyll b that may be present is removed by saponifying it to chlorophyllin by shaking with 30 to 50 c.c. of methyl-alcoholic potassium hydrate. The chlorophyllin is removed by repeated washing with water.

The ether solution of xanthophyll is dried with sodium sulphate, evaporated to 30 c.c., and 200 to 300 c.c. methyl alcohol added. Complete removal of the ether is effected by evaporating down further and filtering the hot solution. On cooling, xanthophyll is deposited in the form of crystals forming shining plates. Water may be added to make the separation of the xanthophyll complete. The yield of xanthophyll from 2 kilos of dried nettle leaves is 0·8 gram.

(10) *Purification of Carotin.* The carotin is easily obtained. The extract containing it, obtained as indicated in section 5, is evaporated *in vacuo* at 40°C., and the oily residue treated with 300 c.c. of 90% alcohol. The carotin begins to separate out immediately in shiny steel blue crystals. Crystallisation is complete on standing in the cold.

Any colourless impurity present in the crystalline mass is dissolved in petrol ether. 200 to 300 c.c. of this are therefore added and the carotin filtered from it. The purification is completed by treatment with a mixture of two parts of petrol ether to one part alcohol. The result is a yield of 0·25 gram of carotin from 2 kilos of dried nettles.

E. Simple Laboratory Experiments on the Leaf Pigments.

As Willstätter truly points out, the experiments described in text-books of plant physiology for the demonstration in class work of the properties of chlorophyll are quite inadequate.

In preceding sections of this chapter, we have described Willstätter's methods for the extraction in quantity of the leaf pigments. It is quite obvious, however, that the length of time and large amount of material and reagents required for these extractions, render their use scarcely possible for ordinary class work. We have therefore brought together in this place a number of easily performed experiments which amplify the collection of examples given in Willstätter and Stoll's book.

The performance of these experiments will not only lead the student to clearer ideas about the chemistry of the leaf pigments, but will also give an opportunity for that chemical and physical manipulation which is becoming increasingly necessary to the plant physiologist, but for the practice of which there is no great opportunity in most other parts of a plant physiology course.

Preliminary. It will be found of great convenience for class work to collect in the summer every year a quantity of nettle leaves. These are dried at air temperature; they are spread out on sheets of paper and a sheet of paper placed on top of them to prevent dust from falling on them, and to prevent undue exposure. They are then ground up finely and dried completely for several days at a temperature of 30° to 40°C., in an incubator.

The powder so obtained is kept in a stoppered bottle. So prepared, the powder retains for a long time the leaf pigments unaltered in quantity and quality.

Any further drying required must be carried out by placing the leaf powder in a vacuum desiccator over sulphuric acid. This procedure is necessary for instance when it is required to show that pure solvents do not extract the pigments from dry leaf powder. In this case it is also necessary that the solvents should be as water-free as possible. Ordinary solvents may have to be redistilled over quick-lime, calcium filings, etc.

Experiment 1. Extraction of the pigments. Required: small Buchner funnel with flask and a water pump; 20 c.c. 85% acetone or 90% alcohol.

Two grams of leaf powder are sucked to a filter paper on the Buchner funnel and a small quantity of the solvent added. This is allowed to soak into the powder for a few minutes. The fluid is then sucked through with the pump. The operation is repeated until all the 20 c.c. of solvent has been added, when the powder is sucked dry. A deep blue green solution, with red fluorescence, is obtained which contains all the four pigments from the leaf. Usually the powder will still be coloured green as the extraction is not generally complete.

Experiment 2. Transfer of the pigments from an acetone solution to ~~an ether, or to~~ a petrol ether, solution. Required: 1 separating funnel; about 10 c.c. ether and 10 c.c. petrol ether; 5 c.c. acetone extract of leaves.

Five c.c. of the acetone extract obtained in Experiment 1 are poured into double the quantity of ether contained in a separating funnel. An equal quantity of distilled water is added, this being poured gently down the side of the funnel in order to avoid the formation of emulsions. In the course of a few minutes, the ether layer separates out and now contains the pigments. The lower layer, which is slightly green, is run off. The addition of distilled water and subsequent removal of the lower layer is repeated about four times, in order completely to remove the acetone from the ether solution. If the ether solution should have become at all emulsified, it can be cleared by shaking with anhydrous sodium sulphate and filtering.

A petrol ether solution may be obtained in the same way by using 10 c.c. of petrol ether in place of ether.

Experiment 3. Demonstration of the two green pigments. Required: 10 c.c. petrol ether solution of mixed pigments; 10 c.c. 92% methyl alcohol; 2 separating funnels.

The petrol ether solution from the last experiment is shaken with 10 c.c. 92% methyl alcohol. Two layers are formed of which the petrol ether layer contains chlorophyll a and the methyl alcohol layer chlorophyll b. The solution of chlorophyll a is blue green while that of chlorophyll b is a purer green, but the colour difference between them is diminished owing to the presence of the yellow pigments, of which carotin is in the petrol ether, and xanthophyll in the methyl alcohol.

A characteristic difference between the two green pigments is to be found in the phase which appears on saponification with methyl

alcoholic potassium hydroxide. This phase-test is best carried out in ethereal solution. The methyl alcoholic solution is therefore poured from the separating funnel into another and, as described in Experiment 2 transferred to an ethereal solution. The petrol ether solution and ether solution are then used for phase tests as described in Experiment 4.

The difference in the absorption spectra of the two chlorophylls is not easily observed unless the solutions are very pure.

Experiment 4. Saponification of the green pigments. Required: 5 c.c. of an ether solution containing the pigments. The petrol ether and ether solutions containing chlorophylls a and b obtained in Experiment 3; 10 c.c. methyl alcoholic potash.

An ether solution of chlorophyll does not react with weak alkali as being an ester it is without acid properties. If however, strong alkalis are used, a brown colouration appears which changes back later to green.

Pour a little of the ether solution from Experiment 2 into a test-tube and in a pipette take a little strong solution of potash in methyl alcohol (obtained by dissolving 30 gms. potassium hydroxide in 100 c.c. methyl alcohol). Place the lower end of the pipette at the bottom of the test-tube and allow the potash to run in below the chlorophyll solution. At the interface between the solutions there appears immediately a brown coloured layer which diffuses on shaking. In about ten minutes it changes back through an olive green colour to pure green. The chlorophyll has been saponified to the potassium salt of the acid chlorophyllin. This salt is insoluble in ether, so if water is added to bring about a separation of the two layers, the green colour is no longer present in the ethereal layer.

The brown phase produced in this saponification of a mixture of the two chlorophylls is the resultant of a yellow phase produced by chlorophyll a and a brown-red phase produced by chlorophyll b. The phase test should therefore also be carried out separately with the petrol ether solution containing chlorophyll a and the ether solution containing chlorophyll b obtained in Experiment 3.

It should be observed that if water is added directly the brown phase appears, the greater part of the green pigment is soluble in ether, and will again give the brown phase on treatment with alkali.

Note Willstätter's theory of the lactam ring to explain the appearance and disappearance of the brown phase.

The phase test also applies to the chlorophyllides and phæophytin and to the phæophorbides. It is not given with allomerised chlorophyll.

This allomerisation takes place in alcoholic solution particularly when water-free (see Section B of this chapter). The chlorophyllides are also very easily allomerised and lose thereby their power of crystallisation. Small quantities of water and of acids protect the substances against allomerisation, while alkalis increase the velocity of the reaction.

Experiment 5. Allomerised chlorophyll does not give the brown phase test.

Dissolve a little crude chlorophyll, obtained by evaporating an ether solution, in absolute alcohol. Add a little alkali, and perform the phase test from time to time till at last the brown phase no longer appears.

Experiment 6. Separation of the green and yellow pigments. Required: 5 c.c. ether solution of pigments; 2 c.c. 30% potassium hydrate in methyl alcohol; 5 c.c. ether.

Shake 5 c.c. of the ether solution of the pigments with 2 c.c. of the strong alkali. After the green colour has reappeared, slowly add 10 c.c. water and then add a little more ether. On shaking the test-tube two layers are produced of which the lower watery-alkaline one contains the saponified green pigments, while the carotin and xanthophyll are contained in the upper ethereal layer.

This test is employed in the examination of the purity of a chlorophyll preparation. If all the yellow pigments have been removed, the ethereal layer in this experiment should remain colourless after saponification of the chlorophyll.

Experiment 7. Separation of the two yellow pigments. Required: The ethereal solution of yellow pigments from Experiment 6; 10 c.c. petrol ether; 30–50 c.c. 90% methyl alcohol; 1 separating funnel.

The ether layer obtained in the last experiment is washed with water in a separating funnel and evaporated down to 1 c.c. It is then diluted with 10 c.c. petrol ether and next mixed with 10 c.c. 90% methyl alcohol. The methyl alcoholic layer is removed and the petrol ether layer is again treated with methyl alcohol and the methyl alcoholic layer again removed. This process is repeated until the methyl alcohol is no longer coloured. The methyl alcohol contains the xanthophyll, the petrol ether the carotin.

It may be recalled that the yellow pigments in solution greedily absorb oxygen. Some observers, either unaware of this or assuming that the chlorophyll they used was free from yellow pigments without applying tests to prove it (Experiment 6), have mistakenly stated that chlorophyll greedily absorbs oxygen.

In solution the two yellow pigments appear very similar. They can, however, be distinguished by means of their absorption spectra. (See section C of this chapter).

Experiment 8. Phytochlorin and Phytorhodin. Required: 5 c.c. ether solution containing both chlorophyll components (Experiment 2); 3 c.c. 30% potash solution in methyl alcohol; hydrochloric acid of various concentrations; separating funnel.

Five c.c. of an ether solution containing both chlorophylls a and b are evaporated to dryness in a test-tube, and the residue treated with 3 c.c. of boiling, concentrated potash solution in methyl alcohol, and boiled gently for half a minute. A liquid with red fluorescence is produced, which consists of a solution of the potassium salts of *isochlorophyllins*. The solution is diluted with double its volume of water and concentrated hydrochloric acid is added until the solution is just acid. The liquid is then shaken with ether in a separating funnel; the dissociation products produced by the previous treatment go over to the ether solution which thus acquires an olive-brown colour.

The ether solution is shaken twice, each time with 10 c.c. 4% hydrochloric acid, and the green-blue acid layer is separated and neutralised with ammonia and shaken with more ether, which then contains in solution phytochlorin e, the derivative of chlorophyll a The phytochlorin e gives to the ether an olive-green colour.

The ether layer remaining in the funnel after the separation of the green-blue acid layer is now extracted with 10 c.c. 12% hydrochloric acid. The green acid solution so obtained is diluted with water and shaken with ether which then becomes coloured red and contains phytorhodin g, the derivative of chlorophyll b.

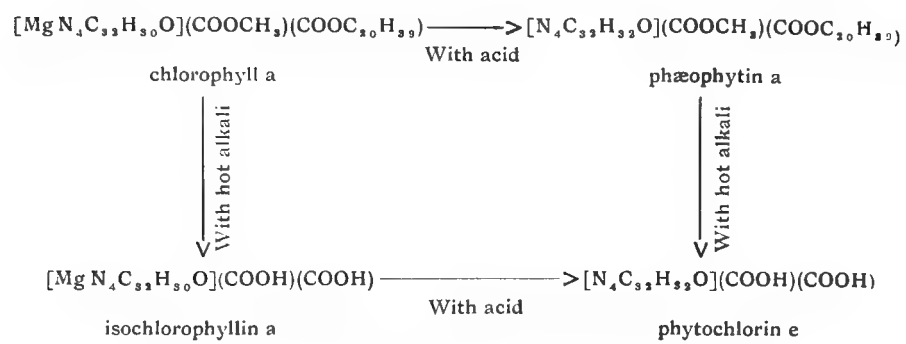
It should be noted that saponification with hot alkali as in this experiment, produces changes in the chlorophyll compounds different from those produced by saponification in the cold.

The potassium salts of the chlorophyllins which are produced by gentle saponification in the cold are not fluorescent, and under the action of acids pass over into the weakly basic phytochlorins f and g and phytorhodins k and i.

By saponification with hot alkali isochlorophyllins are formed which are fluorescent. They are complex magnesium compounds of phytochlorin e and phytorhodin g. On the addition of acid to the isochlorophyllins, these two important dissociation products are themselves formed.

The process can also be effected by addition of acid first and subsequent saponification with hot alkali.

The following scheme may help to make clear the relations between these various derivatives in the case of chlorophyll a. An exactly similar scheme may be made for the case of chlorophyll b.



The relation between chlorophyll and isochlorophyllin is not as simple as that expressed in the above scheme as the alkali not only saponifies the two ester groups but also produces an alteration in the lactam ring grouping, as indicated by the appearance of the brown phase.

Another difference between saponification with cold alkali and with hot alkali is that during the latter process the yellow pigments are destroyed. If water is added after the saponification and the solution shaken up with ether, the ether should remain colourless.

We have gone into the explanation of the changes taking place in this experiment in some detail, because importance attaches to the two dissociation products phytochlorin e and phytorhodin g. It was the formation of these substances that led Willstätter to the discovery that phæophytin, and also chlorophyll, is a mixture of two components. The experiment, moreover, is also of importance as a modification of the method is used in the quantitative estimation of the green pigments in the leaf.

Experiment 9. Substitution of other metals for the magnesium in chlorophyll.

Two c.c. of an ether solution of chlorophyll are shaken with a

little 20% hydrochloric acid and then washed with water in a separating funnel. In this way is produced in ether solution, a magnesium free chlorophyll derivative, phæophytin. The solution is evaporated down on a water bath and the residue dissolved in 5 c.c. alcohol. Note the olive green colour of the solution. This is heated and a grain of copper acetate is added. The colour changes back to a brilliant green, but without the chlorophyll fluorescence. A copper compound of chlorophyll has been produced very similar to the magnesium compound, but much more stable.

For spectroscopic examination of this substance, see Experiment 15.

Phæophytin combines very easily with acetates of some metals to form intensely coloured stable compounds. Ferric acetate gives, even in the cold, a greenish blue solution with a weak fluorescence. Zinc acetate gives a blue green solution with strong fluorescence.

Not only phæophytin but all the chlorophyll derivatives devoid of magnesium, such as phæophorbide, phytochlorin, phytorhodin and the various porphyrins behave similarly towards the salts of certain metals (copper, zinc and iron) and form complex compounds all very stable in acid and alkaline media. The formation of these complex compounds is accompanied by such noticeable changes in colour that even the smallest traces of certain metals can be discovered in this way. Hence it is very difficult to prepare the magnesium free chlorophyll derivatives absolutely pure, as even the zinc from the walls of glass vessels may disturb the molecule; for the same reason spatulas of ignoble metals must not be used.

Also solvents may disturb the molecules of these derivatives owing to the impurities in them. Thus 'pure' methyl alcohol often contains a small quantity of copper which would be sufficient to affect the magnesium free derivatives. Willstätter uses this property in order to test the purity of methyl alcohol as regards copper, by dissolving some phytochlorin e in the methyl alcohol. After standing for some time, the chlorophyll derivative is carried over into ether and the excess of phytochlorin removed by washing with 10% hydrochloric acid. As the copper compound of phytochlorin e is stable in presence of this strength of acid, it remains in the ether layer to which it gives an intense blue-green colouration.

The spectrum of these derivatives is also quite distinct from the metal free substances and more like the chlorophyll spectrum. See Experiment 15, b and c.

The compounds formed by the magnesium free derivatives with other metals are unstable towards acids, and require other conditions for their formation. Thus phytochlorin e in methyl alcoholic solution gives, with water-free barium hydroxide in excess, a barium compound. Even compounds with the alkali metals can be formed in a similar way, the compounds with potassium being the least stable of them all.

The magnesium compound occupies a place in the middle of the series as regards stability, the two extremes being the copper and potassium compounds.

Experiment 10. The action of chlorophyllase.

Fresh leaves of a species rich in chlorophyllase (Heracleum, Galeopsis) are finely divided and put in a 70% acetone solution, 3 c.c. of solution being used for every gram of leaf powder. The chlorophyll, by means of the chlorophyllase, is dissociated into phytol and the acid chlorophyllide. This can be demonstrated after about a quarter of an hour if the solution is diluted with water, transferred to ether and shaken with 0·05% sodium hydroxide. The sodium hydroxide takes up more colouring matter the further the enzyme action has progressed.

Experiment 11. Destruction of chlorophyllase.

If fresh leaves of a species rich in chlorophyllase are first steeped in boiling water for a few minutes before they are placed in the acetone solution, unaltered chlorophyll is extracted which does not react with dilute alkali.

The action of the enzyme chlorophyllase consists in either an alcoholysis (in alcoholic media) or hydrolysis (in aqueous media). For instance, in methyl alcoholic media, the phytyl group is replaced by a methyl group according to the equation

$$(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_{20}H_{39}) + CH_3OH = C_{20}H_{39}OH + (C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOCH_3).$$

Phytol. Methyl chlorophyllide.

In aqueous solutions hydrolysis takes place with the formation of free acid chlorophyllide

$$(C_{32}H_{30}ON_4Mg)(COOCH_3)(COOC_{20}H_{39}) + H_2O = C_{20}H_{39}OH + (C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOH).$$

Chlorophyllide.

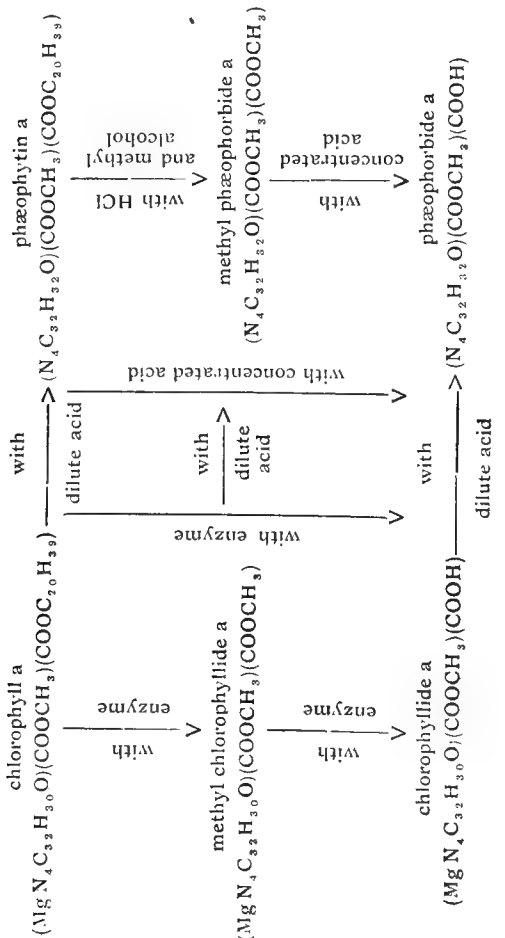
Experiments 10 and 11 demonstrate the hydrolysis of chlorophyll and also indicate that it is an enzyme action. Chlorophyllase is a

very stable enzyme; it is not even destroyed by boiling in alcohol for a short time. But if leaves are boiled in water the enzyme is destroyed.

It should be noted that the enzyme on account of its insolubility remains in the leaf when this is extracted with solvents.

By treatment with acids, magnesium is removed from the chlorophyllides with production of the corresponding phæophorbides. Thus methyl chlorophyllide a $(Mg\,N_4C_{32}H_{30}O)(COOCH_3)(COOCH_3)$ gives methyl phæophorbide a $(N_4C_{32}H_{32}O)(COOCH_3)(COOCH_3)$.

The relations of these various substances may be made clear by the following scheme taken from Willstätter.



Experiment 12. Microscopic examination of ethyl chlorophyllide.

Prepare sections of fresh Heracleum leaves and mount them in a drop of 90% alcohol. Leave the slide under a bell jar containing a dish of alcohol. The section slowly dries in the course of half a day or a day. It is then examined under the microscope when there will be observed the characteristic triangular and hexagonal crystals of ethyl chlorophyllide (crystalline chlorophyll).

Experiment 13. Production of methyl chlorophyllide in the leaf.

Sections may be used as in the preceding experiment, or a piece of a leaf may be employed. In the latter case a test-tube with 4 c.c. 75% methyl alcohol is taken and 1 gram of fresh leaf is added to it. The leaf first becomes a darker green and then during the course of a few hours becomes yellowish. On holding the leaf to the light there can be observed with the naked eye a number of black points. If sections of the leaf be cut and examined under the microscope, these spots appear as aggregates composed of rhombohedral crystals, occurring only in certain cells.

Experiment. 14. Extraction of ethyl chlorophyllide.

Two grams of dry Heracleum leaf powder is left for a day in a test-tube containing 6 c.c. 90% alcohol. The extract is then filtered through a small Buchner funnel and the powder on the filter washed with a little acetone. The filtrate is washed with the same quantity of ether, and then with water. The ether solution is transferred to a separating funnel and washed with water, and then concentrated on a water bath to ½ or 1 c.c., and 3 c.c. petrol ether is added. On standing, the ethyl chlorophyllide is precipitated in the form of crystalline aggregates. It is freed from yellow pigments by shaking with a *little* ether, and can be further purified by redissolving in ether and precipitating again with petrol ether.

Experiment 15. Spectroscopic examination. Required: small spectroscope and glass vessel with parallel sides of about 1 cm. in width; source of light (incandescent burner or Nernst lamp or sunlight).

The following absorption spectra may be examined:—

a. Chlorophyll spectrum from acetone extract obtained in Experiment 1. The extract is diluted with about five times its volume of 85% acetone. The spectrum shows a main absorption in the red at the Fraunhofer line C. Then follow, towards the violet, three absorption bands decreasing in intensity, and the end absorption in the

blue to violet parts of the spectrum.

b. Phæophytin. By adding a drop of strong hydrochloric acid to the extract used in *a*, the magnesium is removed from the complex containing it and phæophytin formed.

There is now an intense absorption in the green just before the line E.

c. Copper compound with phæophytin (see Experiment 9). The intense absorption in the green disappears and the spectrum is very similar to the chlorophyll spectrum.

d. Carotin and xanthophyll. The absorption spectra of the yellow pigments has been described in section C. There is one band in the blue, another in the indigo blue and the end absorption in the violet. Unless the correct concentration is used there will either be complete absorption or none. By altering the concentration it should be possible to obtain the correct strength of solutions for observing the bands.

Spectroscopic analysis is, of course, very useful in work with chlorophyll and its derivatives, as most of the pure substances have characteristic spectra. But in class work where it is difficult to obtain even moderately pure substances, it will scarcely be possible to go much further in this matter than we have indicated in the preceding experiment. It should however be noted that one of the crucial tests for chlorophyll is its spectrum, as the breaking down of the magnesium-containing complex alters this.

Experiments on the state of aggregation of chlorophyll.
Experiment 16. Formation of a colloidal solution of chlorophyll.

Evaporate down 10 c.c. of the acetone extract as obtained in Experiment 1 to about 2 c.c. A colloidal solution of chlorophyll is then made by pouring this acetone solution into a large volume of distilled water (20 to 100 c.c.) the liquid being continually stirred. This operation can be most conveniently done by taking the acetone solution in a pipette and allowing it to run out of the pipette while the latter is used as a stirring rod in the water. Note the change in colour to a purer green, and the disappearance of fluorescence.

The principle involved in this method of preparation of colloidal chlorophyll consists in the replacement of the solvent (acetone) by a medium (water) in which the solute (chlorophyll) is insoluble.

Thus a colloidal solution of sulphur can be similarly made. Sulphur is slightly soluble in warm alcohol, but insoluble in water.

If an alcoholic solution of sulphur is poured into a large volume of distilled water, a sulphur sol is produced.

Experiment 17. To show the difference between a true and a colloidal solution of chlorophyll.

Evaporate 10 c.c. of the acetone extract to complete dryness and test its solubility in ether, petrol ether and benzene. Now add these solvents to some of the colloidal solution prepared in the last experiment, and note that the chlorophyll does not dissolve in any of these solvents. If, however, some salt solution, e.g., a little magnesium sulphate be added, the chlorophyll is precipitated from its colloidal state and is now soluble in ether and other solvents.

Experiment 18. To show that chlorophyll in the plant is probably in the colloidal condition.[1]

Some nettle powder is carefully dried, e.g., by keeping it at 30°C. to 40°C. in an oven, and then further drying in a vacuum desiccator over sulphuric acid. Small quantities of this dry powder are put in test-tubes and different pure water-free substances such as acetone, ether, benzene and absolute alcohol are added. Note that these solvents are not coloured by the chlorophyll. It can be demonstrated that the extracted pigment is easily soluble in any of these substances.

Repeat the experiment with nettle powder moistened with a few drops of water, and note that the solvents are immediately coloured.

Experiment 19. Pure solvents are able to extract chlorophyll from fresh leaves.

Crush 10 grams of fresh leaves of nettle, horse-chestnut or elder in a mortar with some clean sand, and put the crushed material on a filter paper in a Buchner funnel. Add 20 c.c. pure acetone and suck it through by means of a water pump. Repeat this several times. The pure solvent is here able to extract the pigment.

It can also be observed that the leaf substance after extraction is brown, owing to the action of oxydases on the leaf. If therefore, the leaves are dipped first in boiling water, these oxydases are destroyed, and the leaf substance after extraction remains colourless.

Experiment 20. Treatment of fresh leaves with boiling water changes the condition of the chlorophyll.

[1] Although Willstätter regards the facts upon which this experiment is based as a proof of the colloidal condition of chlorophyll in the leaf, in the writers' opinion such a simple explanation will not cover the facts.

Dry a quantity of leaves which have been put in boiling water and examine their solubility as in Experiment 18. Note that the chlorophyll in this powder is soluble in pure solvents.

It should be noted that colloidal chlorophyll is an electronegative suspensoid, and that it is a very excellent substance for demonstrating the properties of such colloids. It might even be worth while for this purpose to prepare a small quantity of pure chlorophyll (a + b) (see section D of this chapter).

In most suspensoids it is difficult to see when the precipitation has taken place, but here where any precipitated chlorophyll can immediately be extracted by ether, the method is open for quantitative work of high accuracy.

By the use of chlorophyll can be demonstrated such properties of colloids as the salt concentration required for precipitation, the effect on this of the valency of the precipitating ion, the stabilising effect of alkali and the reverse effect of acids, the action of protective colloids, etc.

Crude colloidal chlorophyll contains, of course, a good many accompanying substances which vary in composition and quantity, so comparable data are not obtainable by the use of such chlorophyll.

F. Variations in the Quantity of the Leaf Pigments in Different Plants and under Different Conditions.

The part of Willstätter's work with which we have dealt so far concerns the characteristics of the pigments, their chemistry, and the methods for extracting them. Willstätter has further devised methods for the quantitative extraction and separation of the four pigments. Here, as in the aspects of the leaf pigments we have already considered, we are in the fortunate position of being able to neglect all earlier work on the subject, for it is now obvious that the methods employed by workers before Willstätter are imperfect and must give erroneous results. Thus, for instance, it is essential to separate the green and yellow pigments in order to obtain quantitative data as to the amount of chlorophyll present; and again other substances liable to be extracted with the pigments, particularly small quantities of plant acids, will cause considerable alteration in the pigments. These considerations apply equally to

the colorimetric and spectroscopic methods of estimation hitherto employed.[1]

Willstätter himself has so far used only colorimetric methods, for it is of no value to make measurements of a high degree of accuracy before possible errors in the extraction and separation of the pigments have been eliminated. When this has been done Willstätter suggests that quantitative spectroscopic analysis may prove very useful as a further refinement in the quantitative estimation of the pigments.

We shall give in some detail Willstätter's latest methods for the quantitative estimation of the pigments, more particularly as Willstätter in his latest publications (1915 b, 1915 c) applies them to plant-physiological work.

It is scarcely necessary to emphasise the extreme importance of obtaining reliable methods for the determination of the quantities of pigments in leaves. While Willstätter's earlier work in this respect, which is published in his book (1913), was mainly done in order to test the validity of his methods, yet he made also some estimations to determine whether there was any regularity in the variations in the quantity of the pigments in leaves, and these estimations yielded figures very suggestive with regard to the physiological function of the pigments. Now Willstätter has definitely taken up the plant physiological aspect of this question, but it must not be forgotten that his work in this regard has so far been very limited and undertaken from a purely chemical point of view. It remains for the plant physiologist and ecologist to take up the methods and apply them in their various departments of research.

Of course the technique of these methods is not to be acquired without some trouble and practice, but their employment appears at present the only way to reliable results.

It is not our intention to give an historical survey of the considerations which led Willstätter to the methods he ultimately adopted. They were developed for the purpose of comparing the pigment-content of various extracts and preparations. Thus, if for

[1] For instance, in reference to a recent paper by Jacobson and Marcinlewski (1912) where the authors claim to have shown that climatic conditions play an important part in regard to the production of one or other of the chlorophyll components, Willstätter points out that some of the errors committed by these workers were (1) only a fraction of the chlorophyll present was extracted, (2) an unknown portion of the extracted pigment was precipitated as phæophytin, and (3) only a portion of the phæophytin was isolated.

instance, one should want to compare the amounts of chlorophyll (a + b) in two different crude chlorophyll extracts, the simplest thing to do is to separate the green from the yellow pigments by saponification with alkali, and then to compare colorimetrically the two chlorophyllin solutions so obtained. Further, by the saponification of a solution of known chlorophyll content, which can be used as a standard, it is possible to obtain an approximate value for the chlorophyll content of the two extracts.

If, however, one requires to find the ratio of the two chlorophyll components, their derivatives phytochlorin e and phytorhodin g must be separated from one another and separate comparisons made.

i. Methods.

We shall describe first a simple method for the quantitative estimation of chlorophyll in a chlorophyll extract, and then the more exact method used for the estimation of the four pigments in fresh leaves.

(a) *Quantitative Estimation of Chlorophyll (a + b) in a Chlorophyll Extract.*

Ten c.c. of an acetone, or alcohol, extract is diluted to 100 c.c. with ether and 10 c.c. of this is poured into a separating funnel and diluted with a further 40 c.c. of ether.

An ether-diluted alcohol extract can be shaken at once with 4 to 5 c.c. methyl alcoholic solution of potash and the brown phase will appear. If the solution contains acetone, however, this must be completely removed from the solution before saponification, as chlorophyllins are destroyed by acetone. In this case a little methyl alcohol is added and the solution washed thoroughly with water.

After reappearance of the green colour, water is slowly added during gentle rotation of the separating funnel and the aqueous chlorophyllin solution is run into a 200 c.c. measuring flask. The ether solution of the yellow pigment is washed with a little more water to effect complete extraction of the chlorophyllins, and the watery layer added to that in the flask. The whole is made up with alcohol to 200 c.c.

The chlorophyllin solution is then estimated in a colorimeter by comparison with the standard solution.

(b) *Quantitative Estimation of Pigments in Fresh Leaves.*

1. *The Extraction of the Pigments.* The chief feature of the quantitative estimation of the pigments in fresh leaves is the primary treatment of them with watery acetone, and subsequent extraction with acetone containing only a low percentage of water.

The first treatment with weak acetone softens the leaves, removes plant acids, inhibits enzyme action, and at the same time removes no chlorophyll.

The details of the method are as follows:—In a mortar, 25 cm. in diameter, are put 40 grams of fresh leaves with 50 c.c. 40% acetone; the leaves are quickly mashed with 0·5 gram of quartz sand. This serves the double purpose of facilitating disintegration of the leaf and of further diluting the leaf substances.

There are now added 100 c.c. 30% acetone, and the whole filtered on a Buchner funnel through a thin layer of talc, which keeps back the slimy protoplasmic matter.

After sucking the residue dry with the pump it is washed with 30% acetone until the filtrate runs off colourless. The watery acetone is then sucked through.

The leaf substance is now treated with pure acetone and again sucked dry. This is repeated until the acetone has removed all the pigment. Complete extraction will require from 400 to 600 c.c. acetone; towards the end of the extraction 5 to 10% of water is added to the solvent.

As the extract is obtained it is poured, in quantities of 100 to 200 c.c., into 200 to 250 c.c. of ether, and the acetone washed out with distilled water.

Finally the whole of the ether solution is dried with anhydrous sodium sulphate. The extract is then divided into two equal parts, one of which is used for the determination of the green, the other for the determination of the yellow pigments.

2. *Separation of the Chlorophyll Components.* The ether solution so obtained from 20 grams fresh leaves is converted into phæophytin by treatment with 0·5c.c. 2 N alcoholic hydrochloric acid in a boiling flask.

The ether is then evaporated off, first in a vacuum in the cold and then at 60° for a short time under very low pressure. The residue is dissolved in 1 to 2 c.c. of pyridine and heated on a steam bath. While still heated there is added to the pyridine solution 25 to 30 c.c. of boiling, concentrated potash solution in methyl alcohol. This is

done so rapidly that the boiling of the pyridine solution is not interrupted. The brown phase appears and gives place rapidly to olive-green colour.

A vertical condenser is now fitted to the flask which is heated for two minutes on the steam bath. 5 c.c. water are added through the condenser and the boiling continued for another 1 to 1½ minutes.

The flask is now cooled under the tap and its contents transferred to a 500 c.c. separating funnel with water and some ether. The liquid is then acidified with 20% hydrochloric acid, upon which the colour changes to a dull grey-green. 200 c.c. of ether are added and the funnel shaken strongly for some minutes. A little ammonia is added to the dull coloured, watery layer which is shaken up with small quantities of ether until the last is no longer coloured.

The mother liquor is made alkaline with ammonia on account of the flocks which separate out, and then acidified again in order that the ether may remove only a small quantity of the derivatives.

If the flocks are again washed with ammonia and little pigment thereby goes into solution, no phytorhodin has been destroyed in the saponification; otherwise this has taken too long a time.

Before fractionation of the two chlorophyll derivatives the accompanying substances must be removed.

To achieve this the combined ether solutions are extracted 2 or 3 times with 30 c.c. of 12% hydrochloric acid and then with 10 to 15 c.c. of 20% acid till the acid layer finally separates in a colourless state. Further flocks form at the boundary layer, but they usually contain no green pigment. Besides the carotin, the ether also contains brown coloured substances.

The combined acid extracts are transferred to a 500 c.c. separating funnel with 200 c.c. of ether. They are neutralised with concentrated ammonia during gentle rotation until the watery layer is dull blue-violet in colour. The well-stoppered separating funnel is cooled under the tap and at the same time shaken first gently and then strongly. The watery layer is then usually pale blue and is run into a second separating funnel. By this method of neutralisation the last trace of the derivatives is brought into the ether.

The ethereal solution of derivatives is freed from methyl alcohol and pyridine by washing with 200 c.c. of water three times, with1 to 2 c.c. of 3% hydrochloric acid added, as pure water would remove phytochlorin.

The phytochlorin is now separated by shaking 4 or 5 times with 3% hydrochloric acid, 400 c.c. being used altogether, and then several times with 5% acid until this is only feebly green. The extracts with the stronger acid require to be fractioned. This is effected by neutralising and extracting with 30 c.c. of ether, and then repeatedly extracting the ether with 3% hydrochloric acid until the volume of all the 3% hydrochloric acid extracts is brought up to 500 c.c. This constitutes the phytochlorin solution. The remaining liquid contains the phytorhodin which is extracted 4 or 5 times with 12% hydrochloric acid until the ether remains only slightly reddish.

3. *The Preparation of the Xanthophyll and Carotin Solutions.* The ether extract from 20 grams of fresh leaves is saponified with 2 c.c. of concentrated potash solution in methyl alcohol, the mixture being strongly shaken, first by hand and then for half-an-hour on a mechanical shaker. The liquid is then allowed to stand for some time and if it still fluoresces red it is skaken again and, if necessary, more potash is added. When the saponification is complete the solution is transferred to a small separating funnel and shaken with ether. A further 30 c.c. of ether is then added to the syrupy chlorophyllin salts. After shaking with water the upper ethereal layer contains the yellow pigments, the water the chlorophyllin salts. (Cf. Section E, experiment 6). After separation of the two layers, the watery layer may be again shaken with ether in order to determine whether the whole of the yellow pigments have been extracted.

The two yellow pigments are then separated according to the principle used in experiment 7. The ether solution is washed with water and methyl alcoholic potash to remove traces of chlorophyllins and small quantities of brown, acid, organic substances, and then twice more with distilled water. The ether is then evaporated at ordinary temperature under reduced pressure to a few c.c., and then transferred to 80 c.c. of petrol ether in a separating funnel.

From this the xanthophyll is extracted by repeated extractions with methyl alcohol as follows. (1) 100 c.c. 85%, (2) 100 c.c. 90%, (3) two extractions with 92%. If the last extraction is not colourless, further additions of 92% methyl alcohol are made.

In this way the xanthophyll in the methyl alcohol is separated from the carotin which remains in the petrol ether.

The xanthophyll is transferred to 130 c.c. of ether, by adding the latter to the methyl alcohol followed by slow addition of water and

subsequent separation of the watery methyl alcohol layer. The methyl alcohol is completely removed by further washings with water. The xanthophyll solution is then passed through a filter into a 100 c.c. measuring flask.

The solution is then cleared with a few drops of absolute alcohol and ether added up to the 100 c.c. mark.

The petrol ether solution of carotin is similarly washed, cleared and made up to 100 c.c.

4. *The Standard Solutions. The Chlorophyll Components.* The standard solution of the chlorophyll components are prepared by the saponification of a mixture of the methyl phæophorbides as follows:

0·0369 grams Methyl phæophorbide a (half hydrate)
(12×10^{-5} gm.-mols. per litre);

0·0124 grams Methyl phæophorbide b (water-free)
(4×10^{-5} gm.-mols. per litre).

The mixture is dissolved in 2 c.c. of pyridine and saponified with 35% methyl alcoholic potash in the manner described above, under (2), only as pure substances are used it is unnecessary to go through the treatment for purification with 12 and 20% acid. This gives about 500 c.c. phytochlorin e in ether saturated 3% acid, and of phytorhodin g in 12% acid.

These solutions can be kept for about a week, but after a longer time the colours change somewhat. In such a case the comparisons are better if the experimental solution is allowed to stand for a day.

The Yellow Pigments. The carotin solution is made up with petrol ether and the xanthophyll with ether, as follows:

0·0134 grams carotin in 500 c.c. petrol ether containing a little ether (5×10^{-5} gm.-mols. per litre).

0·0142 grams xanthophyll in 500 c.c. ether (5×10^{-5} gm.-mols. per litre).

The carotin solution can be kept in a well stoppered bottle in the dark for at least three weeks. The xanthophyll solution must be made fresh every day as it bleaches quickly, perhaps on account of impurities in the ether.

Instead of using solutions of the actual pigment it is possible to employ a solution of potassium dichromate as a standard solution. Willstätter gives the following thicknesses of aqueous potassium

dichromate solution and of the yellow pigments as corresponding to one another in colour intensity.

Carotin. 0·0286 grams per litre.	Potassium dichromate solution, 2 grams per litre.
100 mm.	101 mm.
50 mm.	41 mm.
25 mm.	19 mm.

Xanthophyll. 0·0284 grams per litre.	Potassium dichromate solution, 2 grams per litre.
100 mm.	72 mm.
50 mm.	27 mm.
25 mm.	14 mm.

ii. Results.

1. The Total Content of Green and Yellow Pigments.

As far as his observations go, Willstätter finds the chlorophyll content of leaves varies from 0·6% to 1·2% of the total dry weight, the greater number of leaves contain about 0·8% of chlorophyll of which three-quarters is chlorophyll a and one quarter chlorophyll b. Much bigger variations were observed between leaves from the same plant than between the mean contents of leaves from different plants. Shade leaves were found to be much richer in chlorophyll than sun leaves in proportion to the dry weight, but not in proportion to the leaf surface; for shade leaves, as is well known, are often very thin.

The total content of the yellow pigments (xanthophyll and carotin) was found to vary in different leaves from 0·1% to 0·2% of the dry weight, the xanthophyll contributing from 0·07% to 0·12%, the carotin from 0·03 to 0·08%.

Shade leaves were not found to contain a higher percentage of yellow pigments corresponding to their higher content of chlorophyll.

Leaves collected at different hours of the day were examined; the time of day was found to be without influence on the chlorophyll content or the ratio of the pigments. As this is of importance in regard to the function of the pigments in the processes of carbon assimilation we may quote here two tables taken from Willstätter's book to show how slight this variation is.

TABLE I.

Grams of Pigment in 1 kilo. dried leaves at different times of day.

Species.	Chlorophyll.		Yellow Pigments.	
	4 a.m.	5 p.m.	4 a.m.	5 p.m.
Sambucus nigra	8·49	8·30	1·48	1·57
Aesculus hippocastanum ...	9·58	8.75	2·07	1·91
Platanus acerifolia	6·82	6·21	1·06	1·35

TABLE II.

Ratio of Pigments in leaves at different times of day.

Species.	$\frac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$		$\frac{\text{Carotin}}{\text{Xanthophyll}}$	
	4 a.m.	5. p.m.	4 a.m.	5 p.m.
Sambucus nigra	2·77	2·85	0·621	0·512
Aesculus hippocastanum ...	2·89	2·82	0·699	0·699
Platanus acerifolia	3·52	3·34	0·478	0·500

2. *Variations in the Proportions of the Two Chlorophyll Components.*

Willstätter found that the composition of chlorophyll present in different plants, and in sun and shade leaves of the same plant is approximately, though not exactly, constant.

The mean ratio of $\frac{\text{chlorophyll a}}{\text{chlorophyll b}}$ is 2·85, the greatest difference from the mean ·7 to ·8.

The variations appear to be produced by the conditions under which the leaves are growing. Thus it seems that some plants are ill suited for growth in the shade. Leaves of Sambucus for example, living in the shade, show abnormal chlorophyll relations, whereas real shade plants such as the Beech exhibit a normal chlorophyll content.

On the whole, shade leaves contain relatively less chlorophyll a than do normal leaves. For if shade leaves are excluded the average ratio of $\frac{\text{chlorophyll a}}{\text{chlorophyll b}}$ is 2·93 with extreme variations from this mean of ·5 to ·6.

Shade leaves give an average ratio of $\frac{\text{chlorophyll a}}{\text{chlorophyll b}}$ of 2·61 $\pm$ ·55, a number appreciably smaller than the mean for normal leaves.

The time of day is found to have no influence on the ratio of pigments.

3. Variations in the Proportions of the Two Yellow Pigments.

The mean value of the ratio of carotin to xanthophyll was found by Willstätter to be 0·546 ± 0·15 to 0·2.

In the case of the yellow pigments, shade leaves show a wider divergence from the normal than they do in regard to chlorophyll. Thus normal leaves give an average ratio of $\frac{\text{carotin}}{\text{xanthophyll}}$ of 0·603 ± 0·1 corresponding to a molecular ratio of 1 : 1·5 to 2. In shade leaves on the other hand, the average ratio of carotin to xanthophyll was as low as 0·421 ± 0·1.

4. Relation between quantities of Green and Yellow Pigments.

The average molecular ratio of the total amount of green to the total amount of yellow pigment is 3·56, varying from 3·07 in the case of sun leaves to 4·68 in the case of shade leaves.

It will be observed that in the case of shade leaves the ratio of quantity of green to quantity of yellow pigments is raised. An exception to this rule was found in the Plantain. On the other hand in leaves well suited for growth in the shade values as high as 6 have been obtained for the ratio of green to yellow pigments.

It has been shewn above that in shade leaves the amount of chlorophyll a is raised in relation to that of chlorophyll b, while of the yellow pigments it is the xanthophyll which is relatively more abundant in these leaves: that is, of the green pigments the one poorer in oxygen is increased in amount, of the yellow pigments, the one richer in oxygen.

No simple relation could, however, be found between the ratios $\frac{\text{chlorophyll a}}{\text{chlorophyll b}}$ and $\frac{\text{carotin}}{\text{xanthophyll}}$.

G. Final Remarks.

In the preceding pages we have endeavoured to give the outlines of the work of Willstätter and his co-workers, and we have emphasised the value of this work as one of the most brilliant researches in organic chemistry. There has been in the past, and in spite of Willstätter's work there will probably be in the future, much loosely performed work on chlorophyll and involving the use of chlorophyll, so that it may be well to emphasise the criteria of purity of this substance that are now available.

These criteria are as follows:

1. The ash should consist of pure magnesium oxide, and should weigh 4·5% of the weight of chlorophyll used.

2. The phytol content should be one-third of the molecule.

3. The chlorophyll must contain no yellow pigments. On saponification as described in experiment 6, the ether layer must remain colourless.

4. By saponification with alkali, the brown phase must appear (experiment 4) showing that the chlorophyll is not allomerised.

5. Phytochlorin e and phytorhodin g must be given as dissociation products (experiment 8).

6. In solution the chlorophyll must give the same spectrum as leaf extracts (showing there is no phæophytin present which would give absorption bands before the line E and between the lines E and F).

We have earlier referred to Étard's work in which the existence of a huge number of chlorophyll substances is asserted. There has now recently appeared a paper by Albert and Alexandre Mary (1915) in which the authors claim to have synthesised chlorophyll from nitrous oxide and aniline. It is indeed surprising that these workers, as the result of the synthesis of a substance with a green colour and a complex absorption spectrum, should put forward conclusions so completely at variance with Willstätter's work. But perhaps these authors have as much justification for their conclusions as Ewart (1915) who from the observation of a substance with a yellow colour and a simple absorption spectrum possessed by hundreds of substances, deduces the presence of xanthophyll in his preparations. It is perhaps significant that the "pure xanthophyll" extracted by Ewart should have properties different from Willstätter's.

The conclusions of Albert and Alexandre Mary and of Ewart have perhaps as sound a basis as that of Wager (1914), who is of opinion that *chlorophyll* is an auto-oxidisable substance which "in fact could replace pyrogallol in the quantitative estimation of the oxygen in the air." The simple phase test described in experiment 4 in section E of this chapter would have shown in this author's chlorophyll, the presence of the yellow pigments, which are of course autoxidisable (cf. experiment 6).

It may be well here to point out that Willstätter's researches only confirm the observations of the English physicist G. G. Stokes, whose work is mentioned by Willstätter with much respect. A few quotations from Stokes' work will show how near he came to the truth. In a paper published in the Proceedings of the Royal Society of London for 1864 he writes, "I find the chlorophyll of

land plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties. The green substances yield solutions exhibiting a strong red fluorescence, the yellow substances do not. The four substances are soluble in the same solvents and three of them are extremely easily decomposed by acids or even acid salts, such as bis-oxalate of potash, but by proper treatment each may be obtained in a state of very approximate isolation so far at least as coloured substances are concerned."

Although it is a matter of national pride that the discovery of the four leaf pigments should have been made by a British worker, yet on the other hand the almost complete neglect with which later investigators in this country have treated Stokes' work is certainly very discreditable. When the obsession for demonstrating the presence of formaldehyde in the leaf (started by Baeyer's hypothesis in 1870, and first 'experimentally' investigated by Pollacci in 1902) began in this country with the work of Usher and Priestley in 1906, these writers neglected the presence of *two* green pigments and completely left out of consideration the yellow pigments in their theory of carbon assimilation. How much of the recent inconsequent work on the same subject might have been avoided if all these later writers had been aware of, and taken notice of, the work of Stokes.

Again, the extraction and separation of the pigments without the aid of chemical action is due to Stokes. In a paper in the Journal of the Chemical Society for 1864, he says "For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation. Bisulphide of carbon in conjunction with alcohol enabled the lecturer to disentangle the coloured substances which are mixed together in the green colouring matter of leaves."

The use of nettle leaves for extraction of chlorophyll was also recommended by Stokes in a paper published in Transactions of the Royal Society in 1852.

Considering that these observations were only side issues of Stokes' work, it is very remarkable that they should have been so correct. There can be no doubt that he did a great deal more work on chlorophyll than appears from his published work. He announced his intention of publishing work on chlorophyll, but it never appeared, and apparently nothing has so far been found among his papers referring in detail to these investigations.

It is well to remember that Willstätter's work has not exhausted chemical investigation on the subject of leaf pigments. There is a great deal yet which is not clear, and much which is very *hypothetical*, as for instance, the relation between the two green pigments, the reactions occurring in the changes from chlorophyll to chlorophyllin salts, the oxidations and reductions of chlorophyll derivatives, and above all, that which is of the greatest interest to us, the photo-chemistry of chlorophyll. On this last subject we have so far had no publication from Willstätter, although it is evident from his papers that he has been working at it and has realised that the phenomena of carbon assimilation such as we know them in living plants cannot be imitated by experiments with the four pigments "in vitro." Knowledge of the photo-chemistry of chlorophyll will probably help us to estimate the true significance of many of the observations which have already been made on chlorophyll outside the plant.

We have so far mainly dealt with Willstätter's work in organic chemistry, and in a later chapter we shall discuss Willstätter's plant physiological work; before concluding this chapter it must, however, be mentioned that Willstätter's physico-chemical work, that on the state of aggregation of chlorophyll, for example, does not appear so brilliant and convincing as his work in organic chemistry. Although the extension of our knowledge of the colloidal state of chlorophyll must be regarded as a great advance, yet Willstätter's arguments and experiments on this point are not very complete, and he seems intentionally to avoid any detailed discussion of the question. The reason for this may be found in the fact that before the subject is properly attacked, an investigation of the colourless substances which accompany the pigments in the chloroplasts, as thorough and as detailed as that of the pigments themselves, is necessary. It is to be hoped that Willstätter or some other equally capable organic chemist will direct his attention to this subject which so much needs investigation.

Willstätter's work is one of those monumental pieces of research which are of permanent value. In the following chapters we shall deal with another piece of work which will always retain its value—the work of F. F. Blackman on the intake of carbon dioxide by the leaf.

Chapter III.

The Path of Gaseous Exchange.

The passage of carbon dioxide from the outside medium into the leaf in the case of submerged water plants almost certainly takes place by diffusion in aqueous solution through the outer walls of the epidermal cells in the same way that substances will diffuse from cell to cell within the plant.

In the case of lower plants like the mosses the path must be the same, as the surface layer of the leaf is uniform throughout. In the higher land plants, on the other hand, there are two paths by which gases might diffuse into and out from the leaf. There might be diffusion through the cuticle of the epidermal cells as in submerged water plants or mosses, or the diffusion of gases might be principally through the small perforations, stomata, which occur in varying abundance over one or both surfaces of the leaves of higher plants, but which comprise only a fraction of the total area of the leaf. It is a possible alternative that both cuticle and stomata may be utilised for diffusion, in which case it becomes of interest to determine the relative importance of the cuticle and stomata in gaseous diffusion into the leaf.

The work on the paths of gaseous exchange before the researches of F. F. Blackman, like the work on chorophyll before Willstätter's, is all open to the criticism that the experimental methods used were imperfect. It is therefore not to be wondered at that a mass of contradictory results was obtained, and that none of the views of earlier workers had been established. It will be sufficient for us to refer here to the observations of Garreau (1850), Merget (1877-8), Wiesner (1879), Boehm (1889) and Wiesner and Molisch (1889), who have urged that the stomata are the path of gaseous exchange, while Boussingault (1868) and Barthélemy (1868) have advocated the contrary view, that the intake of carbon dioxide takes place through the cuticle. Mangin (1888) took up an intermediate position that diffusion through the cuticle is insufficient to account for the whole of the gaseous exchange. During assimilation he concluded that practically all the gaseous exchange takes place through the stomata as the pressure of carbon dioxide in the external air is insufficient to cause much diffusion through the cuticle.

It is unnecessary for us to go into a detailed description of the results and conclusions of these workers nor into a criticism of

their results. It is enough to say that none of them have furnished indisputable evidence on the matter, and their work becomes chiefly of historical interest after the clearing up of the problem by F. F. Blackman in whose papers on the subject (1895 a, 1895 b) a summary and criticism of earlier work is to be found.

The essence of Blackman's work, is the measurement of the quantity of carbon dioxide passing in and out of the two surfaces of living leaves on which the distribution of the stomata is known. For this work it was necessary to devise a special apparatus by which could be measured the small quantities of carbon dioxide with which one has to deal in such experiments. This apparatus is described in the first of Blackman's papers (1895 a). By its means a current of air either free from carbon dioxide or containing any desired concentration of this gas, is passed over the surface of a leaf in a closed chamber and the intake or evolution of carbon dioxide by the leaf measured. This is effected by estimation of the carbon dioxide in the gas leaving the leaf-chamber by passing this through standard baryta solution which is subsequently titrated against standard hydrochloric acid solution.

For details of the apparatus we must refer to the description in the paper cited above. It is especially noteworthy that although the apparatus is complicated yet the manipulation is exceedingly simple, consisting only in the turning of taps. The different parts of the apparatus are in duplicate so that two different surfaces of a leaf or two different parts of a plant, can be examined under exactly similar conditions at the same time.

Special mention should be made of the plant chamber by means of which the two surfaces of a leaf can be examined simultaneously. This chamber consists of two circular rims of brass, 5 millimetres deep and 36 millimetres in diameter, to one face of each of which is hermetically cemented a plate of thin glass. Through the brass rim are drilled at opposite ends of a diameter two small holes, into each of which a copper tube of 1 millimetre bore is soldered. These form the channels by which the gas enters and leaves the chamber. For convenience of handling one tube is curved half way round the rim of the half chamber so that it lies parallel with the other. The leaf to be examined is slipped between the two half-chambers and hermetically sealed to them by means of wax, and the leaf is then ready for experimentation. For leaves of different forms, plant chambers of different shapes may be used.

Blackman experimented with various kinds of leaves, including

those where the stomata were limited to the lower surface only and those where the stomata were present on both surfaces. Usually the carbon dioxide evolved from the leaf into a current of air free from that gas was the quantity measured, but results were also obtained for the intake of carbon dioxide which show the path traversed by the gas is the same whether it is travelling into or out of the leaf.

In the following table we have summarised the results obtained by Blackman for the ratios of the amounts of carbon dioxide given out from the two surfaces of the leaves of various plants.

TABLE III.

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

The numbers in the foregoing table show how constantly the path of carbon dioxide from the leaf follows the distribution of the stomata. Similar results were obtained in the case of carbon dioxide absorbed in assimilation. Thus, in the cases of *Ampelopsis hederacea*, *Platanus occidentalis* and *Polygonum sacchalinense*, where all the stomata occur on the under surfaces of the leaves all the carbon dioxide was found to enter by the lower surface. None

was taken up by the astomatic upper surface. In the case of *Alisma plantago*, which has stomata on both sides of the leaves, there was found in every experiment performed a constant tendency for the absorption of carbon dioxide to be greater by the upper surface where the stomata are more frequent. Confirmatory results were obtained with *Tropæolum majus* and *Acer platanoides*.

From these experiments the conclusion is drawn that the intake and evolution of carbon dioxide takes place through the stomata. The only alternative explanation is that in the case of leaves with the stomata confined to the lower surface the cuticle on the lower surface is fifty to a hundred times more permeable to carbon dioxide than the lower surface. It seems impossible to suppose this the case, especially as leaves with thin cuticles gave results exactly similar to those obtained with leaves possessing very thick cuticles.

Although accepting Blackman's results in regard to the exhalation of carbon dioxide in respiration, Brown and Escombe (1905 a) are of opinion that his method is not so well adapted to investigations of the intake of carbon dioxide in assimilation, chiefly because the amounts of carbon dioxide dealt with seldom exceeded 0·1 c.c. with a possible experimental error of one-tenth of that amount. Brown and Escombe therefore performed some experiments similar to Blackman's under conditions which admitted the measurement of carbon dioxide taken in by the two sides of a leaf on which the distribution of stomata was known.

The following tables taken from Brown and Escombe's paper exhibit their results.

TABLE IV.

Respiration from the two Surfaces of various Leaves.

Plant.	Time in hours.	Leaf area in sq. cms.	CO_2 evolved in c.cs. Upper / Lower	Ratio of CO_2 evolved. Upper / Lower	Ratio of stomatic distribution Upper / Lower
Canna indica	4·75	28·27	8·41 / 20·76	100 / 246	100 / 246
,, ,,	5·0	28·27	5·55 / 17·90	100 / 322	,,
,, ,,	4·23	28·27	3·04 / 6·40	100 / 210	,,
Rumex alpinus	5·5	59·44	1·03 / 3·60	100 / 286	100 / 269

TABLE V.

Assimilation by the two Surfaces of various Leaves illuminated on Upper Surface.

Plant.	Time in hours.	Leaf area in sq. cms.	CO_2 assimilated in c.cs. Upper / Lower	Ratio of CO_2 assimilated Upper / Lower	Ratio of stomatic distribution Upper / Lower
Colchicum speciosum	5·75	59·44	4·34 / 3·26	100 / 72	100 / 119
Senecio macrophyllus	4·75	28·27	3·90 / 3·60	100 / 92	100 / 126
,, ,,	4·25	28·27	5·80 / 4·20	100 / 72	100 / 126
Rumex alpinus	5·0	59·44	5·70 / 8·90	100 / 144	100 / 269
,, ,,	5·5	59·44	7·50 / 9·81	100 / 130	100 / 269
Nuphar advenum	2·0	76·97	2·20 / 0·00	100 / 0	100 / 0
Catalpa bignonioides	1·85	79·03	0·00 / 4·91	0 / 100	0 / 100
,, ,,	2·3	79·03	0·00 / 8·96	0 / 100	0 / 100

Brown and Escombe's results confirm Blackman's in regard to leaves with the stomata confined to one surface. With leaves bearing stomata on both surfaces, when illuminated on the upper surface there is always less intake of carbon dioxide by the lower surface than might be expected from the relative distribution of the stomata over the two surfaces, whereas the ratio of carbon dioxide respired from the two surfaces follows very closely the ratio of stomatal distribution.

Brown and Escombe explain this result in the following way. During respiration, if there is a steady evolution of carbon dioxide, the rate at which this will escape from the leaf will be independent of the degree of opening of the stomata, for should the stomatal aperture decrease, the partial pressure of carbon dioxide inside the leaf will correspondingly increase and the rise in 'diffusion potential' will counterbalance the effect of diminished stomatal aperture.

In the case of assimilation, on the other hand, the 'diffusion potential' will remain constant, for the partial pressure of the carbon dioxide diffusing inwards varies constantly from 0·0003 atmosphere outside the leaf to zero where there is complete absorption of the carbon dioxide. Hence, if the stomatal opening

varies, the rate of intake of carbon dioxide must also vary and the results obtained may be due to the greater degree of opening of the stomata on the illuminated side. Brown and Escombe also think that as more energy is absorbed by the chloroplasts of the palisade parenchyma, carbon dioxide will be more rapidly utilised in that part of the leaf and consequently the diffusion gradient will be steeper in the intercellular spaces of the palisade into which the stomata of the upper surface open, which will also favour a more rapid intake of carbon dioxide by the stomata of the upper surface.

Further evidence as to the path of carbon dioxide into and out of the leaf has been obtained by investigating the gaseous exchange when the stomata are artificially blocked. It had previously been asserted by Boussingault that the path of carbon dioxide intake was through the cuticle. This conclusion was based on an experiment in which leaves of Nerium were painted over with lard. In one the astomatic upper surface was so covered, in the other the lower surface, with the result that the leaf with its stomata blocked assimilated more. Blackman shows that this result is due to the use outside the leaf of too high a concentration of carbon dioxide (more than 30%).

The results of Blackman's own experiments with leaves of *Nerium oleander* are given in the subjoined table and show clearly the effect of increasing the carbon dioxide concentration.

TABLE VI.

Mean percentage of CO_2 present in each experiment.	CO_2 in c.c. decomposed per unit area.		Ratio of amount of CO_2 decomposed per unit area.	
	Normal leaf.	Vaselined leaf.	Normal leaf.	Stomata blocked.
6	0·07	0·01	1	0·14
6·3	0·055	0·01	1	0·20
7·5	0·046	0·017	1	0·21
14	0·18	0·04	1	0·37
55	0·049	0·067	1	1·3
50	0·043	0·069	1	1·5
97	0·033	0·060	1	1·8

Some further experiments made with *Nerium oleander* show that more carbon dioxide passes through the vaselined under surface than through the unvaselined cuticle of the upper surface, so that coating the leaf with vaseline does not render it impervious to the passage of carbon dioxide.

Injecting the leaf with water has a similar influence on altering the ratio of CO_2-intake by the two surfaces as coating the lower surface with vaseline. By such injection the intercellular spaces are filled with water and diffusion of carbon dioxide can then only take place in solution; hence the stomatal surface no longer possesses such an advantage over the upper surface in regard to the passage of carbon dioxide through it. The following table shows the relative amounts of carbon dioxide respired from the two surfaces of leaves under various conditions.

TABLE VII.

Condition of Leaf.	Relative output of CO_2.	
	Upper surface.	Lower surface.
Normal leaf	1	39
Injected ,,	1	10
Vaselined under surface	1	3

Blackman thus comes to the conclusion that the epidermis with its cuticle is slightly permeable to carbon dioxide, but that under normal conditions, by far the greater part of gaseous exchange takes place through the stomata. Under artificial conditions, such as waterlogging the intercellular spaces or blocking the stomata, the passage of carbon dioxide through the cuticle, though not actually greater, may become of relatively more importance.

An extended series of experiments bearing on the same matter of the path of carbon dioxide into the leaf has been made by Stahl (1894). His method consisted in artificially blocking the stomata on parts of the leaf and showing that after exposure to light, starch formation is limited to the regions of the leaf where the stomata were unblocked. Some similar experiments were made independently by Blackman who confirms Stahl's observations.

The experiments of Blackman, Stahl and Brown and Escombe appear to show conclusively that the path of diffusion of carbon dioxide into the leaf is mainly or entirely through the stomata. But there are three facts which rendered difficult the acceptance of this evidence alone. These facts are (1) the large amount of carbon dioxide absorbed by a leaf during active assimilation; (2) the low partial pressure of carbon dioxide in the atmosphere—the carbon dioxide only amounts to about 3 parts per 10,000 of the atmosphere; and (3) the very small fraction of the leaf surface occupied by the stomata.

Brown and Escombe have shown that the leaf of *Catalpa bignonioides* can absorb from ordinary air 0·07 c.c. of carbon dioxide measured at N.T.P. per sq. cm. of leaf surface per hour. The area of the stomatal openings is only 0·09% of the total leaf surface. Hence diffusion through them must take place at the rate of 7·77 c.c. per sq. cm. per hour.

Now experiments made by Brown and Escombe showed that a normal solution of sodium hydroxide exposed to moderately still air containing about 3 parts of carbon dioxide per 10,000 absorbs this gas at ordinary temperatures at the rate of about 0·120 c.c. per sq. cm. of absorbing surface per hour, and this is only increased to a maximum value of 0·177 c.c. per sq. cm. per hour when the rate at which the air is passed over the absorbing solution is increased.

Hence, if the diffusion of carbon dioxide into the leaf takes place entirely through the stomata, this absorption of carbon dioxide must take place about 50 times as fast as it would by a solution of normal sodium hydroxide of which the exposed surface had the same area as the stomata.

Brown and Escombe were thus led to investigate the rate of diffusion of gases through small apertures in a septum. Their method of procedure was as follows: 200 c.c. of normal sodium hydroxide were placed in a flat-bottomed flask which was left open in comparatively still air containing the normal amount of carbon dioxide. The surface of the liquid was about 10 cm. in diameter. A very steady and uniform absorption then took place at the rate of about 0·25 c.c. carbon dioxide per hour.

In order to obtain a suitably perforate septum between the absorbing liquid and the outer air, the neck of the flask was passed through the bottom of a small glass cup to which it was cemented. The annular space of the cup was then filled with mercury. A flat-bottomed nickel crucible was inverted over the mouth of the flask so that the edges dipped into the cup of mercury, and in this way a perfect mercury seal was obtained. A hole of the desired size was made in the bottom of the nickel crucible.

A number of such pieces of apparatus with variously perforated septa were prepared at the same time, and after displacing the air in them with air freed from carbon dioxide they were exposed to the atmosphere under the same conditions. As a result of these experiments, Brown and Escombe came to the conclusion that with small apertures the rates of diffusion are proportional, not to the areas, but to the diameters of the opening. The following table

summarises their results with regard to carbon dioxide. Similar results were obtained with water vapour.

TABLE VIII.

Diffusion of carbon dioxide through apertures of various sizes.

Diameter of aperture.	CO_2 diffused per hour.	CO_2 diffused per sq. cm. per hour.	Ratio of areas of apertures.	Ratio of diameters of apertures.	Ratio of CO_2 diffused in unit time.
22·7	·2380	·0588	1·00	1·00	1·00
12·06	·09280	·0812	·28	·53	·39
12·06	·10180	·0891	·28	·53	·42
6·03	·06252	·2186	·07	·26	·26
5·86	·05558	·2074	·066	·25	·23
3·23	·03988	·4855	023	·14	·16
3·22	·03971	·4852	·020	·14	·16
2·12	·02608	·8253	·008	·093	·10
2·00	·02397	·7629	·007	·088	·10

In order to explain this result, Brown and Escombe consider first the case of a disc capable of absorbing carbon dioxide and freely exposed to the air. If the latter is perfectly still, convergent streams of carbon dioxide will creep through the air towards the disc to replace that absorbed, and a steady gradient of density will be established, and if surfaces are drawn passing through all the points of the same carbon dioxide density, these surfaces will form 'shells' surrounding the disc. If the disc is a perfect absorbent of carbon dioxide, these shells will vary in density from zero at the absorbing surface to a maximum density which is that of carbon dioxide in air. This will theoretically be at an infinite distance from the disc but is practically reached at a point 5 or 6 diameters from the disc. Now Stefan has examined mathematically the exact converse of this case, namely, evaporation from a circular surface of liquid. Stefan obtained the following formula for the amounts of evaporation from such a surface :—

$$M = 4ka\,\frac{P-p''}{P-p'}$$

Where M is the mass of liquid evaporated in a given time, k the coefficient of diffusion of the vapour, a the radius of the disc of liquid, P the pressure of the atmosphere and p' and p'' the pressure of the vapour at the surface and at an infinite distance from it respectively.

The formula given by Larmor for the absorption of carbon dioxide by a perfectly absorbing disc, assuming the formation of

such shells of equal density is essentially the same. It is:—

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

where Q is the quantity absorbed in any time,

k the coefficient of diffusion of carbon dioxide in air,

ρ the density of atmospheric carbon dioxide,

D the diameter of the disc.

Brown and Escombe explain their results in regard to the rate of diffusion through perforate septa as due to the same cause, namely, that when a gas is diffusing through such a perforate septum, shells of equal density are formed outside the perforation just as in the case of the absorbent disc, and the same 'diameter law' will hold.

The accompanying diagrams show the various systems of shells. Fig. 1 is the case of the shells over a perfectly absorbent disc. The density of the diffusing gas varies from ρ at a remote distance from the surface to zero at the surface itself. In Fig. 2 are represented the shells produced on the inner side of a perforated diaphragm opening into a large space in which the gas is rapidly absorbed and where the density of the gas at the perforation is kept at a maximum by a constant current of air. The density of the gas here varies from ρ at the diaphragm to zero at the surface. In Fig. 3 is represented the case of a perforated septum like the

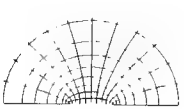


Fig. 1.

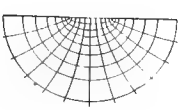


Fig. 2.

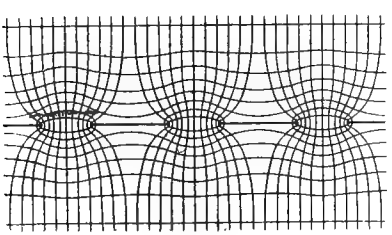


Fig. 4.

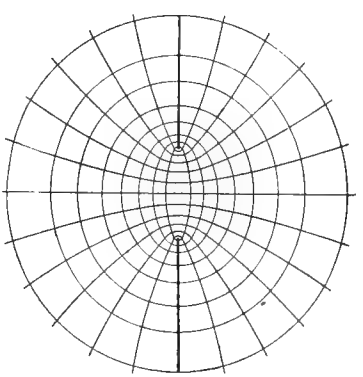


Fig. 3.

FIGS. 1—4.

FIGS. 1 AND 2. Diffusion "shells" formed outside and inside a perforation in a septum.

FIG. 3. Diffusion "shells" outside and inside a perforation in a septum in perfectly still air.

FIG. 4. Lines of flow through a multiperforate septum.

case shown in Fig. 2, but in which there is perfectly still air outside, so that shells are produced outside as well as in. Here the density of the gas will vary from ρ at a remote distance outside to ρ_1 at the perforation to zero at the absorbing surface.

In the case of the leaf in still air, we are dealing with an approximation to this last case, but as in actual fact there will always be more or less movement of air outside the leaf there will be a corresponding approach to the conditions indicated in Fig. 2.

If the partial pressure of carbon dioxide in ordinary air is called P, then in the last case, from Larmor's formula, we have the quantity passing through any shell outside $= 2k(P—P_1)D$, where D is the diameter of the perforation. Similarly, the quantity passing through any shell inside $= 2kP_1D$.

Now when a constant flow is established, these two quantities must be the same so that

$$2k(P—P_1)D = 2kP_1D$$

$$\text{whence } P—P_1 = P_1$$

$$\text{or } P = 2P_1$$

That is, in still air the pressure of carbon dioxide at the perforation will only be one half of the pressure of the gas there when this is kept constantly renewed. Consequently the diffusion gradients will only be half as steep in the former case and the rate of absorption will correspondingly be reduced to half.

Similarly, in the case of the leaf, the rate of passage of carbon dioxide will be increased in the same way when the air outside the leaf is constantly renewed, provided that the cells surrounding the space into which the stomata open are perfect absorbers of the gas. Also, other things being equal, the velocity of flow through the stomata will be proportional, not to the areas of the stomata, but to their diameters.

Since the surface of the leaf is perforated, not by one, but by many stomata, the researches of Brown and Escombe on diffusion through multiperforate septa become of great interest in relation to the intake of carbon dioxide. The multiperforate septa consisted of sheets of celluloid of thickness 0·08 to 0·1 mm. in which a series of holes at definite distances from one another were punched. The septa were fixed to the open ends of glass tubes containing sodium hydroxide. The following table gives some of Brown and Escombe's results. In each case the area of cross sections of the tubes was measured and the diffusion through the perforate septum compared with that down an open tube.

TABLE IX.

Diameter of each hole 0·380 mm.

Length of tube 1·0 cm.

Distance of holes apart in diameters.	Number of holes per sq. cm. of septum.	Percentage area of holes on unit area of septum.	$\frac{\text{Septum diffusion}}{\text{Open tube diffusion}} \times 100.$
2·63	100·00	11·34	56·1
5·26	25·00	2·82	51·7
7·8	11·11	1·25	40·6
10·52	6·25	·70	31·4
13·1	4·00	·45	20·9
15·7	2·77	·31	14·0

It will be observed from these numbers that the obstruction offered to the diffusion of gases by a multiperforate septum is considerably less than the actual obstruction of area. Thus when the area of perforation was less than 3% of the whole area of the septum, the actual diffusion through the perforations was 51·7% of the diffusion taking place through an open tube of the same area of cross section. That is, the diffusion through the septum is nearly 15 times as great as it would be if it were simply proportional to the area of the cross section. As the distance between the holes is increased, the efficiency of the area of the perforations increases until the holes are about 10 diameters apart. In this case and in cases where the distance apart of the holes is increased, the diffusion through the perforations is about 40 times as much as it would be if it were proportional to the area of cross section of the tube.

The accompanying figure (Fig. 4) illustrates what Brown and Escombe imagine to be the lines of equal density and the lines of flow of gas through such a multiperforate septum. The lines of flow of gas diffusing towards the septum will be approximately parallel at some distance from the septum, but as they pass through the perforations they converge, the velocity of flow increasing at the same time owing to the production of ellipsoidal density shells round the opening. After passing through the opening, the lines of flow diverge and as lines of flow from adjacent perforations cannot cross each other (for otherwise there would be shells of different density crossing each other, which is impossible) they must bend round and become once more parallel, the velocity of flow at the same time diminishing. From a consideration of Fig. 4, it is easy to understand how it is that the perforations under favourable conditions of distribution are so efficient for diffusion through them.[1]

Brown and Escombe's results lead them to the general

[1] Brown and Escombe's work deals only with the simpler cases of diffusion through perforate septa. A fuller treatment of the subject in regard to the diffusion of water vapour has more recently been the subject of investigation by Renner (1910).

conclusion that "the interference of the density shells of small holes set at 10 diameters or more apart is small, each hole beyond this limit acting almost independently according to the diameter law."

Now in the leaf of *Helianthus annuus*, for example, the stomata on the under surface are actually about 8 diameters apart. The stomata themselves open into cavities in which shells of diffusion may form. The under surface of such a leaf is therefore a multiperforate septum in which the perforations are so far apart that practically each single opening can exercise its full efficiency as regards diffusion through it, without interference from its neighbours. We may, therefore, expect the diameter law to hold, and the rate of diffusion of carbon dioxide through the stomata to be proportional to the linear dimensions of the stomata.

Assuming the stomata to be circular in shape instead of elliptical as they actually are, Brown and Escombe have worked out the quantity of carbon dioxide capable of diffusing into the leaf under various conditions. Under the most favourable circumstances, when the stomata are wide open and the carbon dioxide in the air outside the leaf is in constant motion so as to maintain the greatest possible pressure of carbon dioxide there, we have the following data:—

Diameter of stoma, 0·00107 cm.

Length of tube, 0·0014 cm.

Number of stomata per sq. cm., 33,000.

Area of cross section of stoma, $9{\cdot}08 \times 10^{-7}$ sq. cm.

Under these circumstances the theoretical value for the quantity of carbon dioxide absorbed by the leaf is 2·578 c.c. per sq. cm. per hour.

If, on the other hand, the air outside the leaf is perfectly still the maximum quantity of carbon dioxide entering the leaf is 2·095 c.c. per sq. cm. per hour.

These values are far higher than the observed quantities of carbon dioxide taken in by the leaf. Thus Thoday (1910) found a leaf of *Helianthus annuus* was capable of increasing in dry weight by about ·17 milligrams per hour per sq. cm. which corresponds to an intake of carbon dioxide of only about 0·14 c.c. of carbon dioxide per hour measured at normal temperature and pressure.

Hence, the stomata, in spite of the relatively small area of the whole leaf surface they occupy, could yet allow the diffusion through them of many times as much carbon dioxide as actually passes through. There is then, every reason to regard the results of Blackman and Brown and Escombe as affording definite proof that the path of intake of carbon dioxide into the assimilating aerial leaf of higher plants is mainly through the stomata.

Chapter IV.

The Factors Influencing the Intake of Carbon Dioxide.

A. General Remarks.

As we have already said in our introductory chapter, carbon assimilation is a complex of processes which probably obey quite different laws. Thus we know that one or more of these processes must be photo-chemical since light is required for carbon assimilation. Consequently one would hardly expect to express the relation between the amount of carbon dioxide used and the various factors which influence the intake of carbon dioxide in a simple way.

It is the great merit of F. F. Blackman that many years ago he called attention to the complexity of the processes of carbon assimilation, and showed that it was impossible to construct such a curve as a temperature-assimilation curve without regard to the possible effects of other factors. The result of Blackman's analysis of the intake of carbon dioxide under various conditions is expressed in his principle of limiting factors, and summed up in his work 'Optima and Limiting Factors' in Annals of Botany for 1905, a paper with which every student of plant physiology should be well acquainted, as the considerations contained therein are so fundamental for all biological processes.

Before F. F. Blackman's publications, investigators dealing with the influence of a factor on any physiological process, spoke of the factor having minimum and maximum values, below and above which the process does not take place and an optimum value at which the process proceeds at its greatest rate. In carbon assimilation there was alleged to be an optimum value of temperature at which assimilation is greatest. Similarly, there was supposed to be an optimum carbon dioxide supply and an optimum illumination for carbon assimilation. The optimum values obtained by different authors did not show any concordance, and Blackman pointed out that the method of experimentation in which, for instance, the influence of carbon dioxide and light are neglected when the effect of temperature is considered, is utterly illogical and cannot be expected to give results of any clear value.

Blackman states the principle of limiting factors as follows: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor."

Thus the rate of carbon assimilation in the leaf may depend on five obvious factors:—

1. Carbon dioxide supply.
2. Water supply.
3. Intensity of illumination.
4. The quantity of chlorophyll.
5. Temperature.

Any one of these might act as a limiting factor in assimilation.

We may quote with advantage an illustration of the operation of limiting factors given by Blackman. A leaf is supposed to have so much light falling on it as would give energy sufficient to decompose 5 c.c. of carbon dioxide per hour. If, now, the leaf is subjected to such a pressure of carbon dioxide that 1 c.c. of carbon dioxide is assimilated by the leaf per hour, there is sufficient energy provided to enable the whole of this carbon dioxide to be assimilated. When the pressure is raised to double the amount, so that 2 c.c. diffuses into the leaf per hour, the energy is sufficient to bring about the assimilation of the whole of the carbon dioxide, and so on until the pressure has been increased to five times its original value. But if the carbon dioxide supply is further increased, no further increase in carbon assimilation will take place as the energy is only supplied at a rate sufficient to allow 5 c.c. of carbon dioxide to be assimilated in an hour. Whatever the value of carbon dioxide supply above this value, the amount of assimilation will always be the same, *i.e.*, the maximum possible for the value of light intensity. The curve connecting assimilation and carbon dioxide supply will therefore be of the form ABC (Fig. 5). On the other hand, if the light intensity be now increased to double its value, it will be sufficient to allow 10 c.c. of carbon dioxide to be assimilated in an hour, and increases in carbon dioxide supply, will result in a steadily increasing carbon assimilation with increasing carbon dioxide supply, until this latter gives an assimilation of 10 c.c. an hour, when illumination will again put a limit on assimilation, and a curve of the form ADE will be obtained. With still stronger light, the curve AFG would be produced. Thus it is impossible to investigate the relation between carbon dioxide supplied and the amount of assimilation without considering the factor of light. Similarly, other factors must be taken into account and care must be taken that a factor other than the one under consideration is not acting as a limiting factor.

The principle of limiting factors is, of course, of general application where a process depends on a number of factors. It is, indeed, rather an elaboration of the 'Law of the Minimum' which had been applied to agricultural problems by Liebig as far back as 1843.

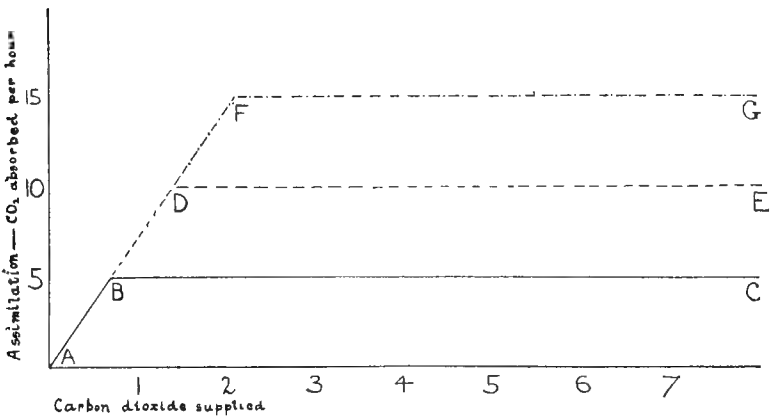


FIG. 5. Scheme to illustrate the action of a limiting factor. (After F. F. Blackman).

The question of the optimum value of a factor requires some consideration.

As regards temperature we have already referred to the van't Hoff rule that for every rise of 10°C the rate of a chemical reaction is doubled or trebled. If this law were followed throughout it is clear that there could be no optimum temperature for assimilation, which would increase more and more rapidly with increasing temperature. As a matter of fact for several plant processes the van't Hoff rule is followed between say 5°C and 29°C, but at higher temperatures it is quite clear that the rate of metabolic change in the organism slows down and the rule does not express the relation between temperature and the process.

To explain this slowing down at high temperatures Blackman introduces a 'time factor.' Thus at 25°C and lower temperatures the initial assimilation rate is maintained unchanged for a considerable time, but at higher temperatures, 30°C and over, although the leaf after exposure to light commences to assimilate at a rate given by the van't Hoff rule, this initial rate of assimilation cannot be maintained but falls off regularly, and the higher the temperature the more rapid the falling off. If then the assimilation at various

temperatures is measured over a considerable time it is certain that a temperature will be found at which assimilation is at a maximum. This apparent optimum temperature will however vary according to the time elapsing between the commencement of assimilation at that temperature and the actual measurement of assimilation owing to the rapid falling off in assimilation due to the time factor.

We shall later discuss the time factor in more detail, but in this place we would comment on the use of such expressions as 'time factor.' It is of course desirable that the same terminology should be used wherever possible in physiology as is employed in pure chemistry and physics. But while analysis of physiological processes has not proceeded far this is not always possible, and it is to the credit of F. F. Blackman that he has introduced the terms 'limiting factor,' 'time factor,' which permit discussion of physiological processes without involving premature assumptions as to their nature.

The work of F. F. Blackman and his pupils has been largely concerned with the influence of the various factors temperature, light and carbon dioxide supply on the rate of carbon assimilation. Before the publication of his 'Experimental Researches on Vegetable Assimilation and Respiration' there had indeed been much work on the influence of these factors on assimilation, but as none of these previous workers had recognised the principle of limiting factors it seems unnecessary for us to discuss their results here. Again the search for an optimum value of the various factors has not helped to elucidate the problems.

We shall first deal with the work of Blackman and his pupils on the relation between assimilation and the chief environmental factors, carbon dioxide supply, light intensity and temperature. Blackman expresses this relation as being such that "the magnitude of this function in every combination of these factors is determined by one or other of them acting as a limiting factor. The identification of the particular limiting factor in any definite case is carried out by applying experimentally the following general principle. When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and of that one alone, will be found to bring about an increase of the magnitude of the function."

We give here (Fig. 6) the curves obtained by Blackman and Smith (1911 b) showing the inter-relationship between carbon assimilation and the three external factors in the case of *Elodea*.

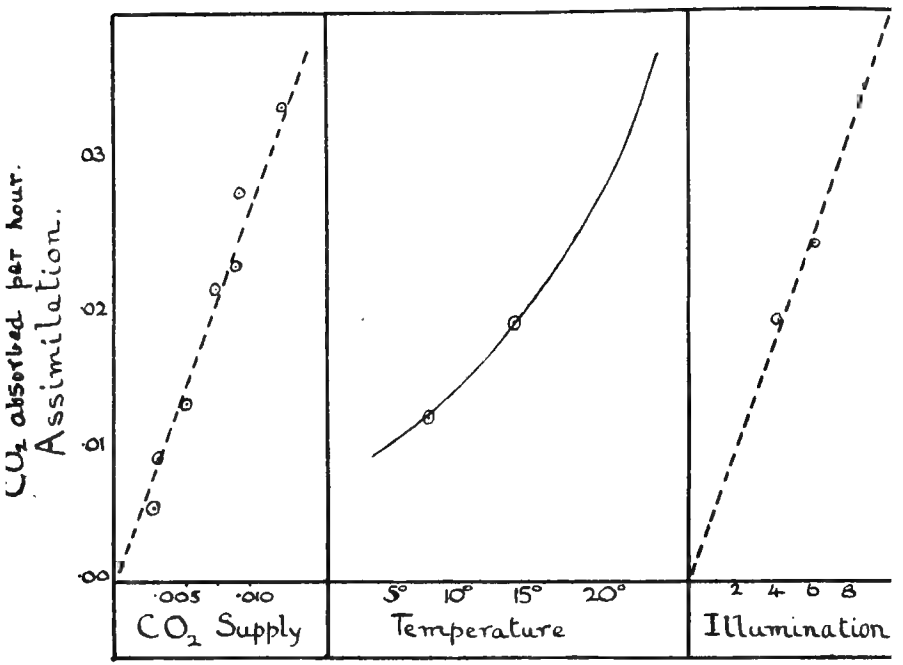


FIG. 6. Inter-relation of environmental factors and assimilation in *Elodea*. (After Blackman and Smith).

The first curve shows the relation between assimilation and carbon dioxide supply when this latter is the limiting factor; the second curve shows the amount of assimilation at different temperatures when temperature is the limiting factor, and the third the relation between assimilation and illumination when the intensity of light is limiting. From these three curves we can obtain the minimum of carbon dioxide supply, temperature and light intensity which are required for any quantity of assimilation.

B. Temperature.

From the researches of van't Hoff it is well known that the relation between temperature and the reaction velocity of a good many chemical reactions can be expressed in a simple way, revealing the fact that in many cases the reaction rate at moderate temperatures is increased 2 or 3 times for a rise of 10°C. On the other hand, animal physiologists have shown that although a smooth temperature-metabolism curve can be constructed which gives the relation between temperature and respiration, yet this curve does not obey the van't Hoff rule. Similar curves have been obtained for plant respiration by Kuijper (1910) and for one aspect of plant growth (Leitch, 1916). Such a curve is shown in Fig. 7 and its relation to true van't Hoff curves with different coefficients (Q_{10}) exhibited. Some consider such a curve as made up of portions of several van't Hoff curves having different constants (Pütter, 1914). Krogh (1916) points out that it is not very probable from *a priori* considerations that the van't Hoff rule should be followed, as we have to do, not with a simple chemical reaction, but with a complex series of reactions possibly taking place in a heterogeneous system. And even if the difference between the heterogeneous system and a system in solution could be neglected, yet the shape of the curve would still be affected if a limiting factor were operative. Thus oxygen pressure in the tissues might be a limiting factor.

Owing to Blackman's recognition throughout his work of the effect of limiting factors, our knowledge of the relation between temperature and carbon assimilation is much clearer. It is recognised by Blackman that in investigating the influence of temperature on carbon assimilation, no other factor must be limiting the rate of the process, as in such a case, the amount of the carbon assimilation is simply dependent upon the value of the limiting factor and is not related to the temperature.

The influence of temperature on carbon assimilation is described in two papers, one by Miss Matthaei (1904) and a second by Blackman and Matthaei (1905). In these papers will be found a general account of the apparatus and method used. Isolated leaves of Cherry Laurel (*Prunus laurocerasus* var. *rotundifolia*) were used in most experiments, while for some, leaves of *Helianthus tuberosus* were employed. The leaves were carefully selected and after

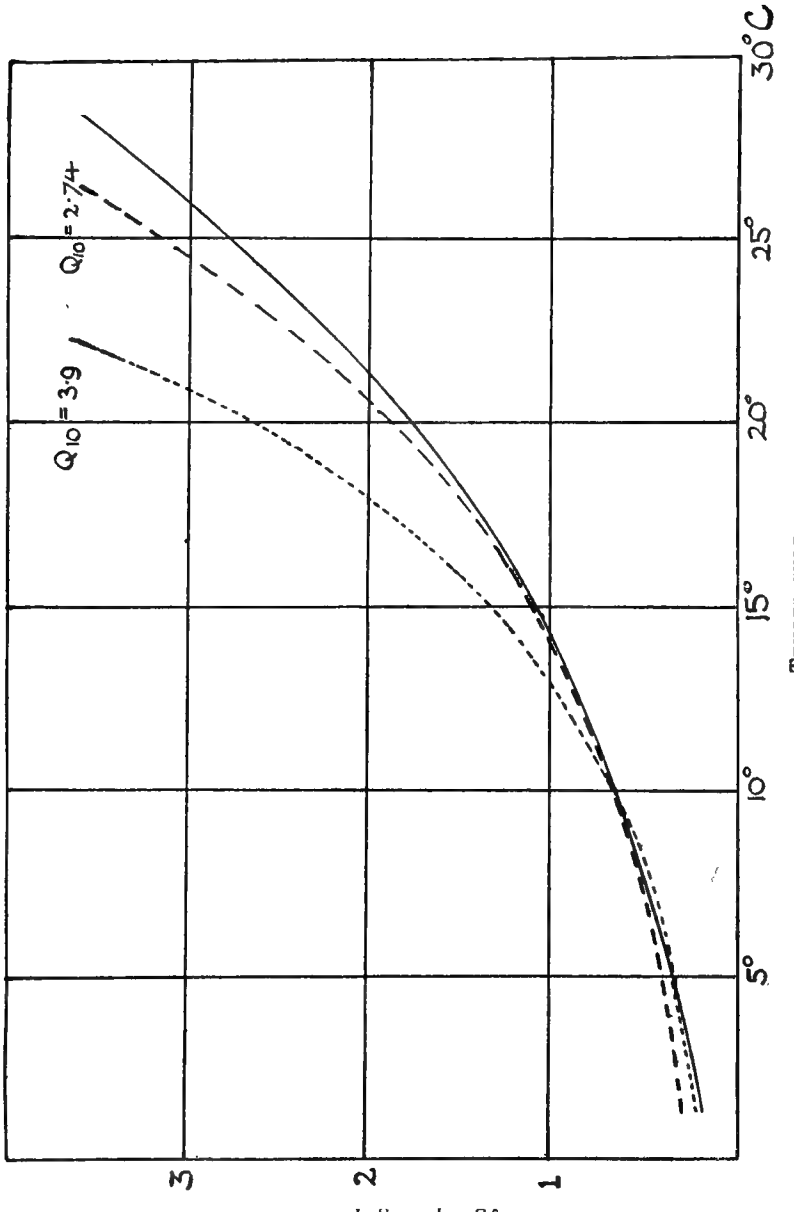


Fig. 7. Temperature metabolism curve of a fish compared with van't Hoff curves. (After Krogh).

picking were kept for 24 hours before experimentation at fairly constant temperature, with their stalks in water in covered beakers exposed to diffuse light. It was found that the previous history of the leaf, especially as regards nutrition and temperature changes, is very important in determining the amount of assimilation. The significance of this we shall refer to later.

For each experiment a fresh leaf was employed and this was kept with the cut end of its stalk in water in order to keep down loss of water from the leaf through transpiration. The leaf was contained in a chamber through which air containing a known quantity of carbon dioxide was passed at a known rate. Analysis of the outflowing gas gave the necessary data for determining the intake of carbon dioxide. As a source of light, incandescent gas or Keith high pressure gas was used. For the work described in the second paper, sunlight alone was used.

It is assumed that assimilation and respiration take place simultaneously in the leaf, and for this purpose the respiration at each temperature was obtained by measuring the output of carbon dioxide in the dark. On adding this to the value of the 'apparent assimilation,' the 'true assimilation' is obtained.

At higher temperatures the respiration is more difficult to estimate, for oscillations occur much too big to be accounted for by experimental errors. Also, during an assimilation experiment, the respiration is constantly changing on account of the assimilation. An approximation to its value was therefore obtained by measuring it in the dark before and after an assimilation experiment and taking the mean value.

Another complication has to be taken into account when high intensities of light are used. It was recognised by Brown and Escombe (1905) that light falling on a leaf would bring about a rise in temperature of the leaf, and they endeavoured to calculate this from a knowledge of other conditions of the leaf. Blackman and Matthaei (1905) show that the values obtained by Brown and Escombe depend on the values of six other quantities which are not all known, and hence values so obtained by calculation are not very likely to be correct.

Blackman and Matthaei therefore made direct measurements of the internal temperature of the leaf by means of small thermo-couples of copper and constantan. One junction was embedded in the midrib of the leaf, and the other kept in a water bath. The internal temperature of the leaf was measured by bringing this water

bath to such a temperature that the E.M.F. of the combination of two thermocouples was zero. The two couples are then at the same temperature and the temperature of the water bath containing one junction is consequently the internal temperature of the leaf.

In this way it was shown that the values obtained by Brown and Escombe for the rise in temperature of the illuminated leaf were much too small. The following table giving some of the values obtained by Blackman and Matthaei shows how much higher the internal temperature of the leaf may be above that of its surroundings when subjected to intense illumination.

TABLE X.

Effect of Light in Raising Internal Temperature of Leaves.

Source of Light.	Relative Intensity of Light.	Temperature of bath containing Leaf Chamber.	Internal Temperature of Leaf.
Keith high pressure gas burners.	13	11°C	15°C
,,	26	11°C	23·7°C
,,	45	13·5°C	30·5°C
Brilliant sunlight in July.	—	18·6°C	22·4°–30·7° C

In all experiments with high light intensities the internal temperature of the leaf was therefore measured.

In all experiments 800 c.c. of air containing from 0·8% to 2·8% of carbon dioxide were passed over the leaf per hour. As this was never used up it was supposed that carbon dioxide was not a limiting factor. The experiment was allowed to run for 1½ to 2 hours before measurements were made, in order to render the conditions constant. The amount of carbon dioxide absorbed during consecutive hourly or two hourly periods was then measured. This gives the value of the 'apparent assimilation' to which is added the value found for the respiration in order to obtain the 'true assimilation.'

Assimilation at Low and Medium Temperatures.

As unit intensity of light was used the light from a single incandescent gas burner when the front of the mantle was 130 cms. from the leaf. With this illumination, assimilation could be detected at as low a temperature as —6°C. With increasing temperature the assimilation rapidly increased up to 3°C, above which, increase

of temperature had no effect on the rate of assimilation. This remained the same right up to 33°C. The accompanying curve (Fig. 8) shows the result obtained with unit intensity of light. It will be observed that it resembles the curves shown in Fig. 5 illustrating the action of a limiting factor. It is evident that this would be the result if the intensity of light were acting as a limiting factor over that part of the curve above 3°C.

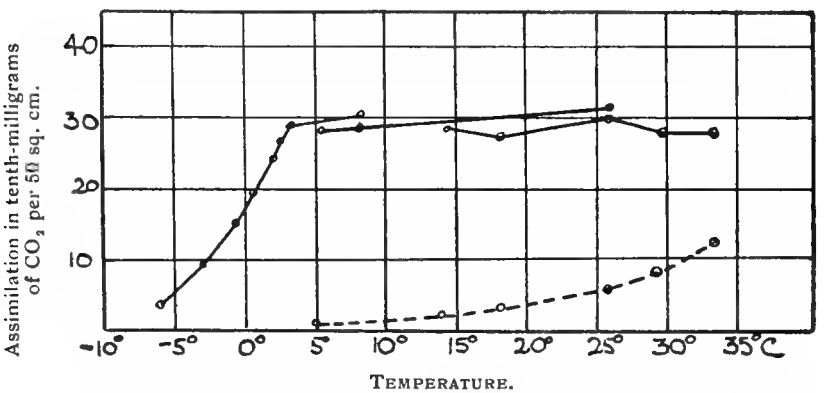


FIG. 8. Curve illustrating effect of temperature on assimilation of Cherry Laurel with unit intensity of light. The broken curve indicates the respiration. (After Matthaei).

Consequently, if the light intensity is doubled, one would expect tne first part of the curve to be much longer, and increase in temperature to produce a corresponding increase in assimilation until this has reached a value twice as great as that given by the horizontal part of the curve when unit intensity of light is employed. Similarly, if the light intensity is still further increased a yet higher temperature has to be employed before the limiting action of light will become evident.

The curves shown in Fig. 9 illustrate this clearly. They show graphically the results obtained by Miss Matthaei for the relation between temperature and assimilation when 1, 2 and 4 units of light intensity were employed. They indicate that provided light is not a limiting factor and the carbon dioxide supply kept constant and in excess, the higher the temperature, the greater the assimilation. Increasing the temperature will, however, produce no change in the rate of assimilation if the light intensity is below a certain value and so acting as a limiting factor.

Assimilation at High Temperatures.

It is thus possible to construct a curve showing the relation between temperature and assimilation when neither light nor carbon dioxide supply is a limiting factor. Above 25°C, however, a fresh complication arises. Below 25°C the amount of assimilation remains constant hour after hour, but above this temperature the rate of assimilation decreases with time. The initial rate of assimilation cannot be maintained. In all the experiments the rate of assimilation during the first 1½ hours was not measured; measurements were then made of the assimilation taking place in successive hours. Finally the leaf was darkened and the respiration measured. The following tables show typical series of results for the assimilation of the leaf at a low temperature and at a high temperature during successive hours.

TABLE XI.

Assimilation of Leaf of Cherry Laurel at 8·8°C.

Area of Leaf 44·6 sq. cms.

Light Intensity.	Time.	Apparent Assimilation.	Real Assimilation per 50 sq. cms. per hour.
Unit	12.30–2.0 p.m.	Preliminary	Preliminary
,,	2.0–4.0 ,,	·00375	·0023
,,	4.0–6.0 ,,	·0037	·00225
Twofold	8.0–9.40 ,,	Preliminary	Preliminary
,,	9.40–11.40 ,,	·00665	·0039
,,	11.40–1.40 a.m.	·0066	·0039
,,	1.40–3.40 ,,	·00645	·0038
,,	3.40–5.40 ,,	·0065	·00385
,,	5.40–7.40 ,,	·0065	·00385

TABLE XII.

Assimilation of Leaf of Cherry Laurel at 37·5°C.

Area of Leaf, 36·0 sq. cms.

Respiration, 0·0019 grams per hour.

Light Intensity.	Time.	Apparent Assimilation.	Real Assimilation per 50 sq. cms. per hour.
45	10.30 a.m.–12.0 noon	Preliminary	Preliminary
,,	12.0 noon–1.0 p.m.	·0154	·0237
,,	1.0–2.0 ,,	·0106	·0176
,,	2.0–3.0 ,,	·00795	·0139
,,	3.0–4.0 ,,	·0059	·0109

These two tables show very clearly the different relation between assimilation and time at temperatures below and above 25°C. Whereas the assimilation proceeds at a constant rate at 8·8°C as long as the experiment is continued, at 37·5°C there is a rapid falling off in the rate of assimilation throughout the experiment. A time factor comes into play. The facts observed in Miss Matthaei's experiments indicate the three following laws in regard to the time factor (Blackman, 1905).

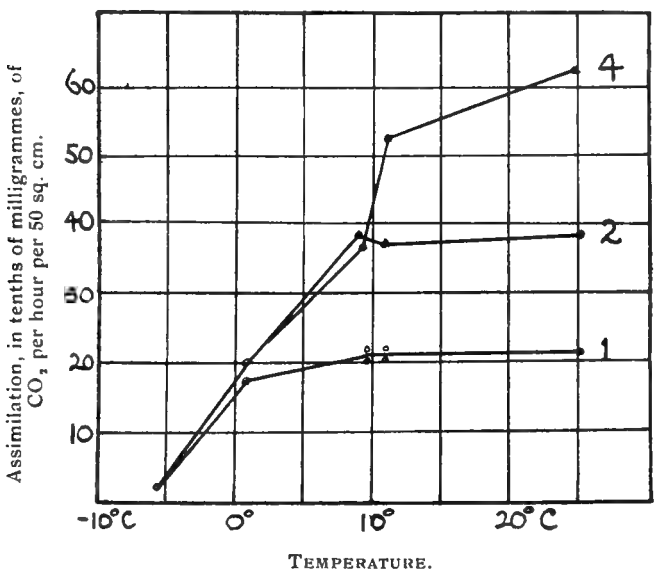


FIG. 9. Curve illustrating the effect of temperature on assimilation of Cherry Laurel under the influence of light of different intensities. 1, unit intensity of light; 2, twofold intensity; 4, fourfold intensity. (After Matthaei).

1. At high temperatures the initial rate of assimilation cannot be maintained, but falls off regularly.

2. The higher the temperature the more rapid is the falling off.

3. The falling off at any given temperature is fastest at first and subsequently becomes less rapid.

It thus becomes impossible to measure the highest possible assimilation at any temperature, but Blackman estimates this initial value of the assimilation by two methods. Firstly, below 25°C no time factor is involved and the assimilation numbers obtained therefore give a correct value of the initial values of the assimilation. The curve obtained from these numbers is a van't Hoff curve in which the temperature coefficient for a rise of 10°C is 2·1. By

assuming that the van't Hoff rule is followed above 25°C we can obtain by calculation the initial values for assimilation at higher temperatures.

Secondly the initial assimilation at a high temperature may be determined from the values actually measured after various times. At any temperature the curve between time and assimilation is plotted and the curve continued back to a point where the value of the time is zero: the value corresponding to this is the initial assimilation.

Blackman's diagram illustrating these methods is shown in Fig. 10. The broken line is the temperature assimilation curve

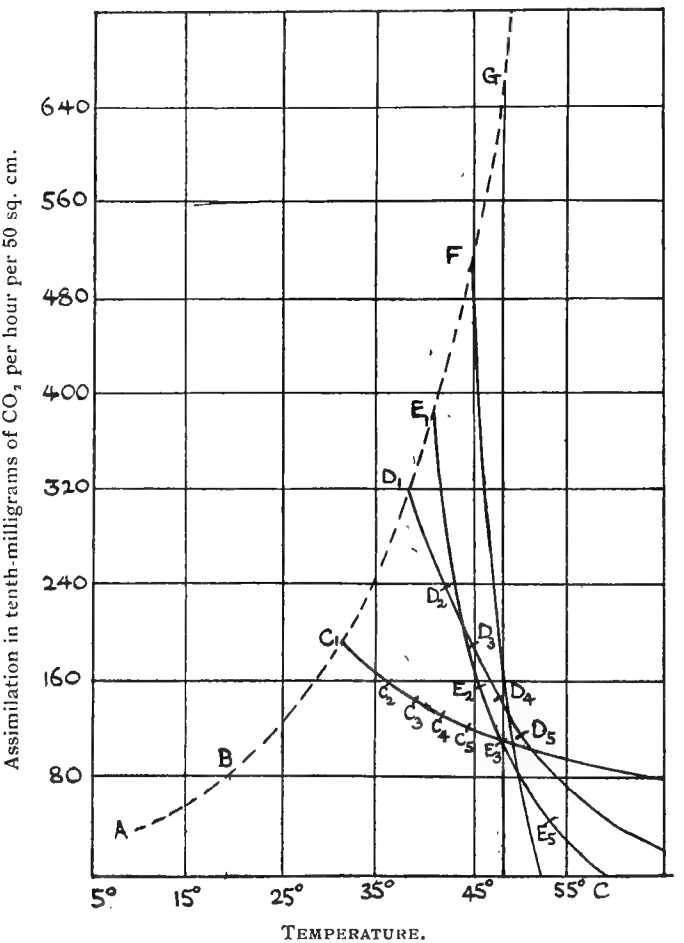


FIG. 10. Curve showing initial assimilation maxima at different temperatures. For further explanation see text. (After Blackman).

obtained by continuing the van't Hoff curve obtained for temperatures below 25°C. As to the second method estimations of the assimilation were made during four successive hours at 30·5°C, 37·5°C and at 40·5°C. The values for assimilation so obtained are plotted against time on the same diagram (the abscissæ now having a time significance) in the curves C_2—C_5, D_2—D_5 and E_2—E_5 respectively, the points C_2, C_3, etc. giving the values of assimilation obtained in the experiments. These curves are continued backwards to a point representing zero time, which gives the initial assimilation. The curves are so arranged in the diagram that the position representing zero time in each case is that also representing the temperature of the determinations, so that if the initial values of assimilation are given by the van't Hoff rule, they will fall on the curve drawn on that assumption. This is shown to be the case, so that as Blackman says, there is satisfactory evidence for a preliminary acceptance of the theory that the initial values of assimilation at high temperatures follow the van't Hoff as well as at low temperatures, although above 25°C the existence of the 'time factor' prevents the direct measurement of this maximum possible assimilation. The suggested form of the assimilation time curves at still higher temperatures are shown at F and G (Fig. 10). At G the temperature is supposed to be reached at which the assimilation falls at once to zero.

Experiments conducted by Blackman and Matthaei (1905) in which natural illumination only was employed show that different leaves may have different temperature coefficients for assimilation. Thus whereas Cherry Laurel has a temperature coefficient of about 2·1, that of *Helianthus tuberosus* was found to be about 2·5. The results are summarised in the accompanying diagram (Fig. 11).

A further point brought out in these researches is that the assimilation rate is affected by the season of the year, thus leaves are more active in February than in April, and from January to March than from October to mid December. These facts are at present unexplained, but they have no effect on the temperature coefficient which is independent of seasonal variation.

Owing to the more rapid falling off of the assimilation with time the higher the temperature, the temperature at which greatest assimilation is observed will depend upon the time which elapses between the commencement of the experiment and the measurement of the assimilation. Thus in the case of Miss Matthaei's measurements the highest value of the assimilation for the first hour after the experiment had run its preliminary 1½ hours is given at 37·5°C,

while for the fourth hour it is given at 30·5°C. Thus the optimum value obtained will depend upon the time that has elapsed between the commencement of the experiment and the measurement of the

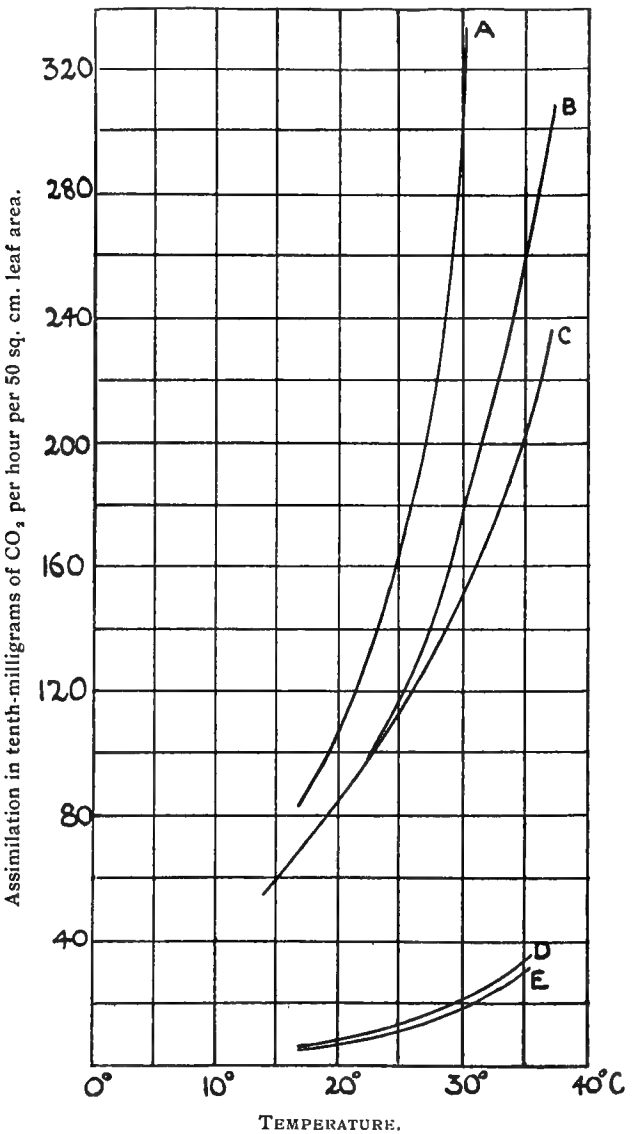


Fig. 11. Curves of assimilation and respiration of *Helianthus* and Cherry Laurel at different temperatures. A, curve of initial assimilation maxima for *Helianthus*; B, the same for Cherry Laurel; C, curve for assimilation of Cherry Laurel two hours after the initial moment of heating to the particular temperature; D and E, temperature-respiration curves for *Helianthus* and Cherry Laurel respectively. (After Blackman and Matthaei).

experiment. Obviously it would also depend on the previous history of the leaf as regards temperature.

In regard to the existence of the time factor Blackman points out that the rate of hydrolytic action of enzymes always shows a marked optimum temperature effect, and he cites Kjeldahl (1879) who showed that malt diastase hydrolysed increasing quantities of starch up to about 63°C after which the action fell off quickly, becoming nothing at 86°C, this being due to the destruction of the enzyme by heat. The apparent production of an optimum is thus due to two opposed processes, the hydrolytic action of the enzyme and the destruction of the enzyme by heat.

This characteristic of enzyme actions was later elaborated by Tammann (1892, 1895) and by Duclaux (1899) and it has come to be called Tammann's principle. Fig. 12, taken from Duclaux, illustrates clearly how the optimum is produced. The curve AB shows the relation between temperature and the enzyme action if the enzyme activity remains unimpaired, the curve CD represents the relation between temperature and quantity of enzyme, and the curve AOE represents the actual curve between temperature and the enzyme action.

Recently some Continental writers (Kanitz 1915, Rahn 1916) have taken the trouble to point out the application of Tammann's principle to Blackman and Matthaei's results. Not only did Blackman himself point out the similarity of his results with those in the case of enzyme actions, but he also recognised that the matter was probably more complex. Thus he says "Physico-chemical finality is not to be attained in this matter, but special research might at least show how far the recorded optima for assimilation and respiration are real metabolic truths and how far they are illusions of experimentation."

With the further criticism of Rahn we need not deal, as it attempts to explain why Blackman did not get a result which as a matter of fact he actually obtained.

Kanitz also criticises Blackman on account of the manner in which the curve of initial assimilation values at high temperatures is obtained. He points out that the number taken by Blackman for the temperature coefficient of assimilation in the case of Cherry Laurel was quite arbitrary; that it might have been 2·4 equally with 2·1. However, an examination of Miss Matthaei's figures are sufficient to show that the choice of 2·1 as the temperature coefficient between 5°C and 25°C was fully justified. Below 5°C the temperature

coefficient increases with decreasing temperature. This may be due to some other factor coming into play and it is a general phenomenon in life processes (cf. *e.g.*, Krogh's temperature-metabolism curve in Fig. 7). Again in the case of many chemical reactions the temperature coefficient gradually decreases with rise of temperature, and this may perhaps be the case in carbon assimilation. In any case Blackman was more justified in assuming as an approximate temperature-coefficient at temperatures above 25°C the value obtained from 5°C to 25°C than the value between —6°C and 5°C. It must be kept in mind that the theoretical curve of initial assimilation maxima is necessarily an approximation.

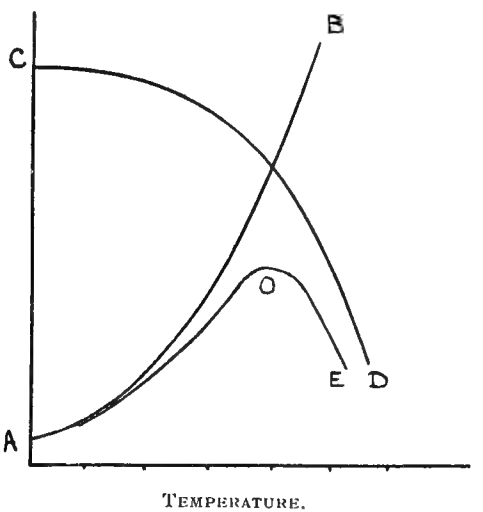


FIG. 12. Curves showing the relation between temperature and rate of enzyme action. (After Duclaux).

Kanitz further criticises the method of obtaining the initial assimilation values obtained by carrying back the time-assimilation curves to zero time. He asserts that the numbers so obtained give the amount of assimilation which actually takes place during the first hour of the experiment, whereas the number actually required is the assimilation which would take place in an hour if the initial rate of assimilation remained constant throughout that hour. This criticism also is due to imperfect consideration of Blackman's curves. The first measurements of assimilation were made by Miss Matthaei from 1½ hours to 2½ hours after the experiment was started. The number so obtained can be taken as approximately representing the rate of assimilation 2 hours after the commencement

of the experiment. Blackman actually continues his curves back for 2 hours, not $1\frac{1}{2}$ hours, and the value so obtained should therefore represent approximately the initial rate of assimilation. Kanitz's criticism is thus founded on a misconception.

The further criticism of Blackman's work offered by Kanitz is based on the assumption that Blackman's construction (Fig. 10) and the application of the time law for enzyme action are mutually exclusive. This is evidently not so. Blackman himself realised the resemblance between his results and those obtained for enzyme actions, but preferred to express his results in a non-committal form to making assumptions which were unproved by experiment.

With the criticisms of Blackman's construction offered by some other continental writers we need not deal, as they amount to no more than the expressions of personal opinion.

Kanitz points out how from a consideration of Duclaux's curve (Fig. 12) it is obvious that the position of the optimum is no fixed point, but must depend on the quantity of enzyme present, which will also depend on the previous history of the system: on its previous temperature, on the velocity with which it is brought to the optimal temperature, etc. It is interesting in this connection to recall that Miss Matthaei found it necessary, in order to obtain uniform results, to take particular care that all leaves used in her experiments were subjected to the same treatment for the 24 hours between their removal from the tree and the commencement of the experiment.

It may also be mentioned here that some investigators regard the optimum temperature as the highest temperature which can be maintained continuously without a depression of the function resulting, and recently Miss Leitch (1916) has adopted this idea for the case of growth. It is clear, however, that if it is really Tammann's principle or something strictly analogous to it that is involved in 'time factors' in carbon assimilation and growth, the position of the optimum, as Kanitz points out, is not a definite fixed point, but depends upon other factors which will only remain constant if the plants are subjected to the same previous history.

C. Light.

The effect of light on the intake of carbon dioxide has been indicated in the previous section of this chapter. Reference was there made to Miss Matthaei's experiments in which it was shown that intensity of light may limit the intake of carbon dioxide, in

which case increase of temperature produces no effect on the rate of assimilation. The results have already been shown graphically in Fig. 9.

Blackman and Matthaei (1905) have also made extensive series of observations on assimilation under different conditions of natural illumination which show strikingly the influence of light as a limiting factor. The general arrangement of the experiments was similar to that employed previously and referred to in the previous section of this chapter. The leaves experimented upon were contained in a leaf chamber as before through which a current of carbon dioxide was passed such that the supply of carbon dioxide never limited the intake of the gas. The experiment summarised in the following table may be regarded as typical.

TABLE XIII.

Assimilation by a Leaf of Helianthus tuberosus under Natural Illumination.

Area of Leaf 70·1 sq. cms.

800 c.c. of air containing 2·5% CO_2 passed over the leaf per hour.

Date: July 30th, 1904.

Temperature 18·0° to 18·3°C.

Assimilation at this temperature when light is not limiting is ·0093 grms. per 50 sq. cms. per hour.

Time P.M.	Illumination.	Temp. of Bath.	CO_2 in grams absorbed by leaf.	Real assimilation in grams CO_2 per 50 sq. cms. per hour.
12.30–1.30	—	—	Preliminary	Preliminary
1.30–2.30	Heavy laden clouds	18·2	0·0011	0·0015
2.30–3.30	Violent thunderstorm at first; then slowly clearing up	18·3	0·0032	0·0030
3.30–4.30	Brighter; no rain	18·3	0·0073	0·0059
4.30–5.30	Sun at first, then clouded over; storm driving up	18·3	0·0050	0·0043
5.30–6.30	Overcast, steady rain; 6·10, heavy storm	18·0	0·0007	0·0010

It will be observed that the intake of carbon dioxide is in none of these measurements near the value given when light is not limiting, and in each case the assimilation must be a measure of the light only. The assimilation shows marked variations parallel with the light conditions.

Besides a large number of similar determinations by Blackman and Matthaei made at various temperatures with light the limiting factor when natural illumination was employed, a few confirmatory measurements were made with the water plant *Elodea* by Blackman and Smith (1911 b); the results have already been shown graphically in this chapter (see Fig. 6). These writers have also shown that Pantanelli's results (1903) rightly interpreted, indicate that assimilation is directly proportional to the intensity of light used until either carbon dioxide supply or temperature becomes a limiting factor. The earlier experiments of Reinke (1883) also support the conclusion.

From the results of his experiments Blackman concludes that where temperature and carbon dioxide supply are in excess the rate of assimilation is proportional to the intensity of illumination. There is thus for every temperature a minimum value of the light intensity which is sufficient to allow the maximum assimilation rate to take place at that temperature always presuming no other factor is limiting. By using perforated screens in front of the leaf to cut off part of the sunlight, Blackman and Matthaei were able to show what proportion of sunlight was required to give the maximum possible assimilation at 29·5°C. Thus it was shown that in bright sunlight during the middle of the day in August the maximum assimilation possible at 29·5°C in the case of Cherry Laurel was given by 0·36 of full sunlight and in the case of *Helianthus* by 0·69 of full sunlight. It would be expected that *Helianthus* was therefore capable of a much higher rate of assimilation than Cherry Laurel at the same temperature, and this is indeed the case (cf. Fig. 11).

Indeed Blackman and Matthaei show that when light is the limiting factor equal areas of different plants equally illuminated produce the same amount of assimilation. Blackman and Smith (1911 b) have shown that the same law holds with water plants (cf. Fig. 13).

With the bearing of these results on the general question of energy in regard to assimilation we propose to deal later.

D. Carbon Dioxide Supply.

The influence of carbon dioxide supply upon assimilation has been investigated by Blackman and Smith in the case of submerged water plants. *Elodea* was chiefly employed for this purpose, but experiments were also made with a water-moss *Fontinalis*, and a few isolated observations were also made with *Ceratophyllum* and *Potamogeton*.

In order to examine the intake of carbon dioxide by submerged water plants, a stream of water containing carbon dioxide dissolved in it was passed over the leaf. By estimations of the carbon dioxide that had passed over the leaf in a definite period the rate of intake of the gas could be measured. The procedure employed with land plants was somewhat modified. A description of the method is given in the eighth paper in Blackman's series of researches (Blackman and Smith, 1911 a).

With small-leaved plants such as *Fontinalis* and *Elodea*, sprigs of the plant were used instead of single leaves as in experiments with Cherry Laurel or *Helianthus*. In most of the experiments medium temperatures and medium illumination were employed. The value taken for the carbon dioxide supply is the mean value of the carbon dioxide concentrations of the liquid before and after passage through the chamber. The 'real' assimilation was calculated as in the experiments with land plants already described.

The results obtained with both *Fontinalis* and *Elodea* are shown in the accompanying curves (Fig. 13). In the weaker

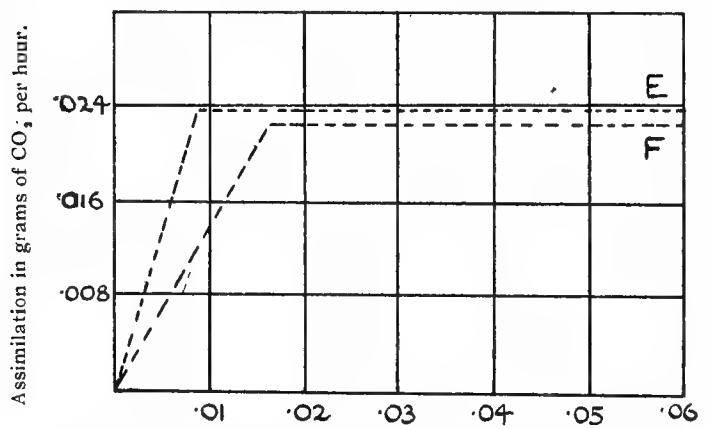


FIG. 13. Curves illustrating the influence of the magnitude of carbon dioxide supply on assimilation. E, *Elodea*; F, *Fontinalis* (after Blackman and Smith).

solutions of carbon dioxide, the assimilation increases directly with increase of carbon dioxide supply. Neither light nor temperature are limiting. In each case, however, a point is reached where increase in carbon dioxide supply is no longer accompanied by a corresponding increase in assimilation. The latter remains constant however much the carbon dioxide supply is increased. Here, either temperature or illumination is limiting the rate of assimilation.

In order to obtain a greater assimilation, either the light or the temperature would have to be increased. In this particular case it can be shown by increasing either light or temperature that it is light which is the limiting factor. Some earlier experiments of Treboux (1903) and Pantanelli (1903) also show clearly the proportionality between carbon dioxide supply and assimilation until light becomes a limiting factor.

Another noteworthy point is that where carbon dioxide supply is the limiting factor, *Fontinalis* assimilates only about half as much carbon dioxide as *Elodea* for any particular concentration of carbon dioxide. It suggests that the difference is due to there being less obstacle to the diffusion of carbon dioxide up to the chloroplasts in *Elodea* than in *Fontinalis*. Moreover, as values obtained with *Ceratophyllum* and *Potamogeton* are of the same order as those obtained with *Elodea*, it appears to be a class distinction between Bryophytes and Phanerogams.

It is to be observed that no depression of assimilation occurs even with such a high concentration of carbon dioxide as 0·0536%. This is 33·92% of saturation, and constitutes an environment as rich in carbon dioxide as is an atmosphere containing 30% of the gas. With higher concentrations, however, the 'real' assimilation becomes much depressed. This is not to be regarded as evidence that there is 'a primary optimal amount' of carbon dioxide for assimilation. Blackman regards the depression of assimilation in high concentrations of carbon dioxide as due to a narcotic effect of the strong carbon dioxide upon protoplasm, which has been previously shown by many workers (cf. Chapin, 1902). The depression has no direct relation to carbon assimilation. Blackman thus concludes that "in the curve expressing, in any given light, the relation of assimilation to the whole range of CO_2-concentrations from zero to saturation, we may separate off the falling end-part of the curve as an effect of narcotic poisoning. The third and last phase thus contrasts with the first two phases, which are specific assimilation effects, the first rising in a straight line where the CO_2 is limiting and the assimilation proportional to it, and the second a horizontal line where the assimilation is limited by the light (or the temperature) and is independent of increase of the CO_2-supply."

E. Chlorophyll Content.

So far we have dealt with environmental factors. It has been seen from Blackman's analysis that the laws governing the intake of carbon dioxide in relation to these factors cannot yet be expressed in simple physical and chemical terms, but the experimental facts so far obtained can be conveniently expressed in terms of the action of limiting factors.

Nor would it seem any more probable that an enquiry into the relation between an internal factor, *e.g.*, chlorophyll, and the intake of carbon dioxide should yield results of any greater physico-chemical definiteness. In this section we shall deal in some detail with investigations made with a view of determining the relation between assimilation and chlorophyll content, and more particularly with the recent work of Willstätter and Stoll (1915) At present only preliminary accounts of the extensive work of these investigators are available; consequently it is difficult to form a correct judgment of the value of the work and the validity of the arguments put forward in support of the hypotheses advanced.

There is a strong contrast between Willstätter's and Blackman s expression and generalisation of experimental results. While Blackman carefully avoids premature conclusions and tries to find non-committal expressions which will embody all his experimental results, Willstätter advances a simple definite hypothesis and attempts to obtain experimental data which will support his theory.

In this section we shall only give Willstätter's experimental data, in a later chapter we shall deal with the various theories of carbon assimilation which he has advanced.

The main result of his work is a demonstration of the complexity of the processes of carbon assimilation; an opinion which has often been expressed by earlier workers. For instance Pfeffer (1897) says that the chloroplasts "are only capable of assimilatory activity when all the component parts co-operate in an appropriate manner, and that the final result is produced not by a single reaction but by the agency of a complicated and self-regulatory mechanism."

Ewart (1896, 1897) and Pantanelli (1903) have published data which also tend to show that chlorophyll is not the only internal factor in the processes of carbon assimilation.

Willstätter, however, expresses this opinion in a far more dogmatic way, postulating that the chloroplast is the seat of a photochemical reaction and that the product formed in this reaction is subjected to an enzymatic action which takes place at the boundary between chloroplast and plasma. In this latter process oxygen is supposed to be evolved. His contention is that the efficiency of the assimilatory process depends not only on the amount of chlorophyll but also on the amount of enzyme, and in his investigation he has examined extreme cases where either chlorophyll or enzyme are in excess.

The very great importance of Willstätter's work lies in the fact that for the first time quantitative estimations of the pigments have been made; in an earlier chapter we have stated in some detail the methods employed by Willstätter and also pointed out how unreliable were the estimations of all of the earlier workers. The principle used in the analysis is the saponification of the leaf extract with alkali and the subsequent abstraction of the yellow pigment with ether. The chlorophyllin solution is then compared colorimetrically with standard solutions. Thus the disturbing influence of the yellow pigments is avoided; however, it must be pointed out that the information obtained only holds for the chromogen complex; as regards the phytol part of the chlorophyll molecule which is split off in the saponification we do not get any information.

Willstätter's experience from earlier investigations where the pigments were estimated in leaves collected at various times of the day and at various seasons led him to the conclusions that the amount of pigment is not altered during the processes of assimilation; this view is confirmed here, for Willstätter finds no appreciable difference in the amount of pigments as the result of assimilation.

Of course it has been assumed before Willstätter's time that the assimilation varies with the amount of chlorophyll, but it had not been possible definitely to estimate the chlorophyll content or to differentiate between the part played by the chlorophyll and the part played by the plasma.

Thus, for instance, Weber (1879) found that equal areas of the leaves of different plants under the same conditions had different assimilatory powers. Haberlandt (1882, and see 1914) explained Weber's results by determining the number of chloroplasts per unit area in the plants used by Weber and showing that there is a parallelism between the assimilatory activity and the number of chloroplasts. His results are exhibited in the following table.

TABLE XIV.

Relation between Assimilatory Activity and Number of Chloroplasts.

Species.	Assimilatory Activity per unit area.	Number of Chloroplasts per unit area.
Tropoeolum majus	100	100
Phaseolus multiflorus	72	64
Ricinus communis	118·5	120
Helianthus annuus	124·5	122

But of course there is no evidence or even probability that all chloroplasts contain the same amount of chlorophyll, so that this attempt to correlate assimilatory activity with quantity of chlorophyll is extremely crude.

While we have no criticisms to offer in regard to Willstätter's chemical analysis of the pigments, it appears from his preliminary account that the experimental arrangements in his assimilation experiments may be open to considerable criticism. As, however, he promises a detailed paper in which "many remarkable details in the experimental arrangement" are to be described, it seems desirable to defer such criticism to a later period.

The main principle of his method of experimentation is the same as that used by earlier workers, *e.g.*, Kreusler (1885–1890) and Blackman. The noteworthy features of Willstätter's method are:

(1) The carbon dioxide is determined by weight in an absorption apparatus.

(2) *The high intensity of illumination.* He uses a ½ watt Osram lamp of 3000 candle power at 15–25 cm. distance from the leaf chamber (corresponding to a light intensity of 48,000 to 130,000 lux).

(3) *The rapid stream of carbon dioxide* (4·5 litres per hour).

(4) *The method of temperature measurement.* The temperature of the *gas* in the leaf chamber is measured (presumably by a mercury thermometer); it is obvious, in view of Blackman's experiments, that this is indeed very unsatisfactory, particularly when such high light intensities are employed.

The experimental conditions used by Willstätter are such "*that the assimilation of a normal well assimilating leaf cannot be increased by increasing the carbon-dioxide concentration or the light intensity.*" The temperature, which is kept constant, generally 25°C, is "favourable to assimilation."

Expressing this in terms of Blackman's principle it must mean that neither light nor carbon dioxide is a limiting factor. The amount of carbon dioxide assimilated can then only depend on internal factors and the temperature.

The ratio between the quantity of chlorophyll and the carbon dioxide assimilated in a certain time is termed by Willstätter the assimilation number (assimilation number $= \frac{\text{amount of } CO_2 \text{ assimilated in one hour.}}{\text{chlorophyll content.}}$)

Approximately constant values of the assimilation number would indicate that the assimilation depended only on the amount of chlorophyll, if variable values are obtained it means that other factors come into play.

It is to be regretted that only the tables which *illustrate* the conclusions are given by Willstätter; none of the preliminary work necessary for the justification of the conclusions is quoted.

Normal Leaves.

In Table XV we give the results obtained by Willstätter for normal leaves.

TABLE XV.

Assimilation Numbers of Normal Leaves.

Concentration of CO_2, 5%.

Rate of Gas Current, 4·5 litres per hour.

Species.	Temp.	Light Int. in Lux.	Wt. of Leaves gm.	Dry Weight gm.	Leaf Surface sq. cm.	Chloro-phyll content mg.	CO_2 ass. in 1 hr. gm.	Ass. No.
Rubus Eubatus ...	25°	48000	5·0	1·80	356	16·2	0·094	5·8
Syringa vulgaris ...	25°	48000	12·0	3·45	371	14·1	0·091	6·5
Sambucus nigra ...	25°	48000	8·0	2·20	343	17·8	0·117	6·6
Ulmus	25°	48000	8·0	2·35	421	13·0	0·089	6·9
Prunus Laurocerasus	30°	75000	10·0	3·40	—	12·2	0·098	8·1
Primula	30°	75000	10·0	0·90	—	11·4	0·105	9·1
Hydragea opuloides	30°	75000	10·0	1·20	—	9·2	0·060	6·5
Pelargonium zonale	30°	75000	10·0	0·96	—	12·5	0·093	7·4

From the numbers given in this table there would seem to be a rough parallelism between the amount of chlorophyll and assimilation. But in all the cases given the leaves were in the same stage of development and all were rich in chlorophyll. On the other hand leaves from the same plant, but in different stages of development, exhibit much wider variations in the assimilation number, as the following table shows.

TABLE XVI.

Assimilation Numbers of Leaves from the same Plant, but in different Stages of Development.

25° 5% CO_2 48000 lux.

Species.	Age of the Leaf.	Weight of Leaves gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll mg.	CO_2 ass. in 1 hr. gm.	Ass. No.
Acer pseudo-platanus	4–6 leaf at the top of the branch 23rd June	6·0	2·0	358	5	0·059	11·8
	leaves from the base of the branch same day	6·0	2·15	469	24	0·124	5·2
Tilia	young light green leaves 25th June	8·0	2·05	421	5·2	0·074	14·2
	lower dark green leaves 26th June	8·0	2·55	530	22·5	0·148	6·6
Taxus baccata	young branch 27th June	20	5·65	—	27·6	0·131	4·7
	last year's branch 28th June	20	7·05	—	47·5	0·102	2·1

It will be observed from this table that the chlorophyll content increases with the age of the leaves; so does the assimilatory power, but not in the same degree. Consequently the assimilation number decreases.

Further data in regard to the variation of the assimilation number with the state of development of the leaves are given in the following table.

Table XVII shows clearly that after a time, although the chlorophyll content increases, yet the assimilation number diminishes. From this fact Willstätter concludes that the chlorophyll is present in excess, while some other internal factor (enzyme) is limiting the rate of assimilation.

Autumn Leaves.

In autumn leaves the conditions are very complex. The general rule is that with decreasing chlorophyll content the assimilation decreases as is shown in Table XVIII. Leaves rich or poor in chlorophyll give the same assimilation number.

TABLE XVII.

Assimilation Numbers of Leaves of the same Species at different Times in Spring.

25° 5% CO_2 48,000 lux.

Date.	Species.	Weight of Leaves gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll mg.	CO_2 per hr. gm.	Ass. No.
29th April ...	Aesculus	8	1·68	211	8·1	0·090	11·1
7th May ...	Hippo-	8	1·65	374	12·1	0·146	12·1
3rd June ...	castanum	8	2·35	386	19·8	0·127	6·4
4th May ...	Tilia	6·0	1·31	344	5·0	0·053	10·6
12th May ...	cordata	6·0	1·29	463	6·9	0·110	16·0
5th June ...		6·0	2·11	421	17·3	0·123	7·1

TABLE XVIII.

Assimilation Numbers of Autumn Leaves.

Date.	Species.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll content mg.	CO_2 ass. per. hr. gm.	Ass. No.
14th July ...	Sambucus nigra	8·0	2·05	359	18·8	0·116	6·2
14th October	Sambucus nigra (leaves easily detached)	8·0	1·64	356	8·2	0·049	6·0
2nd November	Populus pyramidalis (dark green leaves)	8·0	2·55	376	15·2	0·152	10·0
2nd November	Populus pyramidalis (yellow green leaves)	8·0	2·35	363	3·9	0·031	7·9

In many cases, as will be seen from the next table, the assimilation number increases at the beginning of autumn and diminishes later.

TABLE XIX.

Assimilation Numbers of the same Species at different Times in Autumn.

Date.	Appearance of Leaf.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll content mg.	CO_2 ass. per hr.	Ass. No.
30th July ...	deep green thin	4·0	1·55	271	19·7	0·080	4·1
17th September	green	4·0	1·55	336	12·5	—	6·6
5th October ...	green with yellow spots	4·0	1·45	344	7·8	0·064	8·5
19th October ...	almost yellow	4·0	1·35	337	2·1	0·010	4·8

Some leaves which remain green until they fall off keep their assimilatory power, so that in spite of the lateness of the season and high chlorophyll content, the assimilation numbers are still high. Yet in other cases where the chlorophyll content of the leaves in autumn is still high the assimilation numbers are remarkably low, while there is a considerable difference between the assimilation numbers of young and old leaves from the same plant. These results are given in Tables XX and XXI.

TABLE XX.

Autumn Leaves with high Chlorophyll Content and Normal Assimilation Numbers.

Date.	Species.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll content mg.	CO_2 ass. per hr. gm.	Ass. No.
26th October ...	Cydonia japonica var. Moerlosii (falling leaf)	10·0	4·50	301	16·3	0·119	7.3
27th October ...	Clerodendron trichotomum (fallen leaf)	6·2	1·28	211	9·3	0·115	12·3
14th October ...	Lonicera tatarica (fallen leaf)	4·0	1·20	299	3·3	0·020	6·1

TABLE XXI.

Autumn Leaves with same Chlorophyll Content but different Assimilation Numbers.

Date.	Species.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll content mg.	CO_2 ass. per hr. gm.	Ass. No.
11th November	Ampelopsis tricuspida Veitchii (fresh, green)	7·0	1·50	—	6·8	0·014	2·0
16th October ...	Ampelopsis quinquefolia (older, green)	5·0	1·05	251	6·4	0·006	0·9
17th October ...	Ampelopsis quinquefolia (younger leaves from apex of shoot)	5·0	1·15	325	6·4	0·050	7·9

The low assimilation number after exposure to low temperatures is rapidly increased on exposure to higher temperatures. Thus in the case of leaves of *Ampelopsis Veitchii* gathered at 4°C the assimilation number gradually increased from less than 0·8 to 2·6 after 1½ hours at 25°C, and increased up to about 4 when it remained constant.

Leaves poor in chlorophyll (Yellow Varieties).

Leaves poor in chlorophyll, *e.g.*, yellow varieties with a chlorophyll content of from 15% to 3%, or even less, of that of the leaves of the normal green varieties, exhibit a marked deviation in the proportionality between chlorophyll and assimilation. The numbers given in Table XXII for the yellow varieties are not maximal values of the assimilation, for it was found impossible to use the maximum light intensity without injuring the leaves. Under certain conditions it was found that at 15°C instead of 25°C the absolute amount of carbon dioxide absorbed by equal surfaces of yellow and green varieties can in the former reach a value as high or even higher than that taken in by the leaf of the normal variety.

TABLE XXII.

Assimilation Numbers of Leaves of Green and Yellow Varieties of Elm.

Ulmus, 5% CO_2, 3000 candle power at 35 cm. distance (24,000 lux).

Variety.	Temp.	Weight of Leaf gm.	Dry Weight gm.	Chloro-phyll mg.	Leaf Surface cm^2.	CO_2 ass. per hr. gm.	CO_2 per sq. metre gm.	Ass. No.
Chlorophyll poor ...	25°	8·0	2·0	0·95	321	0·075	2·3	79
do.	15°	the same leaf	2·0	0·95	321	0·056	1·7	59
Chlorophyll rich ...	25°	8·0	2·35	13·0	421	0·089	2·1	6·9
do.	15°	8·0	2·35	13·0	421	0·059	1·4	4·5

Willstätter points out that from this table it may be observed that the assimilation of chlorophyll-poor and chlorophyll-rich leaves is very similar at lower temperatures, but that the quantity of assimilation per unit quantity of chlorophyll is much greater in the yellow varieties.

Willstätter seems to indicate that the conditions in the yellow varieties are of great importance in judging the factors which influence the assimilation. As temperature variations do not influence the assimilation in such leaves, while decrease in light decreases the assimilation, he concludes that in this case the enzymatic system is more developed than the chlorophyll system, which thus controls the rate of assimilation. The reaction of the chlorophyll system being photochemical it may be assumed to have a temperature coefficient not far from unity, while the enzymatic process has a temperature coefficient of a magnitude of 2 to 3. In the normal leaf the chlorophyll system is more developed and the enzymatic process limits the rate of assimilation. Consequently

here variations in light intensity are without influence on the assimilation, while temperature variations influence the rate of assimilation considerably. However, no experimental results are given which justify these conclusions, and however interesting they may be they can at present only be accepted as postulates.

Etiolated Leaves.

The complexity of the processes involved in carbon assimilation is also made clear by examination of the behaviour of etiolated leaves.

On this subject observations were made by Miss Irving (1910 at Blackman's suggestion. Blackman assumed that in the case of etiolated leaves "the whole assimilatory apparatus might be efficiently developed except the green pigment, and as this increased by degrees, so the power of photosynthesis would increase. Thus the amount of chlorophyll present would then be the limiting factor for assimilation, and interesting data might be looked for relating the amount of pigment present to the amount of photosynthesis that could be effected."

This expectation was not confirmed by the preliminary experiments carried out by Miss Irving, who found not only that etiolated shoots possessed no power of assimilation, but that shoots that had developed a considerable green colour did not possess the power.

In Miss Irving's experimental arrangement the plants were supplied with their own respiratory carbon dioxide, and the light to which they were exposed was the feeble light from a north window.

Willstätter, using a much stronger light intensity (48,000 lux) and 5% carbon dioxide, obtained results which justified Blackman's expectations that chlorophyll is really a limiting factor. There is a possibility that in Miss Irving's experiments light was a limiting factor.

TABLE XXIII.

Assimilation Numbers of Etiolated Leaves becoming Green.

Phaseolus vulgaris.

Temperature 25°C, 5% carbon dioxide. Light intensity, 48,000 lux.

Light.	Appearance.	Weight of Leaf gm.	Dry Weight gm.	Leaf Surface cm^2.	Chloro-phyll content mg.	CO_2 ass. in 1 hour gm.	Ass. No.
Not previously illuminated	Pure yellow	5·0	—	—	<0·1	0·007	>70
11th June, after 6 hrs. illumination	Greenish yellow	4·4	—	—	0·3	0·040	133
29th May, after 2 days' illumination	Yellow-green	5·0	0·70	216	4·0	0·096	24
31st May, after 4 days' illumination	Grass green	5·0	0·74	260	7·8	0·104	13·3

It will be observed that the assimilation numbers are very high, indicating that it is the chlorophyll which is limiting assimilation.

Chlorotic Leaves.

Willstätter has also examined chlorotic leaves and finds, in spite of the low chlorophyll content, comparatively low assimilation numbers showing that the chlorophyll is only partially utilised. Of course one would not expect if one of the essential elements (iron) were wanting, that the assimilatory apparatus should be properly developed. Willstätter considers that the fact that in chlorotic leaves even a small amount of assimilation takes place, makes the assumption of B. Moore (1914), that iron plays an important part in the assimilatory process, even more improbable than ever.

Although it is premature to attempt to summarise the results of Willstätter's plant physiological work, it seems reasonable to conclude that under certain circumstances when no other factor is limiting, the amount of chlorophyll determines the intake of carbon dioxide by the leaf. Further it is clearly brought out by Willstätter's experiments with leaves in different states of development, yellow varieties, etiolated leaves, etc., that besides chlorophyll other internal factors are operative. This idea is not novel, as it has been expressed for instance by Pfeffer and Blackman, but Willstätter, for the first time, determines the relation between quantity of chlorophyll and assimilatory activity. The novelty which Willstätter claims for his researches lies in the exposition of carbon assimilation as consisting of two different processes, one photochemical and one enzymatic, the complete experimental proof of which Willstätter has not yet brought forward. For plant physiologists there should be nothing new in this view, as from Blackman's experiments it was seen that carbon assimilation had a temperature coefficient between 2 and 2·5 and consequently the photochemical reaction must be coupled with chemical reactions. But it would indeed be noteworthy if the famous German chemist should succeed in convincing plant physiologists that in what Blackman terms the "katalytic honeycomb of the cell" only two processes are concerned in assimilation.

Willstätter's theories of carbon assimilation, both those expressed in his book and in his more recent papers, we shall refer to later.

Chapter V.

The Products of Carbon Assimilation.

A. General Remarks.

In this chapter we propose to deal with the production of substances in the leaf as a result of the assimilatory processes. We shall confine our attention here to a consideration of the substances known to be produced; later we shall deal with the theories of carbon assimilation advanced to explain the production of these substances.

The substances which are known to be produced as a result of carbon assimilation are oxygen and carbohydrates, and the evolution of oxygen by the green plant in sunlight was one of the earliest known facts of plant physiology.

Although a few workers have made investigations on the laws governing the evolution of oxygen in sunlight, notably de Saussure (1804), Bonnier and Mangin (1886) and Maquenne and Demoussy (1913), yet the subject is one which has probably received less attention than any other aspect of carbon assimilation, and of recent years seems scarcely to have attracted that attention which it deserves.

The formation of carbohydrates in the leaf as a result of assimilation was not recognised until the classical researches of Sachs (1862) established this fundamental fact. Since then the production of carbohydrates in the leaf has been the subject of almost continual research, notably by English workers, yet it cannot be claimed that even now our knowledge of the organic products of carbon assimilation is very definite.

Although the production of oxygen and of carbohydrates in the leaf are merely two different aspects of the same process or set of processes, yet the two have always been investigated quite independently of one another. It is therefore convenient to discuss these two questions separately, and we shall therefore in the following section of this chapter review our present knowledge regarding the evolution of oxygen from the assimilating leaf, before passing on to consider the various questions raised in connection with the formation of organic material.

B. The Evolution of Oxygen.

That green parts of plants under the influence of light evolve oxygen, was established by Priestley, Senebier and Ingenhouss towards the end of the eighteenth century, but it was de Saussure

(1804) who first attempted to obtain quantitative data as to the relation between the amount of oxygen evolved and the carbon-dioxide absorbed by the assimilating plant.

Saussure found that the volume of oxygen evolved in a given time by the plant was less than the volume of carbon dioxide absorbed, and he came to the conclusion that part of the oxygen of the carbon dioxide was used in assimilation. It is strange that Saussure should be so often quoted as the discoverer of the fact that the volume of oxygen given out by the assimilating plant is equal to the carbon dioxide absorbed. Thus even Sachs (1882 or see 1887) says "As an essential point, it is at the same time to be insisted upon here that the volume of oxygen evolved is equal to the volume of the carbon dioxide taken in, as de Saussure and, later and more exactly, Boussingault have already established." It is therefore worth while to quote Saussure's conclusion in his own words, "Il résulte de toutes ces expériences, que les plantes, en décomposant le gaz acide carbonique, s'assimilent une partie du gaz oxygène qui y est contenu."

As Saussure's work is perhaps not always easily accessible, it may be worth while to give his actual results here.

Saussure placed a suitable number of plants in a large vessel containing an artificial atmosphere comprising about 21% oxygen and the rest nitrogen, to which was then added carbon dioxide. The plants were then exposed to sunlight on a number of successive days (6 to 18) and at the end of the period the gas in the vessel was analysed.

The following numbers were obtained with 7 plants of periwinkle (Vinca minor).

	Before.	After.
Nitrogen ...	4199 c.c. ...	4338 c.c.
Oxygen ...	1116 ,, ...	1408 ,,
Carbon dioxide	431 ,, ...	0 ,,
	5746	5746

Oxygen evolved	292 c.c.
Carbon dioxide absorbed ...	431 ,,
Oxygen absorbed by plant ...	139 ,,

In the following table are summarised all Saussure's experiments on this subject.

TABLE XXIV.

Oxygen evolved in Assimilation (de Saussure).

Species.	Oxygen evolved.	CO_2 absorbed.	Oxygen absorbed.
Vinca minor	292 c.c.	431 c.c.	139 c.c.
Mentha aquatica ...	224 ,,	309 ,,	86 ,,
Lythrum salicaria ...	121 ,,	149 ,,	27 ,,
Pinus genevensis ...	246 ,,	306 ,,	60 ,,
Cactus opuntia	126 ,,	184 ,,	57 ,,

Such experiments as these, of course, take no account of the respiration of the plants which is certainly going on in the intervals between the illuminated periods and is generally assumed to continue concurrently with assimilation during the illuminated periods as well. The same criticism is to be levelled against the experiments of Boussingault (1864) and others, who obtained a ratio of oxygen evolved to carbon dioxide taken in, of approximately unity, and to those of Schloessing (1892, 1893), who obtained numbers for the $\frac{\text{oxygen}}{CO_2}$ ratio considerably greater than unity (1·05 to 1·33). Similar numbers were also obtained for lichens by Jumelle (1892), and for mosses by Jönsson (1894).

It was Bonnier and Mangin (1886) who attempted to separate the gaseous exchanges due to assimilation and respiration. For this purpose they employed four different methods.

1. By successive exposure of the same green tissue to darkness and light in a closed vessel and measurement of the change in content of oxygen and carbon dioxide of the vessel in which the tissue is enclosed during each period, it is possible to obtain data for the gaseous exchange due to assimilation alone.

Thus if in any time

c′ is the carbon dioxide evolved in the dark and

o′ ,, ,, oxygen absorbed in the dark

The respiratory coefficient $\frac{CO_2}{O_2} = \frac{c'}{o'} = r.$

Similarly if in the same time

o is the oxygen evolved in the light and

c ,, ,, carbon dioxide absorbed in the light

Then the total oxygen produced by the assimilatory process in the given time is $o + o'$

and the total carbon dioxide absorbed in assimilation is $c + c'$

And the true assimilatory coefficient is

$$\frac{o + o'}{c + c'} = \frac{O}{C} = a.$$

2. The second method employed by Bonnier and Mangin is based on Bernard's observation (1878) that by the use of chloroform the assimilation may be suppressed and respiration alone takes place. By comparison of the gaseous exchanges taking place in two similar quantities of leaves exposed to light under the same conditions, but in which one was anæsthetised with ether, and the other not, the gaseous exchange due to assimilation may be estimated.

3. Bonnier and Mangin's third method is based on the suppression of assimilation by removal of all carbon dioxide from the neighbourhood of the leaves. Two similar vessels contain equal weights of similar leafy tissue; one of the vessels contains concentrated barium hydroxide solution, the other an equal volume of pure water. In the former, not only is the carbon dioxide of the atmosphere removed and assimilation prevented, but the carbon dioxide evolved in respiration is absorbed by the baryta. So that, as in the second method, the difference between the oxygen content and carbon dioxide content of the two vessels at the end of the experiment, gives the true values for oxygen and carbon dioxide evolved and absorbed respectively in assimilation.

4. The fourth method depends on the measurement of the gaseous exchanges in branches of the same plant which are unequally green. Thus a yellow branch of *Euonymus japonicus* on exposure to light evolved 2·89 units of carbon dioxide and absorbed 2·11 of oxygen, while in the same time a green branch evolved 2·27 of oxygen and absorbed 0·54 of carbon dioxide.

The four different methods gave concordant results. The results obtained by Bonnier and Mangin for a number of species at different times of the year are shown in Table XXV. It will be observed that the true assimilatory coefficient is always greater than unity, whereas the respiratory coefficient is below unity. The consequence of this is that the apparent assimilatory coefficient, which neglects the respiration, is always lower than the real

assimilatory coefficient. Thus Bonnier and Mangin explain the fact that Boussingault obtained a ratio of $\frac{O_2}{CO_2}$ of about unity.

TABLE XXV.

Assimilatory Coefficients for Different Leaves (Bonnier and Mangin).

Species.	Month.	Real Assimilatory Coefficient $\frac{O_2}{CO_2}$	Respiratory Coefficient $\frac{CO_2}{O_2}$	Apparent Assimilatory Coefficient.
Tobacco	November	1·12	0·73	1·00
Ivy	,,	1·09	0·86	1·00
,, ...	,,	1·08	0·80	1·01
Bramble	,,	1·06	0·84	0·91
Ivy	December	1·06	0·84	0·88
Butcher's Broom	,,	1·08	0·78	0·92
Broom	March	1·16	0·87	1·09
Pinus sylvestris	,,	1·17	0·80	0·88
,, ,,	,,	1·12	0·85	1·04
Chestnut	June	1·06	0·83	0·99
Lilac	,,	1·06	0·96	1·05
,, ...	,,	1·05	0·93	1·02
Holly	February	1·24	0·75	1·13
Chestnut	April	1.16	0·82	0·91
Broom	February	1·16	0·85	0·92

In an extended series of observations on the respiration and assimilation of succulents, Aubert (1892) has obtained similar values for the assimilatory coefficient of ordinary plants, but much larger values for succulents. He concludes that the $\frac{O_2}{CO_2}$ exchange due to assimilation is greater than unity for all plants. For ordinary plants the ratio is not very far removed from unity, but for succulents it may be much larger. For ordinary plants the ratio varied from 1·05 to 1·23, numbers which agree closely with Bonnier and Mangin's observations. Some of the values obtained by him for succulents are given in the accompanying table. The values quoted for *Sedum Telephium* and *Opuntia tomentosa* show that at different times the ratio for the same plant may vary greatly.

Having regard to the peculiar metabolism of succulents, however, the relation of these assimilatory coefficients to the assimilatory process is doubtful.

TABLE XXVI.

Assimilatory Coefficient for Succulents (Aubert).

Species.	Date	Temperature.	Assimilatory Coefficients.
Aloe spinosa	23 July	24°C	2·45
Crassula arborescens ...	2 ,,	32°C	3·57
Mammilaria Newmanniana ...	,,	,,	3·51
Opuntia tomentosa	,,	,,	4·68
,, ,, ...	23 ,,	24°C	7·59
Sedum carneum	2 ,,	32°C	1·55
,, reflexum	,,	,,	1·40
,, Telephium	,,	,,	1·24
,, ,, ...	23 ,,	24°C	1·34

Recently Maquenne and Demoussy (1913) have called in question Bonnier and Mangin's results. As with all other workers on this subject, these investigators used a closed vessel as plant chamber connected to a reservoir containing 8 or 10 parts of carbon dioxide to 100 of air, from which the leaf chamber was filled after evacuation.

The leaf chamber was exposed to light and after a convenient time the gas in the chamber was analysed.

The respiratory and assimilatory coefficients of a large number of species were measured; the results are given in the following table.

TABLE XXVII.

Respiratory and Assimilatory Coefficients (Maquenne and Demoussy).

Species.	Respiratory Coefficient.	Apparent Assimilatory Coefficient.
Ailanthus	1·08	1·02
Aspidistra	0·97	1·00
Aucuba	1·11	1·10
Begonia	1·11	1·03
Cherry Laurel	1·03	0·97
Chrysanthemum	1·02	1·01
Dahlia...	1·07	1·07
Haricot	1·11	1·12
,, ...	1·07	1·07
Ivy	1·08	1·00
Lilac	1·07	1·03
Lily	1·07	1·00
Mahonia (autumn)	0·95	0·99

TABLE XXVII—*continued.*

Species.	Respiratory Coefficient.	Apparent Assimilatory Coefficient.
Maize	1·07	1·05
Oleander	1.05	1·01
Pea	1·07	1·04
Pear	1·10	1·08
Poppy	1·09	1·09
Privet	1·03	1·02
Rhubarb	1·02	1.00
Ricinus	1·03	1·03
Rose	1·02	1·00
Spindle-tree	1·08	1·02
Sorrel	1·04	1·04
Tobacco	1·03	1·04
Turnip...	1·11	1·06
Vine	1·01	0·99
Wheat...	1·03	1·02
Wild Grape	1·00	1·01

From their results Maquenne and Demoussy conclude that the value of the apparent assimilatory coefficient lies between that of the respiratory coefficient and unity, especially as the leaves were probably at a higher temperature during the assimilatory period than in the dark, so that as the respiratory coefficient rises with temperature, higher respiratory coefficients probably correspond with the assimilatory coefficients given.

They therefore conclude that the real assimilatory coefficient approximates to unity.

For if c is the volume of oxygen evolved in assimilation alone, and if d is the volume of carbon dioxide absorbed in assimilation alone,

And if a is the volume of carbon dioxide evolved in respiration, and if b is the volume of oxygen absorbed in respiration during the same period,

$\frac{a}{b} = m$, the respiratory coefficient,

and $\frac{c-b}{d-a}$ = the apparent assimilatory coefficient.

We see that $\frac{c-b}{d-a}$ is between 1 and m.

When, as in the general case $m > 1$

then also $\frac{c-b}{d-a} > 1$

whence $1-\frac{e}{d} < \frac{b}{d}(m-1)$

and when $m < 1$

and $\frac{c-b}{d-a} < 1$

we have $1-\frac{c}{d} > \frac{b}{d}(m-1)$

Now $1-\frac{c}{d}$ is the difference between the real assimilatory coefficient and unity and $\frac{b}{d}(m-1)$ is actually not greater than 0·01 in the first case, nor less than —0·01 in the second. Hence the real assimilatory coefficient differs from unity by a quantity less than 0·01.

From the results we have collected together in this section it becomes quite clear that the relation between the oxygen evolved in assimilation and the carbon dioxide taken in, is by no means definitely determined. Yet this is a matter of great importance in regard to the problems of carbon assimilation, for in the determination of the nature of a reaction or series of reactions, it is of first importance to know the quantitative relation between the initial substances and the products of the reactions.

C. The Carbohydrates of the Leaf.

The presence of starch in the leaf was recognised by von Mohl as long ago as 1837, but it was Sachs (1862, 1864) who identified this starch as a product of assimilation by showing that it appeared in the chloroplasts after exposure to light and disappeared in the dark, and he also showed that chlorophyll was necessary. Sachs' conclusion that starch produced in the chloroplast is the first visible product of assimilation is well known. The method of detection of starch by decolorisation of leaves by alcohol and subsequent treatment with an alcoholic solution of iodine is still the current method of detection of starch in the plant.

It was later recognised that many leaves never elaborate starch and A. Meyer (1885) classified plants into classes according to the quantity of starch their leaves contain. Leaves forming little or no starch were known to yield extracts which reduced cupric solutions and which were optically active, and it was therefore concluded that such leaves contained reducing sugars. In addition the non-reducing disaccharide cane sugar was actually extracted in a crystalline form from leaves of Vine by Kayser (1883).

Brown and Morris (1893) justly pointed out that there was no proof that the cupric reducing substances in the leaf were sugars, and they therefore tested for different sugars. They state that the

only sugars they found were sucrose, glucose, fructose and maltose. Pentoses were tested for, but not found. It is to be regretted that Brown and Morris do not state definitely what tests they applied for the various sugars, as their results have been accepted without question by most later workers. The presence of cane sugar seems quite definitely established, as leaf extracts after treatment with invertase increase in reducing power and change in optical activity, and this change is not very different from that which would result if the increase in reducing power were due to the inversion of cane sugar into glucose and fructose. They were also able to show the presence of maltose by obtaining maltose phenyl-osazone from leaf extracts. Similarly glucose phenyl-osazone is produced, but no evidence is given as to why it is concluded that *d*-glucose and *d*-fructose are the only hexoses present, beyond the fact that they could obtain no other phenyl-osazones. It should be noted that *d*-mannose gives the same osazone as *d*-glucose and *d*-fructose, while the *l* forms of these three hexoses, which are always stated to be absent from the leaf, give a phenyl-osazone of the same crystalline form and the same melting point as the *d* forms of the sugars (see *e.g.*, Tollens, 1914). More recent work, however, has never succeeded in revealing the presence of any hexoses other than *d*-fructose and *d*-glucose, though on the other hand it must be admitted that no definite evidence has so far been brought forward in favour of the absence of all other hexoses. For example, Parkin (1911) was unable to obtain any osazone from extracts of snowdrop leaves other than glucose phenyl-osazone, and hence concludes that galactose and mannose are both absent. This argument is satisfactory for galactose, but it does not hold for mannose, as that sugar gives the same osazone as glucose and fructose.

Moreover, the recent work of Davis, Daish and Sawyer (1916) has cast grave doubt on the presence of maltose in leaves, which these authors find only present in estimable quantity as a result of enzyme action in leaves not instantaneously killed. Contrary too, to Brown and Morris, these later workers conclude that free pentoses are present in leaves.

They base this conclusion (Davis and Sawyer, 1914) on the fact that leaf extracts contain substances soluble in 80% alcohol which are not precipitated by basic lead acetate, which are unfermentable by ordinary yeasts and which exercise a cupric-reducing power after other sugars have been fermented away. There are of course many sugars which would fulfil these conditions,

as the only hexose sugars fermented by ordinary yeasts are *d*-glucose, *d*-mannose, *d*-fructose and less easily *d*-galactose, while numerous disaccharides with cupric-reducing power are not fermented by yeasts, as well as the pentoses, while there is always the possibility of the presence of substances with cupric-reducing properties other than sugars.

The further evidence of the presence of pentoses is derived from the fact that these purified plant extracts on subjection to distillation with hydrochloric acid according to the Kröber-Tollens process (see Tollens, 1914) yield a weight of phloroglucide which would be given by practically the same amount of pentose calculated as a mixture of *l*-arabinose and *l*-xylose. It must be admitted that the concordance is not very striking and Kluyver (1914) has pointed out that the presence of hexoses and disaccharides in such a solution is a source of error, as on distillation with hydrochloric acid these also give small quantities of furfural-like compounds which yield an insoluble phloroglucide, so that the method could not give any very accurate value for small quantities of pentose in presence of large quantities of other sugars. Davis and Sawyer admit the truth of this criticism, but point out that the error actually introduced in this way is small (about 18%).

Nevertheless the evidence that the *only* sugars present in the leaf are sucrose, *d*-glucose, *d*-fructose and pentoses does not carry complete conviction.

Besides sugars and starch Davis, Daish and Sawyer have estimated the complex derivatives of the pentoses, the pentosans, by distillation of the leaf-matter insoluble in alcohol by the Kröber-Tollens method.

The table overleaf may therefore be regarded as summing up our knowledge in regard to the presence of carbohydrates in the leaf.

The absence of *d*-mannose, which is closely related to *d*-glucose and *d*-fructose in chemical constitution and in its behaviour as regards fermentation by yeasts appears to have been generally accepted without the production of any sound evidence in support of the opinion. It is also generally assumed that the *l* forms of the hexoses are completely absent from the leaf. Thus E. F. Armstrong (1913) says: "In spite of frequent search it has never been possible to detect *l*-glucose or *l*-fructose in the leaves of plants, and the work of Brown and Morris leaves hardly any doubt that hexoses of the *d*-series and their polysaccharides are the only products of

assimilation." However, it is not clear how Brown and Morris's work leads to this conclusion. The hexoses of the *l*-series are not fermented by yeasts and there is no evidence in Brown and Morris's paper that these workers tested for hexoses of the *l*-series after the *d*-hexoses had been fermented away. Davis and Sawyer, on the other hand, show that if this is done there still remain cupric-reducing and optically active substances which they conclude are pentoses, and they seem inclined to regard them as a mixture of *l*-arabinose and *l*-xylose, and they support this contention with determinations of pentoses by the Kröber-Tollens method in the way we have already described, but without showing any great concord-

TABLE XXVIII.

Carbohydrates of the Leaf.

Group.	Compound.	Evidence of Presence.
Polysaccharides[1]	Starch ...	Test with Iodine Solution (Sachs). Hydrolysis with Diastase (Brown and Morris) or with Taka-Diastase (Davis, Daish and Sawyer).
	Pentosans ...	Leaf matter insoluble in alcohol yields furfural on distillation with concentrated hydrochloric acid (Davis, Daish and Sawyer).
	Dextrin ...	Leaf matter insoluble in alcohol but soluble in water which possesses optical activity and reducing properties after treatment with taka-diastase occurs sometimes in Potato (Davis and Sawyer).
Disaccharides...	Sucrose ...	Extracted by Kayser from Vine leaves. Inverted with invertase and weak acids (Brown and Morris).
	? Maltose ...	Production of maltose phenyl-osazone from leaf extracts (Brown and Morris). Microchemical test by production of osazone. Presence denied by Davis, Daish and Sawyer.
Hexoses ...	*d*-Glucose *d*-Fructose	Cupric reducing power of plant extracts. Production of glucose phenyl-osazone from plant extracts (Brown and Morris, Parkin).
Pentoses ...	? *l*-Arabinose ? *l*-Xylose	Purified alcoholic extracts of leaves yield furfural on distillation with concentrated hydrochloric acid (Davis and Sawyer). Presence denied by Brown and Morris.

[1] Exclusive of cellulose and pectin substances.

ance between the values obtained by this method of estimation and that of the reducing power.

The problem of determining the different sugars in the leaf is one of extraordinary difficulty owing to the large number of members of the group and the similarity of their properties. At present we cannot regard as settled even the question of what sugars are definitely absent from the leaf. This question is nevertheless of much importance in the quantitative estimation of sugars in the leaf and as the results of such analyses are likely to be used in connection with theories of assimilation, the exact identity of the leaf sugars may be of fundamental importance in obtaining an understanding of the assimilatory process.

It seems to us, therefore, that before forming a final judgment as regards the carbohydrates of the leaf and before accepting in all their details the results of quantitative analyses already made, there is required a thorough investigation that will settle which carbohydrates are present and which are not, as definitely as Willstätter has settled the question of the leaf pigments.

In the following sections of this chapter we summarise the analytical methods employed for quantitative carbohydrate analysis of the leaf and the results obtained by their means, but it should be understood that some of these results may have to be modified when fuller knowledge is obtained of this important but extremely difficult subject.

D. Quantitative Estimation of the Carbohydrates of the Leaf.

The quantitative estimation of the carbohydrates of the leaf was first seriously undertaken by Brown and Morris for *Tropæolum*; their results are given in their well-known paper in the Journal of the Chemical Society for 1893. Since then the most noteworthy contributions to the subject are those of Parkin (1911) on the snowdrop (*Galanthus nivalis* L.) which embodies the results of a careful series of observations extending over several years, and the recent work at Rothamsted of Davis, Daish and Sawyer who have called attention to several sources of error in the methods of earlier workers. With the results obtained by these different investigators we shall deal in the next section of this chapter. We shall here devote a little space to the description of the methods evolved by these various workers for this extremely difficult analysis.

1. Preparation of Material.

In order to obtain correct results in the estimation of substances so liable to change by enzyme action as carbohydrates, special care has to be taken to avoid such change in the preparation of the material for analysis.

Brown and Morris (1893) therefore dried the leaves rapidly at from 75°C to 80°C before estimating starch, and for estimating sugars the leaves were dried on wire-bottomed trays in a steam oven. Parkin (1911) used a similar method. The leaves were air dried at a temperature sufficiently low to prevent discoloration. In both cases the dried leaves were then powdered. That the sugars are extracted unchanged by this method was shown by Parkin by estimating them in material so prepared, and in leaves killed by immersion in liquid air which were subsequently ground up while frozen and then thrown into boiling water (containing a few drops of ammonia to neutralise any acid from the leaf) in order to kill the enzymes.

The following table shows that the two methods give almost identical results.

The numbers for two separate examples (I and II) are given.

TABLE XXIX.

Comparison of Sugars in Air-dried Leaf and in Leaf treated with Liquid Air.

—	Leaf treated with liquid air.		Air-dried Leaf.	
	I	II	I	II
Sucrose	12·84	10·46	12·74	10·42
Reducing Sugars ...	5·94	12·87	5·67	12·38
Total Sugar	18·78	23·33	18·41	22·8
$\frac{\text{Sucrose}}{\text{Hexose}}$	1 : 0·46	1 : 1·23	1 : 0·45	1 : 1·19

From the leaf powder of *Tropæolum* Brown and Morris extracted fat and chlorophyll with ether. The residue was then twice extracted for 24 hours with 80% alcohol at 40°C. The alcoholic extract was used for the estimation of sugars, the residue contained the starch.

In the case of the Snowdrop where the leaf contains no starch, Parkin extracted the sugars by four extractions with cold water

which removed about 97% to 98% of the sugars.

Davis, Daish and Sawyer (1916) consider this method of treatment is unsatisfactory in the case of moderately thick leaves such as that of the mangold, where heating up may be slow and a certain amount of enzyme action is possible before the enzymes are destroyed. They therefore adopt the following method in their work. About 1 kilo. of freshly picked leaf material is dropped in small quantities at a time into 2 litres of boiling 95% alcohol contained in a large zinc beaker to which 20 c.c. of ammonia of S.G. 0·880 is added in order to neutralise the acids present in the leaf. After boiling the alcohol for half an hour the further extraction of the alcohol-soluble contents of the leaf is carried out in an extraction apparatus on the principle of the Soxhlet extractor. The extraction is complete after 12 to 18 hours. The final separation of the extract from the residue is effected in a Buchner press. The residue is dried on paper trays in a steam oven for 18 hours and from it the total insoluble matter, the starch and pentosans are estimated. The alcoholic extract is analysed for total soluble matter, sucrose, maltose, glucose, fructose, and pentoses. It can be kept in a waxed-corked bottle for 3 to 6 months without any change occurring in the sugars if about 10 c.c. to 20 c.c. of toluene are added.

2. *Estimation of Starch.*

Brown and Morris estimated the starch in their dry leaf powder by O'Sullivan's method (1884) which consists in converting the starch into a mixture of dextrin and maltose by means of diastase. The leaf material usually contains tannins, amino-acids, etc. which influence the optical activity and reducing power of a solution and these accompanying substances have therefore to be removed by precipitation by means of basic lead acetate. Davis and Daish (1914) find that this method does not give correct results because some of the dextrin is carried down with the precipitate and so is lost to the analysis. It is estimated that as a result of this the starch estimations in leaf material made by O'Sullivan's method may be 15% to 20% below the actual starch content.

Davis and Daish therefore treat the plant material with taka-diastase which converts starch wholly into maltose and dextrose which are then estimated by measurement of the cupric reducing power and the optical activity. The leaf material is first treated for 24 hours at 38°C with about 20 times its weight of water con-

taining 1% by volume of toluene which removes certain optically active leaf substances, among them dextrin if it is present. The residue, say 10 grams, containing the true starch, is then boiled with 200 c.c. of water to gelatinise the starch. It is then left for 24 hours at 38°C after addition of 0·1 gram of taka-diastase and 2 c.c. of toluene. After destroying the enzyme with 2 drops of concentrated sodium hydroxide and filtering, basic lead acetate is added (about 2·5 c.c.) and the volume made up to 500 c.c. The slight excess of lead is removed by the addition of the exact quantity of solid sodium carbonate necessary. After filtration the reducing power and optical rotation of the solution are determined. From these values the quantity of starch is calculated on the assumption that the only reducing and optically active substances present are glucose and maltose. In measuring the cupric-reducing power of all sugars examined the standard conditions laid down by Brown, Morris and Millar (1897) are employed, and their tables of the reducing power of maltose, glucose and fructose used. Similar tables for *l*-arabinose and *l*-xylose have been compiled by Daish (1914).

3. Estimation of Dextrin ("Soluble Starch").

It was found by Davis and Sawyer (1916) that the leaf material from potato, after extraction with 80% alcohol contains large quantities of a substance readily soluble in water and having a high positive optical rotation. This and the reducing power were determined, and again after treatment with taka-diastase and basic lead acetate, and from the change in reducing power and rotation thus brought about, the dextrin was calculated.

4. Estimation of Pentosans.

These were estimated by Davis, Daish and Sawyer by distilling 1·0 to 1·5 gram of the oven-dried leaf material with hydrochloric acid by the Kröber-Tollens method and weighing the furfural produced as phloroglucide.

5. Preparation of the Leaf Extract for Estimation of Sugars.

Before estimating the sugars in the leaf extract containing them, the alcohol in the case of an alcoholic extract is replaced by water by evaporation of the alcohol and subsequent dilution with water. Davis, Daish and Sawyer evaporate the alcohol under reduced pressure (20–30 mm.) in a special distillation apparatus (Davis, 1913). By this means 3 litres of extract is reduced to 150 c.c. and diluted with water to 500 c.c., a little hot alcohol or toluene

being used to wash out the flask if much chlorophyll or fat is present.

It is now necessary to remove tannins, amino-acids, basic substances, etc. This is effected by addition of the exact quantity of basic lead acetate required to precipitate the whole of these substances. Any excess of lead is removed by hydrogen sulphide (Brown and Morris) or by solid sodium carbonate (Davis, Daish and Sawyer). A solution so prepared can be kept for several weeks if a little toluene is added, provided no excess of basic lead acetate is present and that the solution is *just* alkaline. Any excess of basic lead acetate or much alkali brings about a rapid destruction of fructose.

6. Estimation of Sugars.

The methods employed by various workers for the estimation of sugars vary in details, but the general principles underlying all of them are the same. We will consider first the method used by Parkin, for as this worker concluded that sucrose, glucose and fructose are the only sugars present in the snowdrop leaf, his analysis is simpler than that of Brown and Morris, and Davis, Daish and Sawyer who analysed their extracts for other sugars as well.

(*a*) *Parkin's Method.* The cupric-reducing power and optical rotation of a definite volume of the purified extract is first measured. The cupric-reducing power is due to the hexoses alone, the optical rotation to the hexoses and sucrose together. A further volume of the extract is inverted with invertase and the cupric-reducing power and optical activity again measured. The increase in reducing power and change of optical activity must be due to the inversion of the sucrose, and from these numbers the quantity of sucrose is obtained. From the reducing power and optical activity due to the hexoses, the quantities of fructose and glucose can be calculated.

Parkin also found that after fermentation with brewers' or bakers' yeast, the cupric-reducing power and optical activity became negligible. This indicates the absence of pentoses and the *l* forms of the hexoses.

(*b*) *Method of Brown and Morris.*

(i.) The cupric-reducing power and optical rotation are first measured.

(ii.) For the estimation of cane sugar the solution is inverted with invertase at 50° to 55°C. The increase in cupric-reducing power and change in optical rotation will both give the quantity of sucrose.

(iii.) 50 c.c. of the 1% solution is heated with 3 c.c. of concen-

trated hydrochloric acid for 3 hours on a boiling water bath. This results in the hydrolysis of both the cane sugar and maltose, and the increase in cupric-reducing power and change in optical rotation as compared with the numbers obtained after inversion of cane sugar, give the quantity of maltose.

The cupric-reduction and optical rotation methods do not give concordant numbers for maltose. Davis and Daish (1913) suggest that this is due to the destruction of fructose.

(iv.) The cupric-reducing power and optical rotation of the original purified extract not accounted for by sucrose and maltose are due to glucose and fructose, the quantities of which can be calculated from these values. We discuss the reliability of the glucose and fructose numbers later (pp. 113–114).

(*c*) *Method of Davis, Daish and Sawyer.* These workers estimate sucrose, maltose, glucose, fructose and pentoses. Their methods are essentially the same as those of Brown and Morris, but they eliminate several sources of error and introduce some important modifications.

(i.) As with previous workers the cupric-reducing power and optical rotation of the purified extract is measured. The cupric-reduction is due to glucose, fructose, maltose and pentoses.

(ii.) For the estimation of cane sugar the solution is inverted with dilute acid or invertase. But Davis and Daish (1913) find that with 2% citric acid, inversion of cane sugar is not complete in plant extracts. For this reason they conclude that the earlier results of Campbell (1911), for example, must be completely withdrawn, as an error in the estimation of cane sugar results in an error in maltose and hexoses as well. In the case of Parkin's experiments, however, they consider that any error arising from this cause was small.

Davis, Daish and Sawyer therefore invert the slightly acid solution by boiling it with 10% citric acid for 10 minutes, or by treating it with 1–2 c.c. autolysed yeast (containing invertase) for 24 hours at 38°–40°C. The increase of reducing power or the change of optical rotation both give the quantity of sucrose present. As the two numbers do not give approximately the same value for sucrose, it is assumed that there are optically active substances other than sugars which vitiate the values obtained from optical rotation data. The values obtained by cupric-reduction are therefore the ones assumed to give the true value for cane sugar.

(iii.) In order to estimate maltose, the lead in the extract (p. 111) is completely removed by treatment with hydrogen sulphide. The excess of this is removed with ferric hydroxide. The solution

becomes acid owing to the presence of free acetic acid which is removed by the addition of dilute sodium carbonate solution until the extract is faintly acid to litmus.

Portions of the extract are then fermented with yeasts which do not contain the enzyme maltase, namely, *Saccharomyces marxianus*, *S. anomalus*, *S. exiguus*. Two other portions are fermented with bakers' yeast. The yeast is allowed to incubate for 21 to 28 days at 25°C, by which time fermentation is complete. After addition of alumina cream and filtration, the cupric-reducing power is measured. The differences between the cupric-reducing power of the extracts fermented with maltase-free and maltase-containing yeasts, must be due to the glucose resulting from the hydrolysis of maltose, and so the proportion of maltose in the extracts may be calculated.

(iv.) The pentoses are estimated by distillation with hydrochloric acid and weighing the furfural produced as phloroglucide.

(v.) The reducing power of the maltose and pentose is calculated, and from the reducing power of the original extract, the reducing power of the hexoses can be calculated. The optical activity of the sucrose and maltose is calculated, and that of the pentoses on the assumption that they consist of *l*-xylose and *l*-arabinose in equal proportions. By comparison of these data and the optical activity of the original solution, the optical activity due to the hexoses can be calculated. From this and their cupric-reducing power, the quantities of glucose and fructose can be calculated on the assumption that these are the only hexoses present.

It will be observed that the accuracy of the determinations of glucose and fructose depend upon the accuracy of the following assumptions:—

(i.) That sugars are the only cupric-reducing and optically active substances in the purified extracts.

(ii.) That the only sugars that can be present in the extracts are sucrose, maltose, *d*-glucose, *d*-fructose and pentoses.

(iii.) That the pentoses present are only *l*-arabinose and *l*-xylose, and that these are present in equal quantities.

In regard to this last assumption, Davis, Daish and Sawyer show that the error involved is not very large (about 7%) if the whole of the pentose is either *l*-arabinose or *l*-xylose.

The accuracy of the glucose and fructose estimations also depends upon the accuracy of the following operations:—

(i.) The completeness of the extraction of sugars.

(ii.) The completeness of the inversion of cane sugar.

(iii.) The completeness of the fermentation of sugars other than pentoses by bakers' yeast.

(iv.) The completeness of the fermentation of sugars other than maltose and pentoses by maltase-free yeasts.

Finally the accuracy of the glucose and fructose determinations depends upon the accuracy of the following determinations:—

(i.) The reducing power of the original plant extract.

(ii.) The optical rotation of the original plant extract.

(iii.) The reducing power of the extract after inversion.

(iv.) The reducing power of the extract after fermentation with maltase-free yeasts.

(v.) The reducing power of the extract after fermentation with bakers' yeast.

(vi.) The estimation of pentoses by the Kröber-Tollens method.

As the accuracy of the glucose and fructose determinations thus depends on the accuracy of 13 separate assumptions, operations and determinations, it is not to be expected that the results given for glucose and fructose are likely to have a high order of accuracy. Indeed, Davis (1916) points out that the extracts probably contain optically active substances other than sugars, *e.g.*, amino-acids and amides. As Davis himself says, "the values given as dextrose and lævulose probably do not, in most cases, represent real values"; he therefore prefers to designate them as "apparent dextrose" and "apparent lævulose."

A method by which it may be possible to make more satisfactory estimations of fructose is suggested in a recent publication of Miss Wilson and Atkins (1916). The sucrose is first estimated by measuring the reducing power and optical rotation of the solution of mixed sugars before and after treatment with invertase. After inversion, fructose may then be estimated by oxidising other sugars (glucose and maltose) by means of bromine. Under certain definite conditions, the glucose and maltose are destroyed by this means, and the fructose remains almost entirely unchanged. The method is not very exact, but it is possible that further research on it may render it more accurate, and in any case, the results obtained by its means are not open to all the objections of the indirect method previously described. It is, moreover, considerably more rapid.

Although Miss Wilson and Atkins worked out the method in order to apply it to the analysis of leaf extracts, no account of work involving its use is as yet available.

E. Variations in the Carbohydrate Content of Leaves.

1. *Garden Nasturtium* (*Tropæolum majus*) (Brown and Morris).

Tropæolum majus possesses a leaf which forms much starch. Brown and Morris analysed three sets of leaves of *Tropæolum majus* by their methods indicated in the last section. One set of leaves was picked at 5 a.m. and quickly dried in the steam oven; the second set was picked at the same time and kept in sunshine for 12 hours with the petioles in water before drying; the third set was picked at 5 p.m. after 12 hours insolation. The results of the analysis are given in the following table.

Table XXX.

Variation in Starch and Sugar Content of Tropæolum *Leaves, August 23rd.*

The values are given in percentages of the dry weight.

Carbohydrate.	Picked and dried 5 a.m.	Picked 5 a.m. Kept insolated in water until 5 p.m.	Picked and dried 5 p.m.
Starch	1·23	3·91	4·59
Sucrose	4·65	8·85	3·86
Glucose	0·97	1·20	0·00
Fructose ...	2·99	6·44	0·39
Maltose	1·18	0·69	5·33
Total Sugars ...	9·69[1]	17·18	9·58

In a further experiment one set of leaves was picked and dried at once while another set was placed in water in the dark for 24 hours after picking. The results of the carbohydrate analysis were as follows:—

Table XXXI.

Carbohydrate Content of Leaves before and after 24 hours in the Dark.

Carbohydrate.	Leaves picked and dried at once.	Leaves kept in the dark for 24 hours after picking.
Starch	3·693	2·980
Sucrose	9·98	3·49
Glucose	0·00	0·58
Fructose	1·41	3·46
Maltose	2·25	1·86
Total Sugars	13·64	9·39

[1] This is the number given by Brown and Morris.

From these results Brown and Morris conclude that cane sugar is the first sugar formed in the leaf and that this functions as a temporary reserve which accumulates during active assimilation. When the concentration of cane sugar reaches a certain amount, any excess of sucrose is converted into starch in the chloroplast. The cane sugar, on being translocated from the leaf, is inverted into glucose and fructose, while the starch is hydrolysed and translocated as maltose. That it is not hexoses that are the first sugars formed in the assimilatory process is indicated by the fact that after assimilating all day, leaves still attached to the plant contain no glucose and very little fructose. The cane sugar, on the other hand, has remained almost constant while starch and maltose have both decreased. In the case of the cut insolated leaves it is supposed that translocation is to all intents stopped. Under these circumstances the cane sugar and starch both increase greatly, but the glucose very little.

The results given in Table XXXI indicate that in the dark the cane sugar and starch both decrease in amount, while the glucose and fructose have both increased in amount. As presumably the sucrose is hydrolysed into equal quantities of glucose and fructose, and the latter appears much in excess of the former, Brown and Morris conclude that glucose is largely used for respiration in the leaf.

However, from what we have already said on the reliability of the measurements of glucose and fructose, it is extremely doubtful whether the recorded values of glucose and fructose have any meaning. Moreover, Davis and Sawyer (1916) have been unable to find maltose in *Tropæolum majus* and they conclude that the maltose found by Brown and Morris in their extracts resulted from the degradation of starch by diastatic enzymes after maltase in the leaf had been destroyed.

2. *Snowdrop* (*Galanthus nivalis, L*) (Parkin).

The snowdrop possesses the very usual monocotyledonous characteristic of not forming starch in the leaves. Hence as already indicated, the analysis of sugars is simplified. Parkin's results were obtained from observations made over a number of years. In most cases only the values of sucrose and hexose are given, no attempt being made, except for a special purpose, of distinguishing between the hexoses. Parkin's results are the most clearly stated of all the accounts we have of leaf carbohydrates and it is possible from the numbers he gives, to

realise the degree of accuracy of the results. He realises, for instance, that "this branch of physiological chemistry is as yet in the tentative stage." He prefers to make a large number of analyses with a moderate degree of accuracy to a few with many precautions taken, and he draws conclusions only from wide differences in sugar contents.

As a result of his analyses, Parkin finds that during any single day in spring the percentage of hexose sugars in the leaf remains fairly constant, whereas the sucrose fluctuates greatly, increasing during the day and diminishing at night. Tables XXXII and XXXIII exhibit some of the actual numbers obtained. The values are given in percentages of the dry weight.

TABLE XXXII.

Comparison of Sugars in Snowdrop Leaves picked in the early Morning and in the late Afternoon.

March 7th, 1906, Cambridge.

Maximum shade temperature 19·4°C.
Minimum temperature, previous night 6·1°C.

	9 a.m.	3.30 p.m.
Sucrose	11·22	14.65
Hexose	6·35	5·48
Total Sugars	17·57	20·13

TABLE XXXIII.

Comparison of Sugars in Snowdrop Leaves in the Evening and the following Morning.

March 30th and 31st, 1905, Carlisle.

Maximum shade temperature 9·7°C.
Minimum temperature 3·3°C.

	5·30 p.m.	8 a.m.
Sucrose	15·46	10·84
Hexose	11·41	12·64
Total Sugar	26·87	23·48

As the season advances, the hexose sugars in the leaf increase in proportion to the sucrose, as the following table shows.

TABLE XXXIV.

Seasonal Variation of Sugars in Snowdrop Leaves.

Date of picking.	Time of picking.	Max. shade temp.	Per 100 g. dry leaf.		Sucrose. / Hexose.
			Sucrose.	Hexose.	
Feb. 16, 1906	3 p.m.	9·4°C	19·8	3·56	1 : 0·2
,, 26, 1907	4–5 ,,	7·2 ,,	15·07	2·53	1 . 0·2
Mar. 7, 1906	3.30–4 p.m.	19·4 ,,	14·55	5·69	1 : 0·2
,, 30, 1905	5–6 ,,	9·6 ,,	15·5	11·4	1 : 0·7
Apr. 5, 1906	4–4.30 p.m.	15·6 ,,	14·64	11·17	1 . 0·8
,, ,, 1907	,,	14·4 ,,	14·64	11·61	1 . 0·8
,, 24, 1905	,,	10·6 ,,	14·84	17·29	1 . 1·2
May 4, 1905	3–3.30 ,,	11·7 ,,	10·3	12·78	1 : 1·2

Parkin considers that his results strongly support Brown and Morris' view that sucrose is the first recognisable sugar to appear in the leaf, and that glucose and fructose arise from it by inversion. He also brings forward evidence in support of Brown and Morris' contention that fructose is generally present in excess of glucose. Thus, out of 54 analyses, in 47 cases fructose was in excess of glucose, the proportion $\frac{\text{fructose}}{\text{glucose}}$ varying from $\frac{1}{0{\cdot}4}$ to $\frac{1}{0{\cdot}76}$, while in only 7 cases was the reverse the case and then the glucose was only slightly in excess, the fructose : glucose ratio varying from 1 : 1·01 to 1 : 1·06. As there is a greater tendency for the fructose to be destroyed by the careless use of basic lead acetate, these 7 results are probably due to experimental error. Parkin considers therefore with Brown and Morris, that glucose contributes more readily than fructose to the needs of the leaf.

3. Mangold (Beta vulgaris, *L., var. Sutton's Yellow Globe*).

Davis, Daish and Sawyer have attempted to obtain information in regard to the sugars in leaves and leaf stalks of the mangold at different times of the day and night and at different seasons by an extensive series of analyses carried out at Rothamsted.

Collections of leaves were made from plants growing in the field at 2-hourly intervals over a 24 hour period. Such series of measurements were made at three different times.

I. Stage of early growth. 6 a.m., August 26—4 a.m., August 27, 1913.

II. Stage of intermediate growth. 10 a.m., September 10—8 a.m., September 11, 1912.

III. Final stage of growth. 9 a.m., October 11—7 a.m., October 19, 1912.

In each case, the leaves and leaf stalks were treated separately. In the first series the upper and lower parts of the leaf stalk were dealt with separately; in the second series, the midribs of the leaves were subjected to a separate analysis; in the third stage of growth the midribs and leaf stalks were treated together. Their results are all calculated in terms of the total vacuum dried matter of the leaf.

It may be mentioned at once that Davis and his collaborators found starch was absent from the leaves and petioles of the mangold at all stages of growth except the very earliest. Similarly, no maltose was ever found in either leaves or petioles of this plant at any time. The quantities of other carbohydrates present in the leaf are indicated in the accompanying figures, which are based on the numbers and curves given in Davis, Daish and Sawyer's paper.

Fig. 14 shows the variation in content of the sugars of the mangold leaf found during 24 hours on August 26–27, 1913. The most noteworthy features of these results are:—

1. Both hexoses and sucrose increase rapidly in quantity after daybreak and reach a maximum about mid-day, after which the quantity present falls off fairly regularly and rapidly until the following dawn. Practically the whole of the hexose sugar disappears and about half the sucrose. These changes are closely parallel to the temperature curve (and probably also to the curve of light intensity).

2. The quantity of sucrose is always greater than that of hexose.

3. The variations in the quantity of cane sugar are small, the limits being between 3·11% and 1·5%, whereas the hexoses vary between 0·77% and 2·16%.

4. The quantity of pentosan remains practically constant throughout the day, the fluctuations being probably within the range of experimental error. The same holds for the matter insoluble in alcohol. Davis and his co-workers consider the increase they found in the values of these substances to be really significant, but if this were so, there should be either a sudden fall in these values at sunrise, or the proportion of them in the leaf should go on increasing from day to day. As a matter of fact, in

the second series of these workers, measured a fortnight later in the season, the proportion of pentosan is exactly the same as at the earlier period, while the proportion of matter insoluble in alcohol has actually decreased from about 60% to 50%. Nor is there any better evidence for the second alternative, for at this intermediate stage, where the measurements were taken before and after sunrise, the results obtained show actually a slight increase of these substances after sunrise. The most reasonable explanation is therefore that the differences recorded are simply within the range of experimental error. Similar considerations apply to the variations in the pentose content.

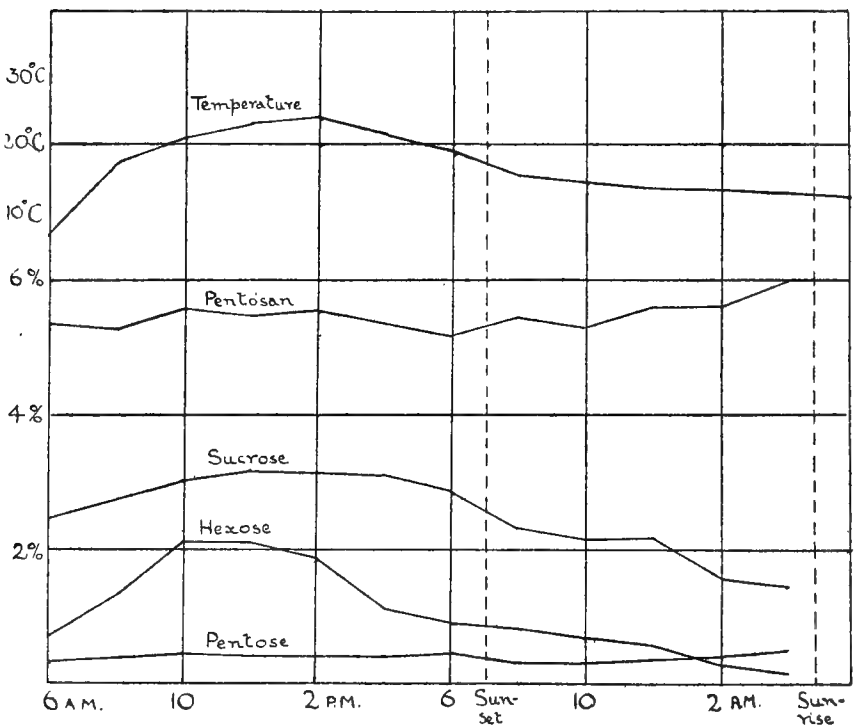


FIG. 14. Variation in Content of Various Carbohydrates in the leaf of Mangold during 24 hours, Aug. 26—27, 1913 (After Davis, Daish and Sawyer).

It is indeed regrettable that Davis, Daish and Sawyer give no data which enable one to judge the likely limits of error of their determinations. They do indeed, under the heading "Probable Error of the Analyses and Methods of Sampling," show that the reduction method and optical rotation method give values for sucrose which differ by about 20%, and they also give the analyses of hexoses

and sucrose in two samples collected at the same time, in which the hexose determinations in the two cases differ by 6%. The only information this gives us is that there is possibly a considerable error due to the variability of different samples, but two samples alone can give us no idea whatever of the actual magnitude of the probable error, which Davis and his co-workers have not determined. It is therefore misleading to give these two analyses under the sub-heading "Error of Sampling."

In Fig. 15 are summarised the results of analyses of leaves in the second period. Here the hexoses are in excess of the sucrose Both curves show synchronising maxima at 2 p.m., 6 p.m. and 2 a.m. Whether these maxima have any meaning, or whether they are merely the result of differences in sampling, it is impossible to say. The fact that the hexoses and sucrose always show unusually high

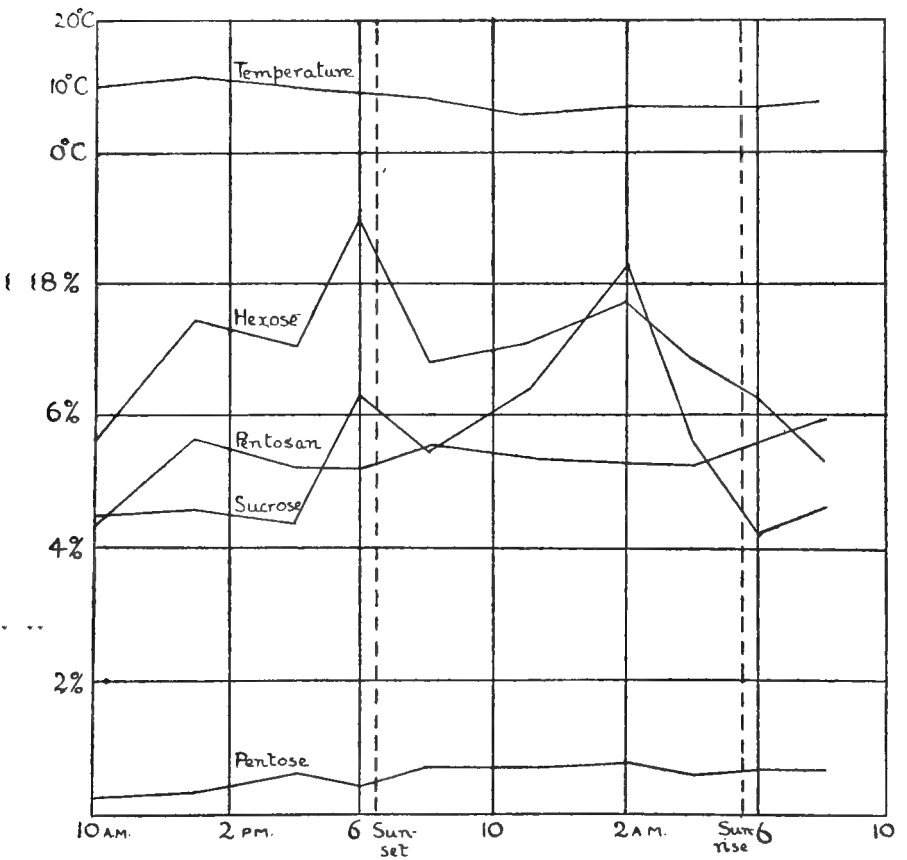


FIG. 15. Variation in Content of various Carbohydrates in the Leaf of Mangold during 24 hours, Sept. 10--11, 1912 (After Davis, Daish and Sawyer).

values in the same samples is suspicious, and the most likely explanation is that in some cases, at any rate, they are apparent maxima and minima, due to errors in sampling. This is especially so in the case of the maxima at 2 p.m., which appear owing to the minima at 4 p.m. Thus, if the minimum value found for hexoses in this case were increased by 7%, the minimum on the hexose curve would disappear, and we have already cited the instance in which the hexose content of two samples collected under similar conditions differed by 6%.

It seems reasonable to conclude from Davis, Daish and Sawyer's figures that the hexose and sucrose in the leaf increase during the day and then gradually decrease during the night. The maximum in these sugars in the middle of the night, at 2 a.m., is extremely difficult to account for on any other ground than error in sampling, for the leaf manufactures no fresh material, and yet the total carbohydrate in the leaf (pentosan, sucrose, hexose and pentose) has increased from 19·53% to 22·13% of the total dry matter of the leaf according to Davis, Daish and Sawyer's complete analysis. These authors suppose this increase is due to the breaking down of a water soluble gummy substance in the leaf into carbohydrates.

The relative variations in sucrose content are similar to those in August, although the percentage of sucrose is more than twice as great. The total hexoses present is about the same amount by weight as sucrose, and is much more than is present earlier in the season.

The pentose content varies little throughout the day; it appears to diminish somewhat during the night.

The results obtained for the last stage of growth are similar to those obtained for the intermediate stage. They are shown graphically in Fig. 16. As before, the sugar content is greater during the day than during the night. In this case, hexoses and sucrose show two maxima during the night, at 7 or 9 p.m. and at 3 a.m. As we have already indicated, the data furnished by the experiments of Davis, Daish and Sawyer are insufficient to enable us to judge whether such night maxima in sugar content actually exist in mangold leaves, or whether their appearance in the curves is simply due to a sampling error.

The results obtained by Davis, Daish and Sawyer in regard to carbohydrates in the mangold leaf may be summarised as follows:—

(i.) All the sugars in the leaf increase in quantity from the first to the final stage of growth.

(ii.) The pentosans form a larger and larger proportion of the matter insoluble in alcohol as the season advances.

(iii.) Of the total sugar, the hexoses form a progressively increasing proportion as the season advances. This point has already been brought forward very clearly by Parkin in the case of the snowdrop (see Table XXXIV).

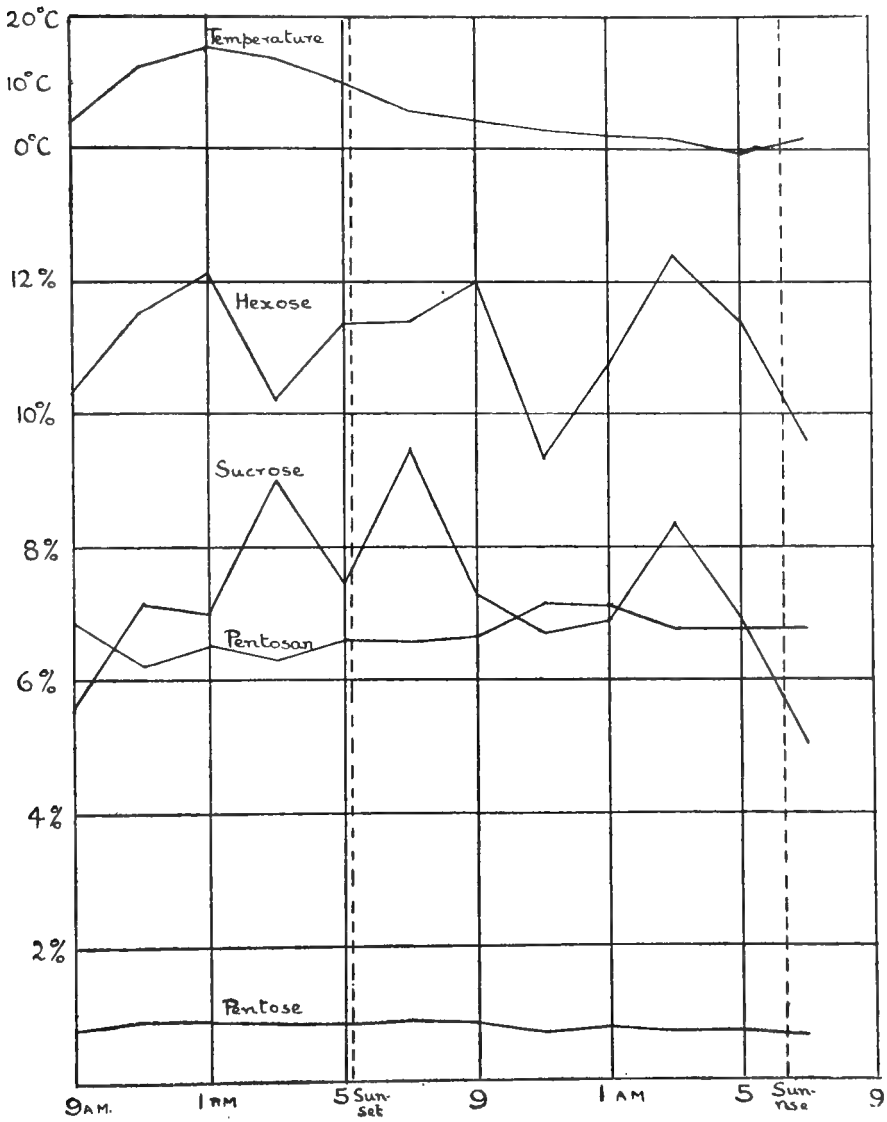


FIG. 16. Variation in Content of various Carbohydrates in the Leaf of Mangold during 24 hours, Oct. 11—12, 1912 (After Davis, Daish and Sawyer).

(iv.) In the first stage of growth, practically all the reducing sugars and about half the sucrose disappear from the leaf during the night. As the season proceeds, a less proportion of the sugar in the leaf disappears each night. It is especially the hexoses which increase in the leaf owing to this. These results are summarised in the following table.

TABLE XXXV.

Seasonal Variations in Carbohydrate Content of Mangold Leaves.

Date.	Temp.	Sucrose.	Hexoses.	Pentoses.	Pentosans.
Aug. 26–27	7·2—23·9°C	1·50—3·11	0·20—2·16	0·36—0·52	5·19—5.96
Sept. 10–11	6·1—10°C	4·24—8·27	5·38—8·90	0·34—0·76	4·42—5·90
Oct. 11–12	−0·6 – 16·1°C	4·98—9·52	9·39—12·41	0·61—0·92	6·21—7·15

The observations made by Davis, Daish and Sawyer on the sugars of midribs and petioles, show that these always contain a higher percentage of sugars than the leaves, and this percentage increases with the season. The hexoses are always much in excess of the sucrose, and the ratio of hexoses to sucrose is always much greater in the petioles than in the leaf lamina. These results are comparable with Parkin's observations that the sugar content of the snowdrop leaf increases from above downwards, and that the ratio of hexose to sucrose also increases. The conclusion drawn from this by both Parkin and the Rothamsted workers is that sucrose is the first sugar formed in the leaf and that this is converted into hexoses for translocation purposes. In support of this they also adduce the fact that the cane sugar is always present in relatively high proportion in the leaf, especially early in the season when it is present in excess of the hexoses. They suppose the cane sugar is gradually inverted by means of the enzyme invertase which is secreted or distributed on the surface of the sieve tubes.

We have already referred to the unreliable character of the determinations of glucose and fructose, an unreliability which is quite realised by Davis, Daish and Sawyer. As, therefore, it is not at all clear what the quantities they term "apparent dextrose" and "apparent levulose" really represent, we do not think any useful purpose would be served by discussing the values they obtain for these quantities.

4. *Potato* (Solanum tuberosum, *var. King Edward VII*).

Davis and Sawyer have made analyses also of the carbohydrates of a leaf which forms starch, that of the potato. The samples were gathered at 2-hourly intervals, from 6 a.m., July 16th to 4 a.m., July 17th, 1914. Separate analyses were made of the stalks. Their results are summarised in Fig. 17. It will be observed that sucrose is the chief sugar present, as was found to be the case also in the early stage of growth of the mangold. The sucrose content of the leaves rises during the day until 2 p.m., and then falls off regularly until dawn the next day. The hexoses show much variation throughout the day and night, and here again it is impossible through absence of data, to judge whether these variations are anything more than differences due to sampling. The hexose content is, on the whole, higher during the day than at night. The pentoses show little variation.

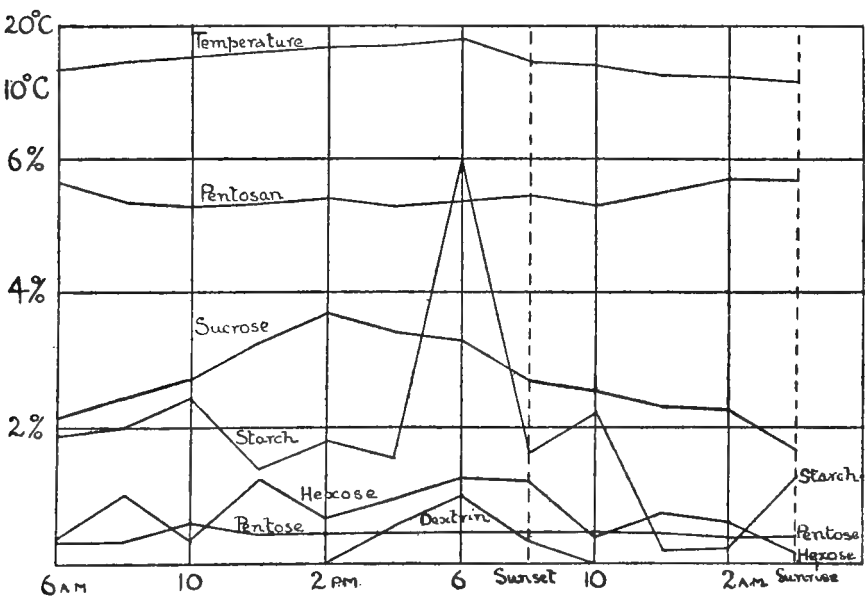


FIG. 17. Variation in Content of various Carbohydrates in the leaf of Potato during 24 hours, July 16—17, 1914 (After Davis, and Sawyer).

The starch content shows a decided maximum in the late afternoon (6 p.m.) and at the same time a quantity of dextrin (soluble starch) is present. By sunset this has almost disappeared and the starch content has rapidly fallen.

As in the mangold, in the leaf stalks of the potato the hexoses are much in excess of the sucrose, and it is reasonable to suppose

that the sucrose is translocated away from the leaves in the form of hexoses.

Even in this plant where the leaves contain abundant starch no maltose is found, either in the leaves or petioles. Davis and Sawyer therefore conclude that the starch, on utilisation by the plant, is broken down into hexoses by a mixture of enzymes similar to that of *Aspergillus oryzæ* which yields taka-diastase. They consider that dextrin and maltose are intermediate stages in the degradation of starch to glucose. Dextrin indeed appears at the period when starch is in large quantity in the leaf. They suppose the enzyme maltase is always present in relative excess in the leaf, and Daish (1916) has shown the presence of maltase in a number of different leaves.

5. *Vine* (Vitis vinifera).

In the leaf of the vine, a plant which stores its carbohydrate as glucose, Deleano (1912) was unable to detect sucrose in the leaf. Davis and his co-workers, on the other hand, state that after taking special precautions in sampling to prevent the leaf enzymes from acting, sucrose is found to be the principal sugar of the leaf. This they regard as supporting their view that sucrose is the first sugar formed in assimilation.

F. Carbohydrate Transformations in the Leaf.

The pioneer researches of Sachs indicated the formation of starch in the chloroplasts of the leaf as a result of carbon assimilation, and it was this investigator who showed the dependence of starch formation on light and chlorophyll. The proof was completed by Godlewski (1873) and Pfeffer (1873) who showed the necessity for an outer atmosphere containing carbon dioxide.

Sachs held the view that starch was the first visible product of assimilation, and he bound himself to no theories concerning possible intermediate products in its formation.

Kayser's work (1883) established the presence of sucrose in the leaf of the vine. It was supposed that starch was converted into cane sugar by diastatic enzymes and that the cane sugar was inverted in the conducting tissue of the leaf. Sachs (1884) also expressed the opinion that the starch is translocated in the form of sugar.

That starch could not always be the first visible product of carbon assimilation became obvious from the researches of A. Meyer (1885) who showed that different species varied greatly in their

capacity for forming starch, many plants not forming it all. In these cases it was shown that the absence of starch was not due to rapid translocation, for no starch was formed even under conditions most favourable to rapid assimilation and accumulation of products. The same investigator showed later (1886) that leaves depleted of starch floated on sugar solutions could form starch from the sugars. Thus almost all leaves formed starch from a 10% solution of fructose, a few from glucose and a very few from galactose. It became reasonable to suppose that starch might possibly be formed in the leaf from sugar.

Confirmatory evidence of this theory was derived from researches on starch formation in the plant, carried out by Boehm (1874, 1876, 1877) and notably by Schimper (1880). Boehm showed that starch in the leaf is not necessarily always the direct result of carbon assimilation. Thus he showed the formation of starch in the leaf as a result of transference of reserve material from other tissues under feeble light intensity, and in an atmosphere devoid of carbon dioxide. Schimper investigated the development of the starch granule. He showed that starch is always formed in a plastid, which might be colourless or green, and although he held that the mesophyll chloroplasts could not elaborate starch from other carbohydrates, further work of Boehm (1883) and that of A. Meyer mentioned above, showed that these chloroplasts could elaborate starch from sugars. These considerations lead to the conclusion that there is no difference between the chloroplast and the colourless amyloplast in regard to their powers of elaborating starch, and it is at least possible that starch formed normally in the chloroplast is formed secondarily and not as a direct result of assimilation.

These considerations were generally held to support Baeyer's suggestion, to which we shall refer in detail later, that the first part of the assimilation process consisted in the formation of formaldehyde which polymerised to hexose, and then gave rise to sucrose or starch. This hypothesis, which involves the view of hexose as the first sugar formed in assimilation, has recently received support from the work of Strakosch (1907), who investigated the distribution of sugars in the leaf and other parts of the sugar beet, by means of microchemical tests depending on the production of osazones. He concludes that glucose is the only sugar present in the mesophyll cells of the leaf. In the veins fructose appears as well, and later, sucrose. Maltose also occurs only in the petiole. Strakosch therefore concludes that glucose is the first sugar formed in assimilation and

sucrose is a later formed product, and he supports this conclusion with an analysis of leaf extract which shows only a negligible quantity of sucrose in the leaf (about one-sixth of the quantity of glucose) while the veins contain nearly five times as much sucrose as hexoses. Strakosch's results are in direct contradiction to those of the English workers, whose work we have summarised in the preceding section of this chapter. Davis, Daish and Sawyer point out that Grafe's test (1905) for fructose, used by Strakosch, is of doubtful applicability in presence of glucose, while the method used to localise glucose and sucrose, which is due to Senft (1904), is, according to Mangham (1915), untrustworthy when sucrose is present together with its hexose constituents. Davis, Daish and Sawyer criticise Strakosch's results as well as Mangham's identification of maltose in plant tissues, on the general ground that little reliance can be placed on such microchemical tests as a means of identifying one sugar in presence of others in plant tissues. The fact that Mangham should claim to distinguish between *d*-glucose and *d*-fructose in the plant by means of the osazone test, when their phenyl osazones are of course identical, is not very reassuring as to the degree of reliability of his results.

Such qualitative observations as those of Strakosch and of Dixon and Mason (1916) in any case cannot have the value of the quantitative researches recorded in detail in the preceding section of this chapter, and we find in all detailed quantitative examinations made of the carbohydrates of the leaf, that cane sugar is always present in the leaf in considerable quantity. We have already pointed out that all the workers who have obtained quantitative data have expressed the opinion that sucrose is the first sugar formed in assimilation, and that this is inverted into hexoses for translocation. When carbohydrate accumulates in the leaf in consequence of assimilation taking place at a greater rate than translocation, the excess of sucrose is supposed to be transformed into starch in *Tropæolum* (Brown and Morris). In the potato, which also forms starch in the leaf, Davis and Sawyer (1916) appear to regard the starch as formed from hexoses, soluble starch, which appears in appreciable quantity in the leaf at the period when starch content is at a maximum, being an intermediate product. This view is based on the intimate relation between the content of hexoses and starch in the leaf throughout the day, a relation, however, which is not very obvious from Davis and Sawyer's published curves (cf. Fig. 17). It must be admitted that the evidence in favour of the production of

either hexoses or sucrose as the first sugar produced in assimilation is scarcely adequate for discussion. In any case Lundegårdh (1914) regards the transformation of sugar into starch and the reverse process as a very complicated one, depending not merely on the concentration of the sugar in the cytoplasm, but also on the quantity of an enzyme, the concentration of which depends on factors at present unknown.

The evidence that hexoses are in excess of other sugars in the conducting tissue of the plant seems definite enough. From this it is concluded that sucrose is converted into hexose by means of invertase in the conducting cells of the plant, and is translocated as hexose sugars. The percentage of sucrose in the petiole is less than in the midribs of the leaf, from which one could expect a diffusion of sucrose away from the leaf. Such a state of affairs would, of course, be produced if the sucrose were inverted in the manner suggested by Davis, Daish and Sawyer in the vascular bundles of the leaf and stem, and they cite in support of this view the observation of Robertson, Irvine and Dobson (1909) that invertase is abundant in the leaf and stem of the beet, although absent from the root. The migration of sucrose, therefore, from a place of higher concentration in the leaf to a place where its concentration is kept constantly lower by the invertive action can be readily understood. Also it is likely that the simpler monosaccharides would diffuse through the plant more rapidly than the more complex disaccharide. The difficulty arises from the fact that the published analyses of Davis, Daish and Sawyer show generally a higher percentage of hexoses in the petioles than in the leaf veins, and one would therefore expect a flow of hexoses towards the leaf and not away from it. On the other hand there is no definite information as to the *concentration* of sugars in the actual conducting cells, and as the hexoses are elaborated into sucrose in the root, it would seem that the concentration of hexoses in the cells of the leaf must rise above that in the root, so that diffusion of hexoses towards the root will take place. There is no doubt that the mechanism of translocation is complex, depending probably on differences of enzyme concentration and possibly also on permeability changes, of which we are at present not merely ignorant of the causes, but also of the nature.

But the form in which carbohydrates are translocated has little bearing on the question of carbon assimilation itself. The inversion of sucrose into hexoses for purpose of translocation, is regarded by Davis, Daish and Sawyer as evidence that

sucrose is a primary product in assimilation. Although it is possible that all the sugar in the mesophyll cells of the leaf is sucrose, and that the hexoses are confined to the vascular bundles, there is no direct evidence of this, and there seems no sufficient reason to conclude that sucrose is the first sugar formed rather than that glucose or other hexoses first appear and that cane sugar is formed from them. It is no more unreasonable to suppose that sucrose should be formed from hexoses in the leaf when these latter reach a certain concentration, than to suppose starch should be formed as a temporary reserve carbohydrate in such leaves as those of *Tropæolum* or Potato, for as starch is a storage form in the potato tuber, and is similarly formed in the leaf, so since sucrose is the storage carbohydrate in the root of *Beta*, being formed from hexoses according to Davis, Daish and Sawyer, there is no reason why it should not also be formed from hexoses in the leaf. The value of evidence in regard to the first sugar formed in the leaf, derived from considerations of the variation in amount of the different sugars, is indicated by the fact that Brown and Morris, Parkin, and Davis, Daish and Sawyer all conclude that sucrose is the first sugar formed in assimilation, while their results on which they base this conclusion differ absolutely. Thus Brown and Morris found that both the sucrose and hexose content diminished during the day; Parkin found the hexose content remained practically constant, while the sucrose varied; Davis, Daish and Sawyer found both the sucrose content and hexose content varying in the leaf, while in the veins the sucrose content remains approximately constant, the hexose varying widely.

We do not wish it to be supposed that we therefore support the view that glucose is the first sugar of carbon assimilation. We hold that the data so far produced from analyses of carbohydrates in leaves and from microchemical examination provide insufficient evidence in favour of or against either theory. While we may regard starch as a secondary product of assimilation, and while also there is good evidence that carbohydrates are translocated, as hexose sugars, in some cases, or to some extent at any rate, and while there is strong evidence that sugars are the first *definitely* known products of the assimilatory process, there is not evidence at present as to which particular sugar is the first one to be produced in the leaf.

Chapter VI.

Energy Relations in Carbon Assimilation.

A. General Remarks.

In the introductory chapter we have referred to the fundamental fact that radiant energy is utilised in carbon assimilation so that compounds of higher energy content are produced from the simpler ones of the surroundings. Beyond this the energy relations of the green leaf are only very imperfectly known, although physical and chemical methods for investigating such energy relations have reached a high degree of development. This aspect of carbon assimilation exhibits perhaps more than any other an unfortunate isolation of effort in research, the various workers on the subject having generally neglected the results obtained by others, both along their own and related lines of investigation.

As in those aspects of the subject we have already dealt with, so here also the complexity of the processes is again evident, and it is difficult to draw definite conclusions. It is possible to measure quantitatively the radiant energy incident on the leaf, and also to measure the amount transmitted. It is, however, by no means easy to determine in what way the energy absorbed is utilised, because we do not know with any approach to completeness how this energy is expended into chemical or electrical energy or heat.

It is generally assumed that the increase in the heat of combustion of the leaf represents that part of the absorbed energy transformed into chemical energy, but it should be pointed out that in so doing, carbon assimilation is taken in its widest possible sense (not carbohydrate assimilation merely) so as to include all the substances formed in the leaf as a result of the photochemical and possible contemporary processes. Thus it is by no means settled as to what extent proteins are formed in the green leaf by photochemical or other chemical actions. In any case an error is introduced if proteins are formed, as their products of combustion cannot be identical with the substances in the leaf from which they are produced, so that their heat of combustion cannot have the same value as the radiant energy used in the leaf in their formation.

We shall first discuss the methods for estimating the amount of material produced in assimilation and the conclusions which have been drawn as to the energy relations of the green leaf from such determinations, before passing on to a discussion of work in which quantitative measurements of both radiant energy and heats

of combustion have been made. Finally, we shall briefly deal with work on the assimilatory power of light of different wave-lengths.

B. Quantitative Estimation of Carbon Assimilation by means of the Products.

In an earlier chapter we have dealt with Blackman's and Willstätter's estimation of carbon assimilation. Both these workers employed a method based on that of Kreusler, in which the intake of carbon dioxide by the leaf is used as a measure of carbon assimilation.[1] The assimilation could also be measured by estimating the increase of carbon content of the leaf, but what is more usually done is to measure the increase of dry weight of the leaf and assume that this is proportional to the increase in carbon content.

Brown and Escombe (1905) in their attempt to determine the energy relations of the leaf, compared the increase of dry matter with the intake of carbon dioxide, but as their results obtained by the two methods were not concordant they came to the conclusion that the dry weight method is untrustworthy. Therefore they only determined the intake of carbon dioxide and estimated the increase in dry matter by calculation. We shall only deal briefly with the extensive researches of Brown and Escombe on this subject, for although they are the first to make quantitative measurements of energy in regard to assimilation and although they clearly indicate the complexity of the energy relations of the leaf, yet the values actually determined by experiment are few and those obtained by calculation are of doubtful value and do not agree with values obtained by direct measurement by other investigators. We have already referred to the divergence between the estimations of the internal leaf temperature made by Brown and Escombe, and the direct temperature measurements made by Blackman and Matthaei.

In order to estimate the dry matter formed, Brown and Escombe multiply the weight of carbon dioxide absorbed by the leaf by a carbohydrate factor of 0·640. This factor they obtain from the analyses in regard to carbohydrate content of leaves of *Tropæolum majus* by Brown and Morris as described in the last chapter. Of

[1] The same method has been employed by Brown and Escombe (1902). We have not dealt with the very interesting results recorded in this paper in regard to carbon dioxide as a limiting factor, as the results are confirmed by F. F. Blackman's later and more complete work on limiting factors. In order to avoid unnecesary length of this review we have in this matter, as elsewhere, confined our remarks to the more complete account where two researches run parallel.

course Brown and Escombe assume that the ratio between the various carbohydrates remains constant and further that carbon dioxide is used only in the production of carbohydrates. It is true that variations in the ratio of the various carbohydrates will make little difference in the carbohydrate factor, and similarly, the error introduced owing to the probable incorrectness of Brown and Morris's analysis (cf. Chapter V) is likely to be small. On the other hand the error introduced by the assumption that the whole of the carbon dioxide is used in carbohydrate formation is likely to be larger, but on this subject our information is very incomplete. It may be interesting to compare this carbohydrate factor with values obtained by experiment for the ratio between increase in dry weight and carbon dioxide absorbed. The following table is due to Krasheninnikoff (1901) and although the values are probably not of a very high order of accuracy, they may give some idea of the variations likely to occur.

TABLE XXXVI.

Increase in Dry Weight of Leaves per gram of Carbon dioxide absorbed.

Bamboo	0·60
Cherry Laurel	0·60
Sugar Cane	0·67
Lime	0·74
Tobacco	0·68

Thoday (1909) compared the increase in dry weight with the increase in carbon content of the leaf in the cases of *Helianthus tuberosus* and Cherry Laurel. His results indicate a considerable variation in the ratio of carbon increase to increase of dry weight in leaves of the same species, but in Thoday's experiments a good many factors are not controlled, and it is impossible to say what causes the variation. We should like to emphasize that in all such cases in plant physiological researches where it is sought to determine the absolute value of a quantity, it is absolutely imperative to determine the probable error of the experiment. This, as far as we know, has not been done in any single instance in work on carbon assimilation.

In regard to the direct determination of the products of assimilation, the principle of this method was first exposed by Sachs in his paper " Ein Beitrag zur Kenntniss der Ernahrungsthätigkeit der Blätter " published in 1884. Sachs' method is well-known.

The dry weight of unit area of one half of a leaf measured at the beginning of an experiment is compared with the dry weight per unit area of the other half of the leaf after its exposure to the required conditions. The difference of the two values is regarded as the weight of products which have accumulated in unit area of the leaf during the experiment. As Sachs found a greater increase in dry weight in detached leaves than in leaves still attached to the plant, he assumed that in the latter case translocation of the products away from the leaf takes place concurrently with assimilation. To obtain the true value for assimilation in attached leaves, Sachs therefore added the loss in dry weight of leaves during the night to the increase in dry weight of the same area during the same time during the day.

Brown and Escombe pointed out that Sachs obtained much higher values for assimilation by his half leaf method than they obtained by direct determination of the carbon dioxide absorbed. They therefore carried out a series of experiments in which the assimilation of the same leaves were measured by both methods. The following table gives the results they obtained for *Catalpa bignonioides*. The results in the last column are obtained by the use of the carbohydrate factor 0·64, to which reference has already been made.

TABLE XXXVII.

Comparison of the Values obtained for Assimilation of Leaves of Catalpa bignonioides *by the Half-Leaf Method and by measuring the Intake of Carbon dioxide.*

Experiment.	Increase in Dry Wt. per sq. decimetre per hour mg. (observed).	CO_2 absorbed per sq. decimetre per hour, ccs.	Carbohydrate formed per sq. dec. per hour, mg. (calculated).
1	9·83	1·41	1·76
2	7·14	1·43	1·79
3	2·60	2·35	2·94
4	7·22	2·33	2·92
Mean	6·69		2·35

It will be observed that the divergence between the results obtained by the two methods is much larger than can be accounted for by experimental error or error in the estimation of the carbohydrate factor. Brown and Escombe attribute this divergence to three sources of serious error to which the half leaf method is liable. These are—

1. Possible changes after assimilation in the power of retention

of water by the colloids of the cell contents when these are dried at 100°C.

2. Differences due to lack of symmetry between the two halves of the leaf in regard to venation and thickness.

3. Alterations of area of the leaf as a result of insolation. Thus if a leaf suffered shrinkage during insolation so that its area as measured afterwards was less than at the beginning of the experiment, the dry weight of unit area would be correspondingly increased. Consequently the values found for the increase in dry weight of unit area would be larger than the true values as, of course, the initial dry weight is measured before insolation on an unshrunken half leaf.

1. The possible error due to changes of composition during insolation which might produce a different water retaining capacity was investigated by Thoday (1909), who measured both the dry weight and carbon content of the experimental and control half leaves and so calculated both the gain in dry weight and of carbon per unit area. Thoday concludes that the correspondence between the increase in dry weight and the starch equivalent of the gain in carbon is sufficiently close to make it clear that fixation of water cannot play an appreciable part in determining the dry weight increase. However, as the starch equivalent of the gain in carbon found in Thoday's experiments varied from 20% less to 40% (and in one extreme case 90%) more than the actual increase in dry weight of the same leaf, it is not clear why Thoday should come to this conclusion from his results. We hesitate therefore to accept Thoday's own opinion that his results indicate that the dry weight method is "not vitiated by any large indeterminable errors such as would arise if varying quantities of water were retained by the colloids of the leaf after drying it at 100°C." The numbers show indeed that changes in composition of the leaf during assimilation will not account for the whole of the discrepancy between the two methods as observed by Brown and Escombe, but they tell us nothing as to whether such change is negligible or not.

2. Brown and Escombe made a number of determinations of the degree of symmetry of the two halves of various leaves by measuring the two halves separately with a planimeter and then drying them to a constant weight. The dry weight per square decimetre of the two halves was calculated and the percentage difference between the dry weight per unit area of the two sides of

the leaf calculated. These differences are summarised in the following table.

TABLE XXXVIII.

Difference in Dry Weight per Unit Area of Opposite Sides of Leaves due to Differences in Symmetry (Brown and Escombe).

Species.	Difference in Dry Weight, Per Cent.
Catalpa bignonoides	3·9
„ „	4·3
„ „	2·3
„ „	5·7
„ „	0·7
Catalpa purpurea	2·3
Catalpa Bungei	1·3
„ „	2·2
Tropœolum majus	0·3
Polygonum Weyrichii	1·1

Similar differences were found by Thoday for some other species and he concludes with Brown and Escombe that this source of error is inherent in the method. He points out that the error arising from this cause may be reduced by using parts of leaves free from big veins instead of whole half leaves. Thus with *Paulownia imperialis* the average percentage difference of four pairs of measurements was 1·4% when the veins were avoided and the average percentage difference of two pairs of measurements was 5·95% when the veins were included.

3. The third source of error suggested by Brown and Escombe is that due to change in area of the leaf during insolation. These investigators measured the area of leaves of *Catalpa bignonioides* before and after insolation and found resulting alterations in area from an increase of 0·14% to a decrease of 3·12%. According to Thoday, leaves of *Helianthns annuus* often diminish in area by more than 5% between early morning and midday if the meteorological conditions are such as to favour rapid transpiration of water.

From such data Brown and Escombe calculate the order of magnitude of the error likely to arise in Sachs' half leaf method. It would thus be quite probable for the error in determination of the dry weight of a half leaf to equal 2%. In such a case Brown and Morris show that with a leaf having a dry weight of 0·5 gm. per sq. decimetre asssimilating 0·002 gm. carbohydrate per sq.

decimetre per hour the error in the increase in dry weight obtained by the half leaf method in an experiment lasting 5 hours would be as much as 100%, whereas the error in the results obtained by measuring the carbon dioxide absorption would amount to no more than 2%. They therefore reject Sachs' method as quite untrustworthy.

As the dry weight method, if it could be made sufficiently accurate, would have its uses we agree with Thoday "that it should not lightly be abandoned." Thoday makes some useful suggestions in regard to decreasing the inaccuracy of the method, but he does not furnish data which enable one to determine the degree of accuracy obtainable when all suggested precautions are taken. It appears to us that the only way of finding this is to make a number of such estimations and determine the probable error of the mean result.

C. The Quantitative Determination of the Heat of Combustion of the Products of Assimilation.

Although the measurement of heats of combustion offers no particular difficulties, very few such measurements have been made in plant physiology. Brown and Escombe assume that the heat of combustion of the products of assimilation is the same as that of glucose, but this assumption is not justified by the values obtained by experiment for the heat of combustion of one gram of material produced in assimilation. It will be seen however from the heats of combustion of various substances recorded in the accompanying table, that measurements of the actual heats of combustion of the products of assimilation might afford helpful information as to the relative proportion of the different products.

Table XXXIX.

Heats of Combustion in Gram-Calories of Various Substances.

Substance	Heat of Combustion per gram.
Ethyl Alcohol	$7{\cdot}18 \times 10^3$
Glucose	$3{\cdot}76 \times 10^3$
Sucrose	$3{\cdot}99 \times 10^3$
Dextrin	$4{\cdot}1 \times 10^3$
Starch	$4{\cdot}1 \times 10^3$
Cellulose	$4{\cdot}2 \times 10^3$
Leucin	$6{\cdot}5 \times 10^3$
Vitellin	$5{\cdot}7 \times 10^3$
Linseed Oil	$9{\cdot}47 \times 10^3$
Olive Oil	$9{\cdot}51 \times 10^3$

Actual determinations of the heat of combustion of the material produced in assimilation have been made by Krasheninnikoff (1901) and by Puriewitsch (1914). They measured the increase in dry weight per unit area per hour by Sachs' dry weight method and also the increase in the heat of combustion per unit area per hour. The increase in heat of combustion per unit increase in dry weight gives the heat of combustion per gram of the products of assimilation. Krasheninnikoff obtained an average value for this of $4{\cdot}4 \times 10^3$ gram-calories. From Puriewitsch's data we have calculated the values for different species set out in the following table. It will be observed that the values agree well with the number obtained by Krasheninnikoff, but not with the value assumed by Brown and Escombe ($3{\cdot}76 \times 10^3$).

TABLE XL.

Heats of Combustion of the Products of Assimilation.

Species.	Increase in Dry Wt. per sq. metre per hr., grm.	Increase in Heat of Combustion per sq. cm. per hr.	Heat of Combustion of Product of Ass. in gm.-cal. per gram.
Acer platanoides	1·2	0·526	$4{\cdot}4 \times 10^3$
Polygonum sacchalinense	2·7	1·41	$5{\cdot}2 \times 10^3$
,, ,,	2·0	0·903	$4{\cdot}5 \times 10^3$

D. The Quantitative Measurement of the Radiant Energy Incident on the Leaf and the Utilisation of this Energy.

A full discussion of the methods used and principles involved in the measurement of radiant energy would be out of place here. The instruments generally employed are of four kinds, the thermopile, the bolometer, the radiometer and radiomicrometer. For a description of these instruments the reader is referred to physical text books, and for a more complete discussion on the relative merits of the various methods, to Kayser's Spectroscopie Baly's Spectroscopy, and Coblentz's "Instruments and Methods used in Radiometry" (1908). Generally speaking, the radiant energy is absorbed and transformed to heat in the measuring instrument, and thus a measure of the total energy obtained, but if by a suitable method a spectrum of the source of light is produced, the same method can be used for measuring the distribution of energy in the different parts of the spectrum.

Although numerous measurements of the radiant energy of the sun have been made by astrophysicists, yet such quantitative measurements of radiant energy as have been made in plant physiological experiments are inadequate, and in plant ecological studies where light may be an all-important factor, such measurements have not even been attempted.[1]

Detlefsen (1888) appears to be the first to attempt energy measurements in regard to problems connected with carbon assimilation. He showed that more energy is used when the leaf is supplied with an atmosphere containing carbon dioxide, than in an atmosphere devoid of this gas. Similar determinations have been made by Mayer (1893) and Ursprung (1903) by the use of a thermopile, but Brown and Escombe (1905) were the first to make an extensive series of measurements of radiant energy in connection with plant physiological problems.

Brown and Escombe measured the intensity of radiation on the leaf by means of a pair of differential platinum thermometers, one bright and the other black, as devised by Callendar (1898). The instrument was rendered self-recording by connecting it with a Callendar's recorder (1899).

The characteristic feature of Brown and Escombe's work on the energy relations of the leaf is that they assume there are certain fundamental properties of the leaf in regard to energy, and they attempt to determine certain physical quantities, such as coefficient of absorption and emissivity, which they regard as constant for all conditions of experiment. But even a superficial consideration is sufficient to tell us that this cannot be the case and the performance of a larger number of experiments would probably have shown these authors what range of variations were likely to be obtained. Thus Puriewitsch insists that the absorption of energy depends on the concentration of carbon dioxide; his results are given in the table overleaf.

The beauty of Brown and Escombe's work lies not in the reliability of the results obtained by measurement or calculation, but in the fact that they are the first, and up to now, the only investigators who have attempted to obtain a complete balance sheet for the leaf in regard to energy.

[1] We do not discuss here the elaborate work of Wiesner (1907), which although interesting in its conceptions of light in respect of plant physiological and ecological problems, does not render much help to the problems under review on account of the inadequate method of energy intensity measurement by means of photographic paper. On the other hand many of the observations recorded may become useful as a basis for future observations.

TABLE XLI.

The Absorption of Radiant Energy by the Leaf in the Presence and in the Absence of Carbon Dioxide (Puriewitsch).

Species.	Date.	Duration of Experiment.	CO_2 in air, per cent.	Ratio of Energy trasmitted in presence of CO_2 to that transmitted in absence of CO_2 per cent.	Ratio of Excess of Energy absorbed by the leaf in presence of CO_2 to that transmitted in absence of CO_2 per cent
Aristolochia Sipho	27 May 1910	11.25 a.m.-12.43 p.m.	1·2	95·0	5·0
		1.1—2.8 p.m.	,,	90·4*	9·6
,, ,,	,, ,,	11.25 a.m.-12.43 p.m.	,,	92·2	7·8
		1.1—2.8 p.m.	,,	95·4*	4·6
Catalpa speciosa	1 June 1910	11.55 a.m.-1·0 p.m.	0·7	99·0	1·0
Acer platanoides	2 June 1910	11.29 a.m.-1.14 p.m.	1·7	98·3	1·7

*The values marked with an asterisk were obtained by means of a Rubens thermopile, the remainder by means of the Bolometer.

Brown and Escombe suppose that the total radiant energy falling on the leaf is used in the following ways;

(1) in assimilation,

(2) in transpiration,

(3) by transmission through the leaf,

(4) by thermal emission (if the leaf temperature is higher than that of its surroundings, as it usually is, this is positive, but if lower the thermal emission is negative, that is, the leaf gains energy from its surroundings).

We have indicated earlier in this chapter that Brown and Escombe estimated the assimilation by calculating the increase in dry weight from the intake of carbon dioxide and the assumed heat of combustion of the products. Thus, in this first determination, two assumptions are made, the accuracy of which is not confirmed by measurement. One of these is the assumption that 1 gram of carbon dioxide absorbed is equivalent to 0·64 gram of dry matter; the other that the heat of combustion of the products is $3·76 \times 10^3$ gram-calories.

The transpiration was determined by weight, and the energy used in transpiration calculated from the heat of vaporisation of water at the particular temperature.

The energy transmitted was calculated from the coefficient of absorption of the leaf, which was found in the following manner. A day of bright sunshine was selected and the intensity of radiation

of sunlight measured. The leaf was interposed above the coils of the instrument for a few minutes and the intensity of radiation again measured. The leaf was then withdrawn when the value of the full intensity of radiation was again recorded on the drum of the self recorder. The ratio of the middle reading to the mean of the first and third readings gives the coefficient of transmission, and the difference between unity and the coefficient of transmission is the coefficient of absorption. The following table shows the coefficients of absorption and transmission found by Brown and Escombe for various species.

TABLE XLII.

Coefficients of Absorption and Transmission of Radiant Energy of Sunlight.

Species.	Coefficient of Absorption.	Coefficient of Transmission.
Helianthus annuus	0·686	0·314
Polygonum Weyrichii	0·647	0·353
,, Sacchalinense	0·691	0·309
Petasites officinalis	0·728	0·272
Silphium terebinthaceum	0·699	0·301
Arctium majus	0·728	0·272
Verbascum olympicum	0·758	0·242
Senecio grandifolius	0·774	0·226

No considerable difference in the coefficient of absorption was found between leaves of the same species of different ages.

Of course, in these determinations the part of the energy reflected from the surface of the leaf is neglected. Brown and Escombe regard the reflected energy as forming a very small fraction of the total incident energy, but having regard to the information available from pure physics it is unlikely to be negligible, as a black cloth, for instance, may reflect 1% of the radiant energy incident upon it.

The difference between the total incident energy absorbed on the one hand, and that used in assimilation and transpiration on the other hand, gives that part of the energy lost by re-radiation, conduction and convection, *i.e.*, that lost by emission.

The following numbers show the results of a typical experiment.

Tropæolum majus, September 11th, 1900.
Leaves in sunlight under canvas screen.
Duration of experiment 4·8 hours.

Assimilation of CO_2 per sq. decimetre per hour ...	1·210 c.c.
Transpiration of water „ „ „ ...	0·1340 gm.
Solar radiation incident on leaf per sq. cm. per min.	0·1282 gm.-cal.
Coefficient of absorption „ „ „	0·700
Solar radiation absorbed by the leaf „ „ „	0·0897 gm.-cal.
Energy expended in assimilation „ „ „	0·0010 „ „
„ „ „ transpiration „ „ „	0·0132 „ „
Total Energy used for internal work „ „ „	0·0142 „ „
Energy lost by re-radiation and convection „ „	0·0755 „ „

In the following table we give the summary of the energy relations as estimated by Brown and Escombe in five such experiments on *Polygonum Weyrichii*.

TABLE XLIII.

Mode of Disposal of Energy by the Leaf of Polygonum Weyrichii.

Total Energy Received 100.

Experiment.	Energy used in Assimilation.	Energy used in Transpiration.	Total Energy expended in Internal work.	Energy lost by Transmission.	Energy lost by Re-radiation and Air Convection.
1	0·42	9·67	10·09	35·31	54·60
2	1·59	53·60	55·19	35·30	9·51
3	1·66	57·01	58·67	35·32	6·01
4	1·32	35·64	36·98	35·28	27·76
5	0·49	52·72	53·21	35·30	11·49

In order to indicate the conditions of experiment, we give overleaf (Table XLIV) the actual experimental data of these five experiments as recorded by Brown and Escombe.

We do not intend to analyse in detail the results obtained by Brown and Escombe, as for reasons already given, we consider that at present it is not possible to obtain absolute values of the energy used in different processes. More light must be shed on the complexity and influence of the various factors before such numbers as Brown and Escombe's can be discussed with profit.

How much importance can be attached to such calculated values is indicated by the estimations of leaf temperature by Brown and Escombe. To obtain this they start with the assumption that

TABLE XLIV.

Experiments on Leaves of Polygonum Weyrichii *under Various Conditions of Insolation.*

Experiment.	Date.	Conditions of Experiment.	Assimilation in c.cs. CO_2 per sq. decimetre per hr.	Transpiration in gms. per sq. decimetre per hr.	Solar Radiation incident on Leaf.	Solar Radiation absorbed by Leaf.	Energy used in Assimilation.	Energy used in Transpiration.	Total Energy used in Internal Work.	Energy lost by Re-radiation and Air convection.
					In gm.-calories per sq. cm. per minute.					
1	June 29, 1900	Intermittent sunlight without any screen.	3·20	0·599	0·6120	0·3959	0·0026	0·0592	0·0618	0·3341
2	June 19, 1900	Full sunshine. Leaves under thin canvas screen.	3·758	1·054	0·1942	0·1256	0·0031	0·1041	0·1072	0·0184
3	June 22, 1900	Intermittent sunlight. Leaves under thin canvas screen.	3·058	0·868	0·1503	0·0972	0·0025	0·0857	0·0882	0·0090
4	July 3, 1900	Intermittent sunshine with some showers. Leaves under canvas screen.	2·271	0·517	0·1431	0·0926	0·0019	0·0510	0·0529	0·0397
5	July 11, 1900	Hot cloudless day. Leaves under thin canvas screen.	1·479	1·291	0·2418	0·1565	0·0012	0·1275	0·1287	0·0278

if a leaf is transpiring in the dark, its temperature will fall until the energy used in transpiration is equal to that which it receives from its surroundings in the same time. In some experiments made by Brown and Wilson (1905) it was found by measuring the temperature of leaves and the loss of water by transpiration, that when there is a temperature difference of 1°C between the leaf and its surroundings, the leaf of *Tropœolum majus*, for example, receives or loses, as the case may be, 0·01427 calories per square centimetre per minute in still air. This is the thermal emissivity. In Brown and Escombe's experiments the total energy lost by radiation is obtained by difference, and the division of this number by the thermal emissivity calculated by Brown and Wilson is supposed to give the difference in temperature between the leaf and its surroundings. As the temperature of the latter is measured, that of the leaf is at once deduced.

We do not propose to discuss Brown and Wilson's method of finding the thermal emissivity, as the method of estimating the leaf temperature is crude, and other factors which may be important, such as respiration and temperature, are regarded as negligible. It is only to be expected that the temperatures so estimated should be far removed from the real temperatures. As Blackman and Matthaei (1905) point out, the temperatures given in Brown and Escombe's tables "for leaves in the sun in the open air are never more than 2°C above the shade temperature of the air, while our few direct measurements with cherry-laurel leaves, brilliantly insolated, indicated 7° to 16°C above the thermometer in the shade." We may add that differences similar to those observed by Blackman and Matthaei and of even greater magnitude have been observed by various workers, notably by Askenasy (1875), Ewart (1897) and Stahl (1909).

Brown and Escombe's results show that only a small proportion of the energy absorbed by the leaf is used in carbon assimilation, but that the actual percentage used for this purpose is a very variable quantity. This conclusion has also been reached by Puriewitsch (1914), who measured the total radiant energy incident on the leaf by means of the bolometer (see Kurlbaum, 1894). The energy used in assimilation was obtained in some cases by direct measurement of the increase of the heat of combustion per unit area of the leaf as described in the previous section of this chapter. Unfortunately, only a few such determinations were made, and the values for the remaining experiments calculated from them.

We may quote the results of a typical experiment of Puriewitsch.

Two leaves of *Acer platanoides* were used. The intensity of radiation incident on the leaf was measured every 10 minutes during the experiment which lasted 6 hours, and the total energy calculated.

The increase in dry weight was obtained by the half-leaf method.

The following results were obtained :—

	Before Insolation.	After Insolation.
Area of half leaf	316·6 sq. cm. ...	316·8 sq. cm.
Dry weight of half leaf ...	1·2494 gm. ...	1·3952 gm.
Dry weight per sq. cm. ...	0·0039 gm. ...	0·0044 gm.
Heat of combustion of 1 gm. dry weight	4300·21 gm.-cal. ...	4313·46 gm.-cal.
Heat of combustion per sq. cm.	16·770 „ ...	18·978 „

Increase of heat of combustion after insolation per sq. cm.— 2·208 gm.-cal.

Total energy incident on leaf per sq. cm. 361·03 gm.-cal.

Quantity of radiant energy used in assimilation 0·6%.

As we have stated before, conclusions in regard to the relation between the intensity of the radiant energy and that part of it used in assimilation, cannot be drawn until our information in regard to the various factors is considerably enlarged. It can, however, be concluded that with high light intensities only a small part of the incident radiant energy is utilised for assimilation. The lowest value, for instance, obtained by Puriewitsch was 0·6% for an average light intensity of 1·003 gm.-calories per minute. But it will clearly be seen from the table below that other factors besides light intensity are operating.

Brown and Escombe's figures exhibit similar variations in the percentage of sun energy utilised in assimilation, but on the whole they are lower. This difference is easily accounted for by the different method employed in measuring the assimilation. Brown and Escombe, as we have shown, used the intake of carbon dioxide in conjunction with a carbohydrate factor and an estimated value of the heat of combustion in order to obtain a measure of the energy used in assimilation. Their method is likely to yield more uniform though perhaps not more accurate results than the half leaf method employed by Puriewitsch, who did not attempt to correct any of the sources of error of the method, which it might

TABLE XLV.

Percentage of Radiant Energy Incident on the Leaf used in Assimilation (Data from Puriewitsch).

Species.	Date.	Duration of Experiment in hrs. & min.	Total Incident Energy per sq. cm. in gm.-cal.	Increase in heat of combustion per s. cm. in g.-cal.	Intensity of Radiant Energy in gm.-cal. per sq. cm. per min.	Percentage of sun energy used in Assimilation.
Acer platanoides	30 May, 1912	6.0	361·03	2·208	1·003	0·6
,, ,,	2 June, ,,	5.0	162·59	1·332	0·542	0·81
,, ,,	13 ,, ,,	6.0	240·33	6·508	0·667	2·7
,, ,,	19 ,, ,,	5.0	202·20	2·630*	0·674	1·3
Helianthus annuus	11 ,, ,,	4.30	132·48	5·977	0·454	4·5
Polygonum sacchalinense	31 May, ,,	1.20	70·85	5·509	0·885	7·7
,, ,,	3 June, ,,	3.0	122·33	5·076	0·679	4·1
,, ,,	16 ,, ,,	1.50	97·62	2·585*	0·887	2·6
,, ,,	17 ,, ,,	2.20	123·18	4·656	0·880	3·7
,, ,,	21 ,, ,,	5.0	136·81	1·540	0·456	1·1
,, ,,	23 ,, ,,	5.0	177·00	4·514*	0·590	2·5
Saxifraga cordifolia	6 ,, ,,	2.20	68·16	3·450	0·487	5·0

*The values marked with an asterisk were actually observed. The remaining values in this column were obtained by calculation.

have been possible to correct.

We may now attempt to correlate the results of Brown and Escombe and of Puriewitsch with those of Blackman on light as a factor in assimilation. It will be recalled that on Blackman's view of limiting factors, if we commence with a very low light intensity, increase in light (radiant energy) will result in a proportionate increase in assimilation until some other factor, such as carbon dioxide supply, is limiting the rate. The curve connecting the light intensity and the rate of assimilation will be of the form already shown in Fig. 5. As regards the *proportion* of the radiant energy used in assimilation, this should remain constant on Blackman's view so long as light is the limiting factor, for the rate of assimilation, and consequently the energy used for it, is directly proportional to the intensity of the light. But when the light is increased so that some other factor is limiting the rate of assimilation, then if that factor remains constant, increase in light intensity will result in a decrease in the percentage of radiant energy used in assimilation.

In many of Brown and Escombe's experiments the intensity of illumination is roughly inversely proportional to the proportion of the energy used in assimilation, but this relation is not by any means

exact or even approximate. It suggests, however, that in some of Brown and Escombe's experiments at any rate, radiant energy was in excess, and some other factor was limiting the rate of assimilation. In a set of experiments they performed in which the proportion of the full radiant energy of the sun utilised in assimilation was compared with that proportion of it so utilised when it was reduced to a fraction of the full energy by means of rotating sectors placed above the leaf, it was always found that reducing the intensity of illumination increased the proportion of energy used in assimilation. This is exactly what one would expect, as in the experimental arrangement of Brown and Escombe, the full intensity of radiant energy falling on the leaf would be likely to be in excess of that required for the carbon dioxide supply.

Similarly, in Puriewitsch's experiments, although unfortunately no data whatever are given in regard to temperature, it seems likely that the radiant energy was not limiting the rate of assimilation. The carbon dioxide supply was low, namely that of the atmosphere. The intensity of radiation was, on the other hand, in all cases moderately high. Under these conditions we should expect that carbon dioxide supply would be the limiting factor and that the variations in the total radiant energy in the different experiments would be without influence on the rate of assimilation. Consequently we should expect that the proportion of the sun energy used in assimilation would vary inversely with the intensity of illumination. Such, however, is not by any means the case, and it is clear we must look for other factors of which no data are given, to explain the results obtained. Puriewitsch does indeed point out that his numbers show that the rate of assimilation falls off with time (cf. Blackman's time factor) but this will not explain his results completely. We have here a particular instance of that lack of correlation of effort to which we have referred in the first section of this chapter, for if Puriewitsch had taken cognizance of Blackman's researches, his experiments might have yielded results of much greater significance. It is only fair to Puriewitsch to point out that he regards his experiments as preliminary.

E. Assimilation in Relation to Radiant Energy of Different Wave-Lengths.

On this subject no satisfactory work has so far been performed, although it has been a favourite subject for investigation for more than a century. On the one hand in no case is the method employed for the

measurement of energy satisfactory, and on the other hand the methods used for measuring assimilation are very crude. Also the fundamental aspects of the problem seem to have escaped the notice of most investigators, in spite of its vital importance. Having regard to the present state of our knowledge concerning radiant energy derived from pure physics, and to what we now know of the processes of carbon assimilation, it ought to be possible to attack the problems profitably.[1]

Concerning the subject with which we deal in this section, there exists a very voluminous literature, to which undue importance is generally given in text-books.

The earliest investigators, as for instance, Senebier (1788) and Dumas (1841) supposed that the blue-violet rays were of most importance in assimilation. Daubeny (1836) and Draper (1844) as well as Sachs (1864 b) and Pfeffer (1871) were of the opinion that the yellow rays were those utilised in assimilation. Lommel in 1871 suggested that the rays most strongly absorbed by chlorophyll, namely those between the B and C lines in the red part of the spectrum, were those most active in assimilation.

Attempts have also been made by Timiriazeff (1877, 1885), Reinke (1884) and Engelmann (1882, 1884) to discover in which part of the spectrum assimilation takes place. They all agree that maximum assimilation takes place in the red part of the spectrum, although they differ as to the exact position. Engelmann, using the most sensitive, though not necessarily the most accurate method, obtains a secondary maximum in the blue-violet end of the spectrum. Timiriazeff and Richter (1902) appear to be aware of the fact that smaller assimilation in the blue part of the spectrum of sunlight may be explained by the less intensity of radiation in that region. Richter and others contend that the assimilation depends only on the energy of the absorbed light and not on the wave length.

The conditions in all these experiments were such that discussion of them is not justified; we mention them here mainly on account of their historical interest.

It does not appear from all this earlier work whether the assimilation would be the same if a leaf were exposed to red or to

[1] We are confronted with the following problems:—

1. The intensities and relative proportions of the different frequencies of radiation incident on the leaf. If sunlight is concerned, astrophysical and meteorological factors are of considerable influence here, but a discussion of this aspect of radiation is outside the scope of this review.
2. The relative absorption by the leaf of radiation of different frequency.
3. The relative proportion of absorbed energy of any particular frequency which is used in assimilation.

blue light of the same energy. This problem has been attacked by Kniep and Minder (1909). They used a Rubens' thermopile for the measurement of radiant energy. Light of different colours were obtained by the use of different filters.

For red a glass filter was used which let through light of wave lengths 620$\mu\mu$—infra red, and a little light of wave length 608$\mu\mu$. The coefficients of transmission given for different wave lengths were—

Wave Length. $\mu\mu$	Coefficient of Transmission.
644	0·846
578	0·00056
546	0·000057
509	0·000

The blue filter let through light of wave lengths 523·8$\mu\mu$—ultra violet. The coefficients of transmission given for different wave lengths were—

Wave Length. $\mu\mu$	Coefficient of Transmission.
546	0·00
509	0·0109
480	0·177
436	0·455
405	0·395
384	0·267
361	0·078
340	0·010
332	0·000

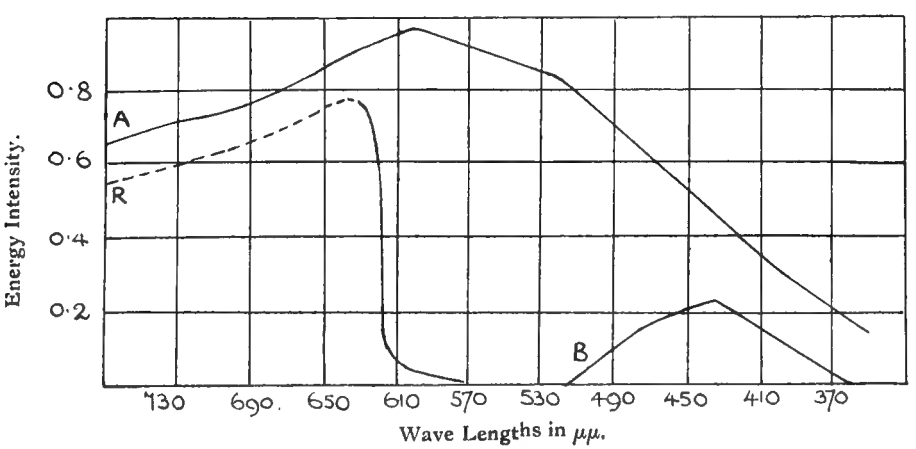


FIG. 18. Energy Intensity in the Normal Spectrum of direct Sunlight after Langley (Curve A), compared with the Energy Intensity in the Spectra of Sunlight after its passage through Red (Curve R) and Blue (Curve B) Filters. The broken part of Curve R is assumed. (After Kniep and Minder).

As a green filter was used a solution obtained by mixing a solution of potassium monochromate with ammonical copper oxide. This solution let through light of wave lengths between 512$\mu\mu$ and 524$\mu\mu$. No quantitative data were obtained in regard to the coefficients of transmission. From those coefficients of transmission measured and from the curve of distribution of energy in the spectrum of the source of light it is possible to construct curves showing the distribution of energy in the various regions of the spectra of the light let through the filters. So, for instance, the distribution of energy in the solar spectrum has been examined by Langley (1882) and Fig. 18 shows the distribution of energy in the solar spectrum and the distribution of energy in the light let through Kniep and Minder's red and blue filters.

Kniep and Minder only performed their experiments on cloudless days at Naples between 11 a.m. and 2.30 p.m. when the intensity of the light and the distribution of energy in the spectrum[1] remained moderately constant. Heat rays were excluded by the use of screens of distilled water.

It is to be regretted that these authors, after having realised the essential facts of energy distribution in the spectrum and after introducing reliable methods, should render their experiments ineffective by using a method for measuring carbon assimilation which is one of the most unreliable. This method, which consists in measuring the rate at which bubbles of gas are given off by an assimilating submerged water plant,[2] has recently formed the subject of an investigation by Kniep himself (1915), who shows how many and serious are the sources of error in it. In order to employ this method, Kniep and Minder had to reduce the intensity of radiation by a series of screens of different substances: water, copper sulphate, potassium dichromate, more screens being used for the red than the blue in order to bring the intensity of the radiation to the same value in the two cases. By doing this, of course they necessarily alter the distribution of energy, and the values obtained for transmission and distribution of energy to which we have already referred, have not much bearing on the actual experiments.

[1] The relative intensity of the light of the blue part of the spectrum is very small in the morning, increases towards mid-day, and falls off again in the evening.

[2] This method, generally known as the "bubbling method," was due, like so much in plant physiology, to Sachs. Accounts of researches in which it was used are to be found in the works of, *e.g.*, Pfeffer (1897), Reinke (1883, 1884), Pantanelli (1903) and Treboux (1903).

The conclusion they draw from their experiments is that blue and red light of the same intensity produce the same assimilation. Green light is incapable of producing assimilation. The absolute intensity of energy incident on their plants is of the order of 0·005 gm.-calories per sq. centimetre per minute, and although it is likely that with this low energy intensity, light is a limiting factor, yet it cannot be assumed that this is so. Kniep and Minder appear to be unaware of Blackman's work on limiting factors, and they give no data relating to factors other than light intensity. It would, therefore, be impossible to draw any valid conclusions from their results, even if their experimental method were beyond criticism.

Investigations along another line have been made by attempting to measure that part of the total energy absorbed by the leaf, which is actually absorbed by the chlorophyll. The experiments of Timiriazeff (1903), in which the absorption of radiant energy by alcoholic extracts of leaves was taken as a measure of the absorption of light by chlorophyll, are clearly of little value, as his leaf extracts would contain far less chlorophyll than impurities. Also the state of aggregation of chlorophyll and its distribution in the leaf are different from those in an alcoholic extract.

Again, the isolated experiments of Brown and Escombe (1905) in which the absorption of radiant energy by the white and green portions of a leaf of *Negundo aceroides* was compared and the difference between the two values attributed to the chlorophyll, is not to be regarded as providing any definite evidence, for it is unfair to assume that the conditions in green and albino parts of a leaf are identical except for the presence of the pigment, and moreover, the considerations we have already put forward in regard to Brown and Escombe's measurements of coefficients of absorption hold equally well here.

Nevertheless, in a comparatively recent publication, Weigert (1911) has accepted Brown and Escombe's result for the *Negundo* leaf, and applied it to work out the efficiency of the assimilatory system for another species. Brown and Escombe had found that in one of their experiments on the energy relations of the leaf, an intensity radiation of 0·5 gm.-calories per sq. centimetre per minute could be reduced to $\frac{1}{12}$ of this amount without diminishing the rate of assimilation; with further reduction of light intensity this became the limiting factor. They estimated the energy used for assimilation at 0·0017 gm.-calories per sq. centimetre per minute *i.e.*, 4·1% of the total incident energy. Now these workers found

in their experiment with the *Negundo* leaf that 4·2% of the total incident energy is absorbed by the chlorophyll. These incidental numbers have been given some importance by Weigert, who assumes from them that $\frac{4·1}{4·2}$ or 98% is the efficiency of the assimilatory system. This result is indeed, as Weigert himself admits, surprising, and he regards the plant as the most ideal photochemical machine which could possibly exist. Presumably Weigert would have been still more surprised if he had chosen a value from another of Brown and Escombe's experiments in which 4·48% of the total energy received by the leaf was used in assimilation. This by his method would have given an efficiency of $\frac{4·48}{4·2}$ or 107%.

But without this absurdity it is clear that numbers obtained from such data are completely valueless.

Chapter VII.

Theories of Carbon Assimilation.

A. General Remarks.

It is significant to note that the contributions to the literature with which this chapter deals, are not the work of those plant physiologists who have built up this branch of their subject: de Saussure, Sachs, Pfeffer, F. F. Blackman. It seems as if those who by years of experience have obtained most insight into the complexity of plant processes have realised that the only way for development lay in bringing to light facts, and endeavouring to determine the laws underlying these facts.

It is remarkable that all the theories of carbon assimilation have not advanced the state of plant physiology in the least; it would not have materially altered our knowledge of plant processes if all that voluminous literature had never appeared. Thus none of the various aspects of carbon assimilation with which we have dealt in the preceding chapters owes anything of its development to any theory of carbon assimilation that has ever been advanced.

It is surprising that no protest has been raised by plant physiologists against the overwhelming tendency to publish theories which have little or no reference to the facts of assimilation by the plant. Spoehr's recent paper, "The Theories of Photosynthesis in the Light of Some New Facts" (1916), is indeed a voice raised in the desert. We should specially like to draw attention to this paper which critically examines one group of theories, those based on the formaldehyde hypothesis. Generally speaking, we agree with Spoehr's statement, that "It can safely be said at the outset that, when critically considered from a physiological view point, none of the existing theories is even moderately well established by observations of facts."

In the following we shall cite the theories and suggestions of various chemists who have directed their attention to the problems of carbon assimilation, namely A. Baeyer, J. H. van't Hoff, M. Siegfried, and R. Willstätter. Only the hypothesis of Baeyer seems to have aroused any interest among botanists, as the literature of the subject sufficiently indicates, and it is not unusual to find Baeyer's hypothesis almost accepted as an axiom in

biological text-books. Here again we agree with Spoehr who expresses as his opinion, "In recent years this hypothesis has largely directed the course of the investigations in this subject, and it seems to the writer, to the detriment of critical and independent thinking on the broader aspects thereof."

B. Hypothesis of Baeyer.

We shall in what follows only deal with Baeyer and his followers as briefly as possible, as those interested in this branch of this subject will have no difficulty in finding ample references to the literature in text-books and journals.

Before Baeyer's time the chemists, for instance, Liebig (1843b), Kolbe and Schmidt (1861), and Berthelot (1864), appear to have been of the opinion that in the process of assimilation organic acids were produced from which carbohydrates were subsequently formed. In the course of a paper, entitled "Ueber die Wasserentziehung und ihrer Bedeutung für das Pflanzenleben und die Gährung," Baeyer threw out a suggestion as to the formation of formaldehyde as an intermediate product of assimilation, a hypothesis which was really based on Butlerow's observation (1861) that trioxymethylene (a condensation product of formaldehyde) on heating in alkaline medium yields a syrupy product with some of the properties of sugars.

We give below Baeyer's suggestion in a translation of his own words.

"The general assumption in regard to the formation in the plant of sugars and related bodies, is that in the green parts carbon dioxide under the action of light is reduced and by subsequent synthesis transformed to sugar. Intermediate steps have been sought in organic acids: formic acid, oxalic acid, tartaric acid, which can be regarded as reduction products of carbon dioxide. According to this opinion, at those times when the green parts of the plant are most strongly subjected to the action of the sun's rays, a strong accumulation of acids should take place, and these should then gradually give place to sugar. As far as I know this has never been observed, and when it is remembered that in the plant sugars and their anhydrides are formed under all circumstances, whereas the presence of acids varies according to the kind of plant, the particular part of it and its age, then the opinion already often put forward, that the sugar is formed directly from the carbon dioxide, increases in probability.

The discovery of Butlerow provides the key, and one may indeed wonder that so far it has been so little utilised by plant physiologists. The similarity which exists between the blood pigment and the chlorophyll of the plant, has often been referred to; it is also probable that chlorophyll as well as hæmoglobin, binds carbon dioxide. Now when sunlight strikes chlorophyll which is surrounded by CO_2, the carbon dioxide appears to undergo the same dissociation, oxygen escapes, and carbon monoxide remains bound to the chlorophyll. The simplest reduction of carbon monoxide is that to the aldehyde of formic acid; it only requires to take up hydrogen,

$$CO + H_2 = COH_2.$$

This aldehyde is then transformed under the influence of the cell contents as well as by alkalies, into sugar. As a matter of fact it would be difficult, according to the other opinion, by a successive synthesis, to reach the goal so easily! Glycerin could be formed by the condensation of three molecules, and the subsequent reduction of the glyceric aldehyde so formed.

The formation of sugar in a more complicated way is not hereby excluded, and it could very well be possible that plant acids under certain circumstances are transformed into this substance, which in a thousand different forms helps to build up the body of the plant.

In what manner the cell content acts in order to effect the condensation of formaldehyde cannot be concluded beforehand, but one can assume that the sugar formed remains bound with it, and later, according to circumstances, splits off into carbohydrate, sugar, starch or glucoside. This is exhibited at least in the life-history of the slime fungi in which at a certain stage, from a mass similar to the cell content, a great quantity of cellulose is suddenly differentiated. In this connection it would be very interesting to examine chemically the slime fungi in various periods of their life, and determine whether they contain free sugar or free anhydrides, or whether from the plasmodium sugar or cellulose could be split off in the same way that this takes place in the natural process of development."

The experimental evidence which has been adduced in support of Baeyer's hypothesis is not of much interest, and in most cases an unjustifiable parallel is drawn between experiments carried out "in vitro" and processes in the cell. So long as our knowledge of the heterogeneous system in which these latter take place is so

incomplete, it is impossible to draw conclusions from experiments in which the conditions are clearly so different.

The experiments in relation to Baeyer's hypothesis fall into three groups:—

1. Experiments on the formation of formaldehyde in (*a*) systems containing carbon-dioxide and water, (*b*) systems containing carbon-dioxide, water and chlorophyll, (*c*) leaves.

2. Experiments on the formation of sugars from formaldehyde.

3. Feeding experiments with formaldehyde.

1. (*a*) The production of formaldehyde from carbon dioxide and water in the absence of chlorophyll certainly seems possible under certain conditions. For instance, Fenton (1907) and D. Berthelot and Gaudechon (1910) have each succeeded in reducing carbon-dioxide to formaldehyde under appropriate conditions, but there is no evidence that these conditions are in the least comparable to those in the plant. References to further work in this connection will be found in the paper by Spoehr already cited, in which this side of the question is critically dealt with.

(*b*) Experiments dealing with the photochemical production of formaldehyde from systems containing carbon dioxide, water and chlorophyll have all been made with crude chlorophyll, and in most cases oxygen has not been removed from the system. In order to repeat these experiments critically we ourselves extracted some pure chlorophyll ($a + b$). The results obtained in the experiments made with this pure chlorophyll are recorded in a paper by Jörgensen and Kidd (1916). It was found that the production of formaldehyde was always due to the oxidation of chlorophyll. In systems containing only carbon dioxide, water and chlorophyll no formaldehyde is produced.

(*c*) Pollacci (1899—1907), Grafe (1906), Kimpflin (1907) and R. J. H. Gibson (1908) contend that formaldehyde can be identified in leaves after illumination, while Curtius and Franzen (1912) contend that other aldehydes are produced, *e.g.*, α β hexylene aldehyde, but in view of the critical experiments of Fincke (1913) and Spoehr (1913) it is clear that under various conditions a large number of substances in the plant will produce aldehydes. Thus experiments of this type do not give support to the formaldehyde hypothesis.

2. In view of the experiments of Butlerow (1861), Fischer (1888, 1889) and Loew (1889), and above all Nef (1910, 1913) it

seems certain that various monosaccharides can be produced from formaldehyde under certain conditions. But these conditions, generally high temperature and alkaline medium, are not the same as those existing in the plant, so that it is impossible to argue from these experiments "in vitro" as to the possible condensation of formaldehyde to sugar in the leaf. Nor do we know of any photochemical or enzymatic reactions which could bring about this change.

3. It has been urged that carbon assimilation should proceed in absence of carbon dioxide if an intermediate product were given as nutrient. Thus Loew (1889) and Bokorny (1888-1911) insist that *Spirogyra* in absence of carbon dioxide, but in presence of the sodium bisulphite compound with formaldehyde can form starch, while Grafe (1909, 1911) and Miss Baker (1913) have urged that plants can so utilise gaseous formaldehyde itself if this is present in the air in a concentration sufficiently low to prevent toxic effects. This only takes place in the light; in the dark formaldehyde is toxic. Spoehr established that formaldehyde vapour mixed with air is quickly oxidised to formic acid in sunlight, so that Grafe's and Miss Baker's experiments could only be used in favour of a formic acid theory of assimilation.

Moreover, the utilisation of a substance by the leaf is no proof that that substance is an intermediate product in carbon assimilation, as we know of several substances which can be utilised by the plant, such as glycerine and sugars not normally found in the leaf, but which nevertheless are not generally supposed to be intermediate products.

Again it has not been found possible to utilise carbon-monoxide in assimilation, as has been shown for example by de Saussure (1804), Boussingault (1868) and Krashéninnikoff (1909).

Thus it is seen, as Spoehr expresses it, that Baeyer's hypothesis, "though alluring on account of its simplicity, is by no means as well established as many writers on the subject would have us believe." Indeed it seems to us that the words of Sachs written 35 years ago (1882, 1887) are as applicable now as on the day when they were written; "whether it is right to claim, with Bertlelot and Kekulé, formic acid or some other member of the formyl group as the first product of assimilation, on account of its simple constitution, I hold as at least very questionable; and it has hitherto been proved by nothing."

C. Suggestion of van't Hoff.

Baeyer's hypothesis is based on the synthesis of carbohydrates in the laboratory by ordinary chemical processes. It is difficult, as we have pointed out, to imagine that the laboratory conditions required for this synthesis should be comparable with those in the plant. Much more stimulating and interesting therefore, is the suggestion of van't Hoff that the reversible enzyme action is a characteristic of many reactions in the plant. It is not clear from the few remarks of van't Hoff in what manner he thought the photochemical reaction and synthetic enzyme reaction should co-operate in the production of carbohydrates. His main interest appears to have centred on the problem as to the substances from which the main products of assimilation could have been synthesized by enzyme reaction. So for instance at a lecture in Düsseldorf in 1898, he said "It was pointed out by Tammann that under the action of emulsin, amygdalin is only partially split and that this hydrolysis proceeds further if the products are removed. Perhaps if he had added a further amount of products of hydrolysis, he might have succeeded in synthesizing amygdalin. Duclaux put forward transformation formulæ, which again suggest the attainment of an equilibrium, and Hill seems to have effected the synthesis of maltose from glucose by means of a yeast enzyme. Unless a ferment undergoes alteration of some kind during its period of activity, it follows, on theoretical grounds, that a condition of equilibrium and not one of total change must be brought about, and that therefore the opposite reaction must be induced. *We are indeed justified in asking the question, whether (by application of the theory of equilibrium), under the influence of zymase and by exceeding a certain limiting opposing pressure of carbon dioxide, glucose might not be formed from alcohol and carbon dioxide*, and moreover whether trypsin may not be able, under conditions prescribed by the theory of equilibrium, to form protein from the products of the hydrolysis, which it brings about under other conditions." (Bayliss' translation, 1914; the italics are our own.)

It is to be regretted that this suggestion has not attracted the attention of plant physiologists as work on the lines indicated by van't Hoff's suggestion, would at least have been likely to result in lasting contributions to our knowledge of plant processes. Such work is of course considerably more difficult that the carrying out of qualitative tests for formaldehyde, which constitutes the bulk of the work done on behalf of Baeyer's hypothesis.

The subject is clearly one which interested van't Hoff deeply, as is seen from his letters and diary (Cohen, 1912), and he intended to subject the problems to an extensive investigation. Bad health, and finally death, prevented him from carrying out this project, and we only possess from his hands two papers on the subject (1909, 1910) entitled "On Synthetic Enzyme Action," neither of which is of interest here.

Van't Hoff's suggestion obtains a new interest in view of Willstätter's discovery that chlorophyll is a double ester of two primary alcohols, and that leaves contain an enzyme which can effect hydrolysis or alcoholysis of chlorophyll, and can also synthesize chlorophyll from phytol and chlorophyllid (Willstätter and Stoll, 1911, 1913). Unfortunately, as we have pointed out earlier, Willstätter's contention that the amount of pigment is not altered during assimilation, only holds for the chromogen complex, and provides no information as regards the alcohol groups. Therefore we have no indication whether chlorophyllase or the alcohol groups play any part in the processes of carbon assimilation.

D. Suggestion of Siegfried.

Siegfried (1905) worked on the action of carbon dioxide on amino-acids and proteins, and came to the conclusion that definite compounds, carbaminic acids and carbaminates are produced. Thus he says that his results "appear to justify the assumption that where carbon dioxide meets protein in the animal organism, carbon dioxide is fixed organically, and that the compounds so produced dissociate again with evolution of carbon dioxide." After discussing the bearing of this conclusion on various processes in animal physiology, such as blood processes, and the working of muscle, he concludes: "Finally, plant physiology also will have to concern itself with this question. Where there is chlorophyll there is also protoplasm. If by the intake of carbon dioxide by the plant carbamino groups are formed, the intake of carbon dioxide will be accelerated. Instead of, or along with the question, how is carbon dioxide reduced, the question must be solved, how are carbon acids reduced."

It will be seen that we have here a suggestion in regard to the processes of carbon assimilation which differs markedly from the Baeyer hypothesis. It is generally assumed that in the first stage of the assimilatory process the carbon dioxide takes part in

a photochemical reaction; in Siegfried's view on the other hand the first stage of carbon assimilation is a purely chemical process, and the photochemical reaction occurs in a complex carbon compound. This suggestion of Siegfried's has been as completely neglected by plant physiologists as that of van't Hoff, although it offers possibilities of connecting carbon assimilation with nitrogen assimilation in a way which is not possible on the Baeyer hypothesis.

Further interest in Siegfried's suggestion should result from the extensive researches of Ciamician and Silber (1901-1915) on the photochemical reactions in complex organic compounds. Willstätter and Stoll (1915b-d) seem unaware of the work of Siegfried, but express almost identically the same view in regard to the accumulation of carbon dioxide in the protoplasm by means of proteins.

E. Theories of Willstätter.

It cannot be said that the development of plant physiology during the last hundred years has been very rapid, nor is the position which it occupies among other branches of botanical science worthy of its importance. This is no doubt largely due to lack of knowledge of the fundamental sciences, physics and chemistry, which must form the basis of all science which is not merely cataloguing or descriptive. As a consequence of this whenever chemists have put forward contributions to the theory of plant processes, their statements have usually been accepted by botanists without reserve. That this has been much to the detriment of plant physiology is evident from a survey of the history of the subject. It is only to be expected that theories of pure chemists on plant physiological subjects should be misleading, when one considers how infinitely more complex are the conditions in the plant compared with the moderately simple laboratory conditions of ordinary chemical experiments. This was true sixty or seventy years ago, and it is true to-day.

Willstätter, whose brilliant chemical work has been, and probably will be in the future, of so much value to plant physiology, has, like the eminent chemists Liebig and Baeyer before him, ventured to put forward theoretical views on the processes of carbon assimilation. Below we give a translation of the first instalment of his theory published in 1906 (p. 64) under the subheading of "The Life of the Plant."

"Plants and animals live by means of the catalytic action of

metals, which they contain in the form of complex organic compounds. They differ chemically by the nature and function of the metal. The life of chlorophyll-containing plants is mainly synthesizing. While biology so far has been incapable of giving an explanation, the proof of the presence of magnesium in chlorophyll from all classes of plants allows the conclusion to be drawn that the assimilation of carbon dioxide is a reaction of the basic metal magnesium, which as well known exhibits great power of combination in complex organic molecules. The intake of carbon dioxide is probably a process similar to the Grignard synthesis. The disintegrating (abbauende) life of blood-containing animals requires for the oxidation of organic substances a carrier (Überträger), particularly iron, which, perhaps, on account of its oxidisability, combines loosely with the oxygen and transports it to a series of comparatively unstable compounds. Besides along these main roads natural development along less important roads and blind alleys may have succeeded in the formation of organisms which live by the action of other metals, *e.g.*, copper, and which have shown themselves less capable of evolution.

It is thus seen that there are essentially two kinds of life, which develop along parallel lines of evolution: synthesizing life with magnesium, and disintergrating life with iron, *i.e.*, reducing life and oxidising life."

We have given a translation of Willstätter's views on life in full. Plant physiologists will probably appreciate them without any comment from us. But we may draw attention to the fact that some people regard iron as a synthesizing agent in life, for instance, B. Moore (1914), who has elaborated a theory of carbon assimilation on this view. We do not deal at length with this theory as it involves the formaldehyde hypothesis, and is open to all the criticisms that may be levelled against that hypothesis and a good many more. Moore, besides attempting to explain life as it is at present, utilises his hypothesis for speculation on the origin of life. It may be well to keep in mind the remarks of Darwin in a letter written in 1863, "It is mere rubbish, thinking at present of the origin of life; one might as well think of the origin of matter." (See Darwin, 1902, p. 257.)

Willstätter, it will be observed, attempts to utilise the work of Grignard in justification of the part he attributes to magnesium in his theory. Grignard however rightly points out how very different are the conditions in any Grignard synthesis from those in the plant.

We give below some remarks of Grignard (1913) on this subject.

" Avec cette extraordinaire faculté d'adaptation aux molécules chimiques les plus diverses, le magnésium ne serait-il pas capable de jouer un rôle très actif dans les synthèses naturelles de la matière organisée ?

Willstätter a, en effet, reconnu que ce métal s'accumulait, pour ainsi dire, dans une substance douée d'une activité catalytique considérable, la chlorophylle.

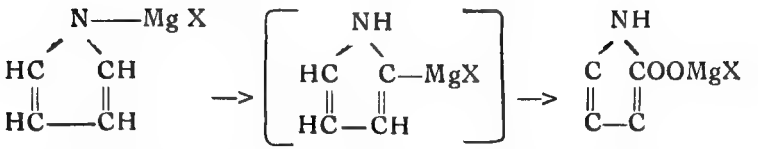
Il a isolé des chlorophylles les plus diverses des combinaisons contenant jusqu'à 3·5% de magnésie. Et il a conclu de ses études qu'il doit se former des combinaisons analogues aux organomagnésiens et que l'absorption du gaz carbonique par la chlorophylle serait tout à fait analogue à une réaction de Grignard. Il est arrivé ainsi à comparer la chlorophylle des feuilles à l'hémoglobine des animaux et à penser qu'il y aurait pour la matière vivante deux cycles de transformation : la vie de synthèse avec l'aide du magnésium et la vie d'oxydation avec l'aide du fer.

Il reste cependant à trouver sous quelle forme le magnésium peut s'approprier à ces réactions, dans un pareil milieu, si différent de celui où nous sommes habitués à le voir triompher. Ce sera le problème de demain."

A further instalment of Willstätter's theory appears in his book (1913, pp. 23–25), which again we give in the form of a translation of Willstätter's own words.

" The rôle of magnesium can be imagined to be the same as in those organo-magnesium compounds discovered by Barbier and Grignard which have attained such importance in organic synthesis on account of their reactive properties. Already in our first communication (1906) on the analysis of chlorophyll a parallel is drawn between the latter and the Grignard compounds. The parallel appeared to be inaccurate and met with contradiction because it took no notice of the difference between the binding of a metal to carbon in the ordinary organo-magnesium compounds, and the substitution with nitrogen in chlorophyll. But we do not consider this difference either distinct or characteristic.

Since our publication, B. Oddo has carried out important investigations on pyrrole magnesium iodide which reacts with carbon dioxide and with acid chlorides resulting in the formation of α substituted pyrroles, α carbopyrrole acid, and alkylpyrrylketone. Probably the N-magnesium derivative is first formed, and this is either transformed into the α-magnesium compound, or reacts as such, *e.g.*



The pyrrole magnesium derivatives have thus—analogous with sodiumacetic ester—behaved like any Grignard body with binding of the metal to carbon.

Chlorophyll can be regarded as of the same class of organo-magnesium compounds, and it seems unjustified to draw a sharp line between magnesium phenyl iodide, pyrrole magnesium iodide and chlorophyll, only chlorophyll is characterised by a greater stability of magnesium towards water than the ordinary organo-magnesium compounds on account of the complex binding of the metal.

This comparison does not require that the pigment in the process of assimilation should take the carbon dioxide into its molecule. This can be prevented by substitution in the magnesium-carrying pyrrole nuclei. Rather the function of chlorophyll may be imagined thus: that the carbon dioxide is attracted by the affinity of the magnesium compounds, and that its reduction is effected by the chlorophyll component a in the process which uses the absorbed light energy. Chlorophyll a is hereby oxidised to chlorophyll b, and this is again transformed to the first component with evolution of oxygen. Between the two components an equilibrium condition is obtained.

It is possible that this evolution of oxygen either takes place direct or that the yellow pigments, carotin and xanthophyll take part in the re-formation of chlorophyll a. As the yellow pigments constantly accompany the green pigments in the chloroplasts, it is probable that they have a function. Perhaps this is to regulate the ratio of the chlorophyll components, perhaps by the withdrawal of oxygen from chlorophyll b by carotin, this oxygen being then evolved from xanthophyll by means of the action of an enzyme."

It will be seen that Willstätter here first defends his conception of chlorophyll as a Grignard body. In spite of his elaboration of this conception, it appears to us that his arguments amount to this: In the Grignard syntheses a good many curious things may happen. Carbon assimilation is a curious process which involves the complex organic magnesium compound chlorophyll. Why should not carbon assimilation be a Grignard synthesis

The second part of his theoretical consideration is more interesting, as for the first time we have a suggestion which involves the presence of two different chlorophylls. Although this fact was brought out by the work of Stokes, all subsequent theorists avoided the difficulty by neglecting it. As Willstätter insists that the absolute value of the green pigments and the ratio between them remains constant,[1] there must be a mechanism which keeps the system in equilibrium. The main agency in this, Willstätter suggests, is the yellow pigments assisted by suitable enzymes.

Willstätter's conception of the chlorophyll apparatus as constituting a system in dynamic equilibrium is of course very interesting. However, as long as our knowledge of photochemical reactions and enzyme reactions in the chloroplasts is as imperfect as it is at present, this theory of Willstätter's cannot be accepted as more than a suggestion.

Finally we shall consider the theories expounded by Willstätter in his latest publications (1915, b-d). One of the suggestions put forward in these papers we have already mentioned (Chapter IV, Section E), namely, the reasonable suggestion that carbon assimilation consists of a photochemical process and an enzymatic process. This conclusion was derived from plant physiological experiments with leaves in various conditions.

Further work with isolated chlorophyll was performed in support of his elaboration of the theory, but it is not clear whether this most recent theory replaces the earlier one we have already dealt with, or whether it is intended to supplement it. At least no mention is any longer made of chlorophyll as a Grignard synthesizing agent, nor is any account taken of the *two* chlorophylls, nor of the yellow pigments. Willstätter considers now that a dissociable compound of chlorophyll and carbon dioxide is formed, but as it is formed in the dark he assumes that the function of light is simply to produce an isomer of higher energy content. This assumption carries in its train a number of equally wild speculations which the reader will find in the translation we give below of the summary of the theory.

"As it is seen from the above, the entrance of carbon dioxide into the chloroplast takes place by means of an absorbing substance. The apparatus acts as a carbon dioxide accumulator, as it brings

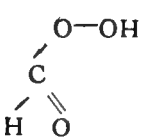
[1] See Chapter II, Section F. Further figures in support of this statement are given by Willstätter and Stoll (1915 b, p. 336) for strongly assimilating leaves under artificial conditions.

the carbon dioxide of the air to a greater concentration, on account of its property to take up more carbon dioxide at lower temperature suited to increase of assimilation under natural conditions. The carbon dioxide wanders on to the place of smallest carbon dioxide pressure.

The real assimilation process we can differentiate into several sub-processes. Chlorophyll takes up carbon dioxide and at the same time forms a dissociable compound. One must suppose that this compound takes up light energy, and thereby undergoes rearrangement into an isomer of greater energy content, which is suited for its own disintegration. A transformation product of carbonic acid which can be split off enzymatically with loss of energy, must be imagined as intermediate product, as the observation recorded in the first chapter makes it very probable that a part of the assimilation process is of enzymatic nature.

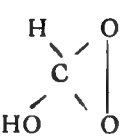
There is only known one isomer of carbonic acid of peroxide nature to which could be ascribed the rôle of intermediate product, performic acid, obtained in solution by J. d'Ans and W. Frey, which easily dissociates into carbon dioxide and water. In the assimilation process one must of course imagine another way of dissociation of the intermediate product, namely, its disintegration with evolution of oxygen.

As for performic acid, various structural formulæ can be considered



Formylhydroperoxide.

and



Formaldehydeperoxide.

so it is quite possible that the intermediate product of photosynthesis bound to chlorophyll is a peroxide other than the known substance, performic acid."

In our opinion this latter part of Willstätter's theory, which is based on his experiments with chlorophyll sols and carbon dioxide, is due to premature and incorrect interpretation of his experimental results. These, briefly, are as follows. In a system consisting of a chlorophyll sol and carbon dioxide, a certain amount of carbon dioxide will be absorbed by the water constituting the dispersion medium, and a further quantity will be used in the production of phæophytin according to the equation

$$C_{55}H_{72}O_5N_4Mg + 2CO_2 + 2H_2O = Mg(HCO_3)_2 + C_{55}H_{74}O_5N_4.$$
chlorophyll a — phæophytin a

but he finds more carbon dioxide is absorbed than can be accounted for by these two processes. For example, 0·505 g. chlorophyll (a + b) was used in 104·02 c.c. water. This absorbed 184·45 c.c. carbon dioxide at 0°C and 747·3 mm. partial pressure, *i.e.*, 6·45 c.c. more than could be accounted for by the water. This is equivalent to 12·6 mg. Of this 7 mg. would be used in the formation of phæophytin according to the equation given above; there thus remains 5·6 mg. unaccounted for.

We do not see any necessity to introduce a mystical and purely hypothetical peroxide in order to explain this result. Indeed, the assumption of the formation of such a peroxide is purely gratuitous in view of the fact that we have absolutely no information in regard to the behaviour of carbon dioxide towards the ester groups of the chlorophyll molecule.[1]

From his experiments on the absorption of carbon dioxide in the dark by living leaves and leaf powder, Willstätter concludes that in the leaf there is a mechanism for absorbing carbon dioxide as the leaves and leaf powder absorb many times as much carbon dioxide as can be explained as due to absorption by the chlorophyll. To explain this he puts forward exactly the same hypothesis that Siegfried had propounded ten years before, without, however, making any reference to Siegfried. It may be interesting to compare with Siegfried's results already cited, the remark of Willstätter (1915 b, p. 345) "It is possible that in the absorption phenomenon described, carbamino compounds of amino acids or of proteins are formed. Preparation work is here presented with a new problem." This last sentence suggests that Willstätter is unaware of Siegfried's work.

[1] It is interesting to note that the solubility of carbon dioxide in some alcohols and esters is much greater than in water, see *e.g.*, Just (1901).

Chapter VIII.

Concluding Remarks.

In the preceding pages we have attempted to give the outlines of one the most fundamental problems of plant physiology. The subject has been attacked from many different points of view and by many different methods, but in our opinion the main interest is not centred in the achievements of any individual investigator. What, in our opinion, is the most important aspect which presents itself in reviewing the facts obtained in recent investigations on carbon assimilation, is the prospect of the development of a new phase of science. This is the prospect that plant physiology is developing into an exact science, utilising the experiences of the fundamental sciences, physics and chemistry, but nevertheless a science, exact and independent, with its own working principles and methods, directing and stimulating the development of the applied sciences, agriculture and horticulture. No prophetic vision is needed to foretell that developments in agriculture and horticulture will follow development in plant physiology as great as those which were produced by physics and chemistry in engineering and other technical sciences.

But such development can only take place if we learn from the past what are likely to be the limitations to successful development.

The present state of the subject is the result of a number of independent investigations, the bearing of which on one another is rather accidental than designed, and in this lack of co-ordination is to be found a reason for the slow development of the subject hitherto. It is clear that the only way to attain a reasonable rate of progress is to institute a much closer and more intimate co-operation between scientific workers attacking the same problems from different points of view and by different methods.

It is generally desirable in a review of this nature to conclude with a brief summary of the present position of the subject. In the case of carbon assimilation it seems to us that it is not so much the complete array of experimental facts obtained in the various researches which is of importance, but the general principles which become clear from a consideration of the whole subject. This is especially so as the subject is in a more or less mobile condition and development and ever-widening scope must follow along sound lines of work based on the principles of the subject.

Blackman has brought out the important relation between environmental factors and carbon assimilation, and has formulated the principle of limiting factors in regard to their co-operation. However, absolute rules cannot be made as to amount of assimilation under any definite environment, owing to the complexity introduced by the existence of unknown internal factors. Willstätter has attempted to analyse the internal factors, and has brought proof that chlorophyll is not the only internal factor, though what other internal factors there are Willstätter's work does not show. Future work will have to investigate the inter-relation between the internal factors as well as the co-operation between the internal and external factors.

However, the internal factors operative at any moment are a product of hereditary factors and environmental factors. It seems likely that an application of the principles of genetics may prove helpful in the analysis of internal factors in assimilation, and this application may give a method for controlling some internal factors.

The aim, at present, of investigations on carbon assimilation is to be able to tell the assimilatory power of a plant with a known history as regards environmental and hereditary factors when it is placed in a known environment. Then it becomes of industrial importance to discover how environmental factors can be modified so as to give the maximum assimilation in relation to the inherited internal factors.

We should like to emphasize that the popular idea that under natural conditions any particular factor, as for instance, light, is nearly always in excess, while some other factor, as for instance carbon dioxide, is nearly always limiting, is not justified. The power of the plant to utilise any environmental factor must undergo diurnal and seasonal variations depending on the interplay of the other factors.

For instance the environmental factors, radiation and temperature, undergo daily and seasonal variations while although in regard to the carbon dioxide supply not much information is to be had, undoubtedly considerable variations occur (see, for example, Krantz, 1909). In this connection may be mentioned the work of Kraus (1911) who successfully shows how great may be the variations in environment over a very small area.

The importance of work on carbon assimilation depends not merely on its value in plant physiology and on its application in agriculture, but also, as we have emphasized in our introductory chapter, for the utilisation of radiant energy. For this reason in

the future plant physiology will acquire the co-operation of photochemists, who already take an interest in the work of the plant physiologist. It is encouraging to find an appreciation of plant physiology by the photochemist Plotnikow (1910), whose words on this subject we may quote in conclusion. "From this one realises how much material, work and patience is required for this problem. But let us hope that this will prove no obstacle for further investigations in this field. Labour loving scientists should not lose heart for penetrating further along the path so far prepared.

A brilliant success must finally crown their labour."

LITERATURE CITED.

Armstrong, E. F. The Simple Carbohydrates and the Glucosides. Second Edition, London, 1912.

Arnaud. Recherches sur les matières colorantes des feuilles; identité de la matière rouge orangé avec la carotine, $C^{18}H^{24}O$. Comptes rendus, 100, 1885, 751-753.

Aubert, E. Recherches sur la Respiration et l'Assimilation des Plantes grasses. Rev. gén. de Bot., 4, 1892, 203-219, 273-282, 321-331, 337-353, 373-391, 421-441, 497-502, 558-568.

Askenasy, E. Ueber die Temperatur, welche Pflanzen in Sonnenlicht annehmen. Bot. Zeit., 33, 1875, 441-444.

Baeyer, A. Ueber die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung. Ber. d. deut. chem. Ges., 3, 1870, 63-78.

Baker, S. M. Quantitative Experiments on the Effect of Formaldehyde on Living Plants. Ann. of Bot., 27, 1913, 411-442.

Baly, E. C. C. Spectroscopy. New Edition, London, 1912.

Barthélemy, A. Du rôle que joue la cuticule dans la respiration des plantes. Ann. Sci. Nat., Bot., 5e Sér., 9, 1868, 287-297.

,, ,, De la respiration et de la circulation des gaz dans les végétaux. Ann. Sci. Nat., Bot., 5e Sér, 19, 1874, 131-175.

,, ,, Du rôle des stomates et de la respiration cuticulaire. Comptes Rendus, 84, 1877, 663-666, see also 85, 1877, 1055.

Bayliss, W. M. The Nature of Enzyme Action. Third Edition, London, 1914.

Bernard, C. Leçons sur les phénomènes de la vie commun aux animaux et aux végétaux. Paris, 1878.

Berthelot, M. Leçons sur les methodes générals de synthèse. Paris, 1864.

Berthelot, D, and Gaudechon, H. Synthèse photochimique des hydrates de carbone aux dépens des éléments de l'anhydride carbonique et de la vapeur d'eau, en l'absence de chlorophylle; synthèse photochimique des composés quaternaires. Comptes rendus, 150, 1910, 1690-1693.

Blackman, F. F. (1895 a) Experimental Researches on Vegetable Assimilation and Respiration. I. On a New Method for Investigating the Carbonic Acid Exchanges of Plants. Phil. Trans. Roy. Soc. London, B, 186, 1895, 485-502.

,, ,, (1895 b) Experimental Researches on Vegetable Assimilation and Respiration. II. On the Paths of Gaseous Exchange between Aerial Leaves and the Atmosphere. Phil. Trans. Roy. Soc. London, B, 186, 1895, 503-562.

,, ,, Optima and Limiting Factors. Annals of Botany, 19, 1905, 281-295.

,, ,, and Matthaei, G. L. C., Experimental Researches on Vegetable Assimilation and Respiration. IV. A Quantitative Study of Carbon-Dioxide Assimilation and Leaf-Temperature in Natural Illumination. Proc. Roy. Soc. London, B, 76, 1905, 402-460.

,, ,, and Smith, A.M., (1911 a) Experimental Researches on Vegetable Assimilation and Respiration. VIII. A New Method for Estimating the Gaseous Exchanges of Submerged Plants. Proc. Roy. Soc. London, B. 83, 1911, 374-388.

,, ,, and Smith, A.M., (1911 b) Experimental Researches on Vegetable Assimilation and Respiration. IX. On Assimilation in Submerged Water Plants, and its

Relation to the Concentration of Carbon Dioxide and other Factors. Proc. Roy. Soc. London, B, 83, 1911, 389-412.

Boehm, J. Ueber die Stärkebildung in dem Keimblättern der Kresse, des Rettigs, und des Leins. Sitzungsb. d. k. Akad. der Wiss. in Wien, 69, 1874, 163-198.

,, ,, Über Stärkebildung in den Chlorophyllkörnern. Sitzungsb.d.k.Akad. der Wiss. in Wien, 73, 1876, 39-66.

,, ,, Ueber Stärkebildung in verdunkelten Blatt-theilen der Feuerbohne. Ber. d. deut. chem. Ges., 10, 1877, 1804-1807.

,, ,, Ueber Stärkebildung aus Zucker. Bot. Zeit., 41, 1883, 33-38, 49-54.

,, ,, Ueber das Verhalten von Vegetabilischen Geweben und von Stärke und Kohle zu Gasen. Bot. Zeit., 41, 1883, 521-526, 537-552, 553-559.

Bokorny, T. Ueber Stärkebildung aus Verschiedenden Stoffen. Ber. d. deut. bot. Ges., 6, 1888, 116-120.

,, ,, Studien und Experimente über den chemischen Vorgang der Assimilation. Erlangen, 1888.

,, ,, Über Stärkebildung aus Formaldehyd. Ber. d. deut. bot. Ges., 24, 1891, 103-106.

,, ,, Uber die Assimilation des Formaldehyds und die Versuche dieses Zwischenprodukt bei den Kohlensäure-Assimilation nachzuweisen Assimilation von Glycerin und Zucker. Archiv. für ges. Physiol., 125, 1908, 467-490.

,, ,, Weitere Mitteilung über CO_2-Assimilation und Ernährung von Pflanzen mit Formaldehyde. Archiv. für ges. Physiol., 128, 1909, 565-586.

,, ,, Ernährung von grünen Pflanzen mit Formaldehyd und formaldehydabspaltenden Substanzen. Bioch. Zeitsch., 36, 1911, 83-97.

Bonnier, G. and Mangin, L. Recherches sur l'action chlorophyllienne séparée de la respiration. Ann. Sci. Nat., Bot., 7e Sér., 3, 1886, 1-44.

Boussingault, J. B. De la végétation dans l'obscurité. Ann. Sci. Nat., Bot., 5e Sér., 1, 1864, 314-324.

,, ,, Étude sur les fonctions des feuilles. Agronomie, 4, 1868, 359-397.

Brown, H. T. and Escombe, F. Static Diffusion of Gases and Liquids in Relation to the Assimilation of Carbon and Translocation in Plants. Phil. Trans. Roy. Soc. London, B., 190, 1900, 223-291.

,, The Influence of Varying Amounts of Carbon Dioxide in the Air on the Phyotosynthetic Process of Leaves and on the Mode of Growth of Plants. Proc. Roy. Soc. London, B, 70, 1902, 397-413.

Researches on some of the Physiological Processes of Green Leaves, with Special Reference to the Interchange of Energy between the Leaf and its Surroundings. Proc. Roy. Soc. London, B, 76, 1905, 29-111.

,, and Morris, G. H. A contribution to the Chemistry and Physiology of Foliage Leaves. Journ. Chem. Soc., Trans., 63, 1893, 604-683.

Morris, G. H., and Millar, J. H. Experimental Methods employed in the Examination of the Products of Starch Hydrolysis by Diastase. Journ. Chem. Soc., Trans., 71, 1897, 72-108.

Brown, H. T., Morris, G. H., and Millar, J. H. Specific Rotation of Maltose and of Soluble Starch. Journ. Chem. Soc., Trans., 71, 1897, 109-114.

,, ,, ,, ,, ,, ,, Relation of the Specific Rotatory and Cupric Reducing Powers of the Products of Starch Hydrolysis by Diastase. Journ. Chem. Soc., Trans., 71, 1897, 115-123.

,, ,, ,, ,, ,, The Solution Density and Cupric-reducing Power of Dextrose, Levulose and Invert-Sugar. Journ. Chem. Soc., Trans., 71, 1897, 275-284.

,, ,, and Wilson, W. E. On the Thermal Emissivity of a Green Leaf in Still and Moving Air. Proc. Roy. Soc. London, B, 76, 1905, 122-137.

Butlerow, A. Bildung einer zuckerartigen Substanz durch Synthese. Ann. der Chemie und Pharmacie, 120, 1861, 295-298.

Callendar, H. L. A Quantitative Bolometric Sunshine Recorder. Brit. Assoc. Report, Bristol Meeting, 1898, 796-797.

Callendar's Recorder and Platinum Thermometer. Engineering, 67, May 26, 1899, 675-676.

Campbell, A. V. The Carbohydrates of the Mangold Leaf. Journ. Agric. Sci., 4, 1912, 248-259.

Chapin, P. Einfluss der Kohlensaüre auf das Wachtum. Flora, 91, 1902, 348-379.

Chodat, R., and Schweizer, R. Nouvelles Recherches sur les Ferments Oxydants—IX. De l'emploi de la peroxydase comme réactif de la photolyse par la chlorophylle. Arch. des Sciences Phys. et Nat., 4e Periode, 39, 1915, 334-338.

Ciamician, G., and Silber, P. Chemische Lichtwirkung, I–XXXIII, Ber. d. deut. chem. Ges., 34-48, 1901-1915.

Coblentz, W. W. Instruments and Methods used in Radiometry. (Bulletin of the Bureau of Standards, Vol. 4, No. 3) Reprint No. 85, Department of Commerce and Labor, Bureau of Standards, Washington, D.C., 1908.

Cohen, E. Jacobus Henricus van't Hoff. Sein Leben und Wirken. Leipzig, 1912.

Cohn, F. Ueber einige Algen von Helgoland, in "Beiträge zur nähren Kenntnis und Verbreitung der Algen" herausgegeben von L. Rabenhorst, Heft 2, 19, Leipzig, 1865.

,, ,, Beiträge zur Physiologie der Phycochromaceen und Florideen. Arkiv f. mikros. Anatomie, 3, 1867, 1-60.

Curtius, T., and Franzen, H. Über die chemischen Bestandteile grüner Pflanzen (Erste Mitteilung). Über den Blätteraldehyd. Ann. der Chem., 390, 1912, 89-121.

,, ,, ,, ,, ,, Über die chemischen Bestandteile grüner Pflanzen; (Zweite Mitteilung). Über die flüchtigen Bestandteile der Hainbuchenblätter. Ann. der Chem., 404, 1914, 93-130.

Czapek, F. in Lotos 59, 1911. References in Czapek (1913) and Willstätter and Stoll (1913).

,, ,, Biochemie der Pflanzen, Band 1 (Zweite Aufl.), Jena, 1913.

Daish, A. J. Methods of Estimation of Carbohydrates. III. The Cupric-reducing Power of the Pentoses—Xylose and Arabinose. Journ. Agric. Sci., 6, 1914, 255-262.

,, ,, The Distribution of Maltase in Plants. II. The Presence of Maltase in Foliage Leaves. Bioch. Journ., 10, 1916, 49-55.

Darwin, C. Charles Darwin: his life told in an autobiographical chapter, and in a selected series of his published letters. Edited by his son, Francis Darwin. London, 1902.

Daubeny, C. On the Action of Light upon Plants, and of Plants upon the Atmosphere. Phil. Trans. Roy. Soc. London, 1836, 149-176.

Davis, W. A. A Simple Laboratory Apparatus for the continuous evaporation of large volumes of liquid 'in vacuo.' Journ. Agric. Sci., 5, 1913, 434-436.

" " Studies of the Formation and Translocation of Carbohydrates in Plants. II. The Dextrose-Lævulose Ratio in the Mangold. Journ. Agric. Sci., 7, 1916, 327-351.

" " and Daish, A. J. A Study of the Methods of Estimation of Carbohydrates, especially in Plant Extracts. A new Method for the Estimation of Maltose in Presence of other Sugars. Journ. Agric. Sci., 5, 1913, 437-468.

" " " " " Methods of Estimating Carbohydrates. II. The Estimation of Starch in Plant Material. The Use of Taka-Diastase. Journ. Agric. Sci., 6, 1914, 152-168.

" " Daish, A. J., and Sawyer, G. C. Studies of the Formation and Translocation of Carbohydrates in Plants. I. The Carbohydrates of the Mangold Leaf. Journ. Agric. Sci., 7, 1916, 255-326.

" " and Sawyer, G. C. The Estimation of Carbohydrates. IV. The Presence of Free Pentoses in Plant Extracts and the Influence of other Sugars on their Estimation. Journ. Agric. Sci., 6, 1914, 406-412.

" " " " " Studies of the Formation and Translocation of Carbohydrates in Plants. III. The Carbohydrates of the Leaf and Leaf Stalks of the Potato. The Mechanism of the Degradation of Starch in the Leaf. Journ. Agric. Sci., 7, 1916, 352-384.

Deleano, N. T. Untersuchungen über die in Weinblättern enthaltenen Kohlenhydrate und stickstoffhaltigen Körper. Hoppe-Seyler's Zeitsch. f. physiol. Chem., 80, 1912, 79-94.

Detlefsen, E. Die Lichtabsorption in assimilierenden Blättern. Arbeit. d. bot. Inst. in Würzburg, 3, 1888, 534-552.

Dixon, H. H., and Mason, T. G. The Primary Sugar of Photosynthesis. Nature, 97, 1916, 160.

Draper, J. W. On the Decomposition of Carbonic Acid by the Leaves of Plants under the Influence of Yellow Light. Phil. Mag., 25, 1844, 169-173.

Duclaux, E. Traité de microbiologie, Tome 2, Diastases, Toxines et Venins. Paris, 1899.

Dumas, J. B., and Boussingault, J. B. Essai de statique chimique des êtres organisés. Paris, 1841 ; 3e édit., 1844.

Ege, R., and Krogh, A. Temperature and Respiratory Exchange in Fishes. Intern. Revue der ges. Hydrobiologie und Hydrogeographie, 7, 1914, 48-55.

Engelmann, T. W. Ueber Sauerstoffausscheidung von Pflanzenzellen im Microspectrum. Bot. Zeit., 40, 1882, 419-426.

" " Untersuchungen über die quantitativen Beziehungen zwischen Absorption des Lichtes und Assimilation in Pflanzenzellen. Bot. Zeit., 42, 1884, 81-93, 97-105.

Étard, A. La Biochemie et les Chlorophylles. Paris, 1906.

Ewart, A. J. On Assimilatory Inhibition in Plants. Journ. Linn. Soc., Bot., 31, 1896, 364-461.

" " Further Observations upon Assimilatory Inhibition. Journ. Linn. Soc., Bot., 31, 1897, 554-576.

" " (1897 b) The Effects of Tropical Insolation. Annals of Botany, 11, 1897, 439-480.

" " On the Function of Chlorophyll. Proc. Roy. Soc. London, B, 89, 1915, 1-17.

Fenton, H. J. H. The Reduction of Carbon Dioxide to Formaldehyde in Aqueous Solution. Journ. Chem. Soc., Trans., 91, 1907, 687-693.

Fincke, H. Nachweis von Formaldehyd in der Pflanzen. Bioch. Zeitsch., 53, 1913, 214-225.

Fischer, E. Ueber die Verbindungen des Phenylhydrazins mit den Zuckerarten III. Ber. d. deut. chem. Ges., 21, 1888, 988-991.

,, ,, and Passmore, F. Bildung von Acrose aus Formaldehyd. Ber d. deut. chem. Ges., 22, 1889, 359-361.

Garreau. Recherches sur l'absorption et l'exhalation des surfaces aériennes des plantes. Ann. Sci. Nat., Bot., 3e Sér., 13, 1850, 321-346.

Gautier, A. Sur la chlorophylle. Comptes rendus, 89, 1879, 861-866.

Gibson, A. H. Natural Sources of Energy. Cambridge, 1913.

Gibson, R. J. H. A Photo-electric Theory of Photosynthesis. Annals of Botany, 22, 1908, 117-120.

Godlewski, E. Abhängigkeit der Stärkebildung in den Chlorophyllkörnern von dem Kohlensäuregehalt der Luft. Flora, 56, 1873, 378-383.

Grafe, V. Studien über den mikrochemischen Nachweis verschiedener Zuckerarten in den Pflanzengeweben mittels der Phenylhydrazinmethode. Sitzungsb. d. k. Akad. d. Wiss. in Wein, Abt. 1, 114, 1905, 15-28.

,, ,, Über ein neues specifisches Formaldehydreagents. Öst. bot. Zeitsch., 56, 1906, 289-291.

,, ,, and Vieser, E. Untersuchungen über das Verhalten grüner Pflanzen zu gasförmigen Formaldehyd. Ber d. deut. bot. Ges., 27, 1909, 431-446.

,, ,, ,, ,, ,, Untersuchungen über das Verhalten grüner Pflanzen zu gasförmigen Formaldehyd II. Ber d. deut. bot. Ges., 29, 1911, 19-26.

Grignard, V. Le Magnésium en chimie organique. Bull. de la Soc. chim. de Paris. 4e Sér., 13, 1913, (Supplement) pp. xxxvi.

Haas, P., and Hill, T. G. The Chemistry of Plant Products. London, 1913.

Haberlandt, G. Vergleichende Anatomie des assimilatorischen Gewebesystems der Pflanzen. Jahrb. f. wiss. Bot., 13, 1882, 74-118.

,, ,, Physiological Plant Anatomy. English Edition, London, 1914.

Herlitzka, A. Über den Zustand des Chlorophylls in der Pflanze und über kolloidales Chlorophyll. Bioch. Zeitsch., 38, 1912, 321-330.

Hoppe-Seyler, F. Ueber das Chlorophyll der Pflanzen. Zeitsch. f. physiol. Chem., 3, 1879, 339-350; 4, 1880, 193-203; 5, 1881, 75-78.

Ingen-Housz, J. Experiments upon Vegetables, discovering their great power of purifying the common air in the sunshine, and of injuring it in the shade and at night; to which is joined a new method of examining the accurate degree of salubrity of the atmosphere. London, 1799.

Irving, A. A. The Beginning of Photosynthesis and the Development of Chlorophyll. Annals of Botany, 24, 1910, 805-818.

Jacobson, C. A., and Marchlewski, L. Studien in der Chlorophyllgruppe XIV. Über die Dualität des Chlorophylls und das wechselnde Verhältnis seiner Komponenten. Bioch. Zeitsch., 39, 1912, 174-184.

Jönsson, B. Recherches sur la respiration et l'assimilation des Muscinées. Comptes rendus, 119, 1894, 440-443.

Jörgensen, I., and Kidd, F. Some Photochemical Experiments with Pure Chlorophyll and their Bearing on Theories of Carbon Assimilation. Proc. Roy. Soc., B, 89, 1916, 342-361.

Jumelle, H. Recherches physiologiques sur les Lichens. Rev. gén. de Bot., 4, 1892, 49-64, 103-121, 159-175, 220-231, 259-272, 305-320.

Just, G. Löslichkeit von Gasen in organischen Lösungsmitteln. Zeitsch. f. physik. Chem., 37, 1901, 342-367.

Kanitz, A. Temperatur und Lebensvorgänge, Berlin, 1915.

Kayser, H. G. J. Handbuch der Spectroscopie, 6 Bande, Leipzig, 1900-1912.

Kayser, R. Ueber Vorkommen von Rohrzucker und einiger seiner Umwandlungsproducte im Organismus der Pflanzen. Landw. Versuchs. Stat., 29, 1883, 461.

Kimpflin, G. Présence du menthal dans les végétaux vertes. Comptes rendus, 144, 1907, 148-150.

Kjeldahl. Recherches sur le ferment production du sucre. Comptes rendus des travaux du lab. de Carlsberg, 1879.

Kluyver, J. Biochemische Suikerbepalingen, Leiden, 1914.

Kniep, H. Über den Gasaustausch der Wasserpflanzen. Ein Beitrag zur Kritik der Blazenzählmethode. Jahrb. f. wiss. Bot., 56, 1915, 460-509.

,, ,, and Minder, F. Über den Einfluss verschiedenfarbigen Lichtes auf die Kohlensäureassimilation. Zeitsch. für Bot., 1, 1909, 619-650.

Kolbe, H. and Schmitt, R. Directe Umwandlung der Kohlensäure in Ameisensäure. Ann. der Chemie und Pharmacie, 119, 1861, 251-253.

Krantz, L. Electrostatisches Düngen. Berichte d. deut. landw. Ges. 1909.

Krasheninnikoff. The Utilisation of Sun Energy by Plants. (In Russian) Moscow, 1901. References in Palladin (1911) and Puriewitsch (1914).

Krashéninnikoff, T. La plante verte assimille-t-elle l'oxyde de carbone ? Rev. gén. de Bot., 21, 1909, 173-193.

Kraus, G. Boden und Klima auf kleinstem Raum, Jena, 1911.

Kreusler, U. Ueber eine Methode zur Beobachtung der Assimilation und Athmung der Pflanzen und über einige diese Vorgänge beeinflussende Momente. Landw. Jahrb., 14, 1885, 913-965.

,, ,, Beobachtungen über die Kohlensäure-Aufnahme und-Ausgabe (Assimilation und Athmung) der Pflanzen. II. Mittheilung: Abhängigkeit von Entwicklungszustand —Einfluss der Temperatur. Landw. Jahrb., 16, 1887, 711-755.

,, ,, Beobachtungen über die Kohlensäure-Aufnahme und-Ausgabe (Assimilation und Athmung) der Pflanzen. III. Mittheilung: Einfluss der Temperatur; untere Grenze der Wirkung. Landw. Jahrb., 17, 1888, 161-175.

,, ,, Beobachtungen über Assimilation und Athmung der Pflanzen. IV. Mittheilung. Verhalten bei höheren Temperaturen; Kohlensäureausscheidung seitens getödteter Exemplare; Kohlensäureverbrauch, wenn Ober und Unterseite der Blätter dem Licht zugewendet. Landw. Jahrb., 19, 1890, 649-668.

Krogh, A. The Respiratory Exchange of Animals and Man. London, 1916.

Kuijper, J. Über den Einfluss der Temperatur auf die Atmung der höheren Pflanzen. Rec. trav. bot. Néerland, 7, 1910, 131-239.

Kurlbaum, F. Notiz über eine Methode zur quantitativen Bestimmung strahlender Wärme. Wiedermanns Annalen der Phys. und Chem., 51, 1894, 591-592.

Langley. La distribution de l'énergie dans le spectre normal. Ann. de chim. et de phys. 5e Sér., 25, 1882, 211-219.

Leitch, I. Some Experiments on the Influence of Temperature on the Rate of Growth in Pisum sativum. Annals of Botany, 30, 1916, 25-46.

Liebig, J. Chemistry in its Application to Agriculture and Physiology. 3rd Edition, London, 1843.

,, ,, (1843 b) Die Wechselwirthschaft. Ann. der Chem., 46, 1843, 58-97,

Loew, O. Ueber Bildung von Zuckerarten aus Formaldehyd. Ber. d. deut. chem. Ges., 22, 1889, 470-478.

,, ,, Nachträgliche Bermerkungen über Formose. Ber. d. deut. chem. Ges., 22, 1889, 478-482.

,, ,, Ueber die Rolle des Formaldehyds bei der Assimilation der Pflanzen. Ber. d. deut. chem. Ges., 22, 1889, 482-484.

Lommel, E. Ueber das Verhalten des Chlorophylls zum Licht. Pog. Ann., 143, 1871, 568-585.

Lundegårdh, H. Einige Bedingungen der Bildung und Auflösung der Stärke. Ein Beitrag zur Theorie des Kohlenhydratstoffwechsels. Jahrb. f. wiss. Bot., 53, 1914, 421-463.

Mangham, S. Observations on the Osazone Method of locating Sugars in Plant Tissues. Annals of Botany, 29, 1915, 369-391.

Mangin, L. Sur la diffusion des gaz à travers les surfaces cutinisées. Comptes rendus, 104, 1887, 1809-1812.

,, ,, Sur la rôle des stomates dans l'entrée ou la sortie des gaz. Comptes rendus, 105, 1887, 879-881.

,, ,, Recherches sur la pénétration ou la sortie des gaz dans les plantes. Ann. de la Sci. Agron. Française et Étrangère, 1888.

,, ,, Sur la perméabilité de l'epidermie des feuilles pour les gaz. Comptes rendus, 106, 1888, 771-774.

Maquenne, L., and Demoussy, E. Sur la valeur des coefficients chlorophylliens et leurs rapports avec les quotients respiratoires réels. Comptes rendus, 156, 1913, 506-512.

Mary, Albert and Alexandre (Physicochemical Studies on the Synthesis of a Chlorophyll Pigment) Mon. Sci. I 5e Sér., 5, i, 1915, 121-128, Abstract in Journ. Chem. Soc., Abstr. 108, i, 1915, 979-980.

Matthaei, G. L. C. Experimental Researches on Vegetable Assimilation and Respiration III—On the Effect of Temperature on Carbon dioxide Assimilation. Phil. Trans. Roy. Soc. London, B, 197, 1904, 47-105.

Mayer, A. G. The Radiation and Absorption of Heat by Leaves. Amer. Journ. Sci., 45, 1893, 340-346.

Merget. Sur les fonctions des feuilles dans les phénomènes d'échanges gazeux entre les plantes et l'atmosphère ; rôle des stomates. Comptes rendus, 84, 1877, 376-379.

,, Sur les échanges gazeux entre les plantes et l'atmosphere. Réponse aux observations critique de M. Barthélemy. Comptes rendus, 84, 1877, 957-959.

,, Des fonctions des feuilles dans le phénomène des échanges gazeux entre les plantes et l'atmosphere. Du rôle des stomates dans les fonctions des feuilles. Comptes rendus, 86, 1878, 1492-1495.

Meyer, A. Ueber die Assimilationsproducte der Laubblätter angiospermer Pflanzen. Bot. Zeit., 43, 1885, 417-423, 433-440, 449-457, 465-472, 481-491, 497-507.

,, ,, Bildung der Stärkekörner in den Laubblättern aus Zuckerarten-Mannit und Glycerin. Bot. Zeit., 44, 1886, 81-88, 105, 113, 129-137, 145-151.

Mohl, H. von. Untersuchungen über die anatomischen Verhältnisse des Chlorophylls. Diss. 1837. (French Trans. Recherches anatomiques sur la chlorophylle. Ann. Sci. Nat., Bot., 9, 1838, 150-166).

Molisch, H. Über den braunen Farbstoff der Phæophyceen und Diatomeen. Bot. Zeit., 63, 1905, 131-144.

Moore, B. The Presence of Inorganic Iron Compounds in the Chloroplasts of the Green Cells of Plants, considered in Relationship to Natural Photo-synthesis and the Origin of Life. Proc. Roy. Soc. London, B, 87, 1914, 556-570.

Nef, J. U. Dissoziationsvorgänge in der Zuckergruppe (Erste Abhandlung) Ueber das Verhalten der Zuckerarten gegen die Fehlings'che Lösung sowei gegen andere Oxydationsmittel. Ann. der Chem., 357, 1907, 214-312.

,, ,, Dissoziationsvorgänge in der Zuckergruppe (Zweite Abhandlung). Über das Verhalten der Zuckerarten gegen Ätzalkalien. Ann. der Chem., 376, 1910, 1-119.

,, ,, Dissoziationsvorgänge in der Zuckergruppe (Dritte Abhandlung). Ann. der Chem., 403, 1913, 204-383.

Oddo, B. Synthesen mittels Magnesium-Pyrrol-Verbindungen. II. Mitteilung. Alkyl-Pyrryl-Ketone. Ber. d. deut. chem. Ges., 43, 1910, 1012-1021.

O'Sullivan, C. On the Estimation of Starch. Journ. Chem. Soc. Trans., 45, 1884, 1-10.

Palladin, V. I., and Stanewitsch, E. (1910 a) Die Abhängigkeit der Pflanzenatmung von den Lipoiden. Bioch. Zeitsch., 26, 1910, 351-369.

,, ,, (1910 b) Zur Physiologie der Lipoide. Ber. d. deut. bot. Ges., 28, 1910, 120-125.

,, ,, Pflanzenphysiologie, Berlin, 1911.

Pantanelli, E. Abhängigkeit der Sauerstoffausscheidung belichteten Pflanzen von äusseren Bedingungen. Jahrb. f. wiss. Bot., 39, 1903, 167-228.

Parkin, J. The Carbohydrates of the foliage leaf of the Snowdrop (*Galanthus nivalis*, L.) and their bearing on the first sugar of photosynthesis. Bioch. Journ., 6, 1911, 1-47.

Pfeffer, W. Zur Frage über die Wirkung farbigen Lichtes auf die Kohlensäurezersetzung. Bot. Zeit., 29, 1871, 319-323.

,, ,, Die Wirkung farbigen Lichtes auf die Zersetzung der Kohlensäure in Pflanzen. Arbeit. bot. Inst. in Würzburg, 1, 1871, 1-76.

,, ,, Ueber die Beziehung des Lichts zur Regeneration von Eiweissstoffen aus dem beim Keimungsprocess gebildeten Asparagin. Monatsb. d. Berliner Akad., 1873, 780-788.

,, ,, Pflanzenphysiologie, Bd. 1, Zweite Aufl. Leipzig, 1897.

,, ,, Physiology of Plants, Vol. 1, English Edition, Oxford, 1900.

Plotnikow, J. Photochemie, Halle a. S. 1910.

Pollacci, G. Intorno alla presenza dell'aldeide formica nei vegetali. Atti dell' Ist. Bot. di Pavia, N. S. 6, 1899, 45.

,, ,, Intorno all' assimilazione chlorofilliana delle piante. Atti dell' Ist. Bot. di Pavia, N. S. 7, 1899, 1-21.

,, ,, L'assimilation chlorophylienne. Archiv. Ital. de Biol, 35, 1901, 151-152.

,, ,, Intorno all' assimilazione chlorofilliana delle piante, Seconda memoria. Atti dell' Ist. Bot. di Pavia, N. S. 8, 1902, 1-66.

,, ,, L'assimilation chlorophyllienne, Deuxième mémoire. Archiv. Ital. de Biol., 37, 1904, 446.

,, ,, Sulla scoperta dell' aldeide formica nelle piante. Rend. R. Accad. dei Lincei., Ser. 5a, 16, 1907, 199-205.

Priestley, J. Experiments and Observations on Different Kinds of Air. 3 vols. London, 1774-1777.

Puriewitsch, K. Untersuchungen über Photosynthese. Jahrb. f. wiss. Bot., 53, 1914, 210-254.

Pütter, A. Temperaturkoeffizienten. Zeitsch. f. allg. Physiologie, 16 1914, 574-627.

Rahn, O. Der Einfluss der Temperatur und der Gifte auf Enzymwirkung, Garüng, und Wachtum. Bioch. Zeitsch., 72, 1916, 351-377.

Reinke, J. Untersuchungen über die Einwirkung des Lichtes auf die Sauerstoffausscheidung der Pflanzen (Erste Mittheilung). Bot. Zeit., 41, 1883, 697-707, 713-723, 732-738.

,, ,, Untersuchungen über die Einwirkung des Lichtes auf die Sauerstoffausscheidung der Pflanzen (Zweite Mittheilung). Bot. Zeit., 42, 1884, 1-10, 16-29, 33-46, 49-59.

Renner, O. Beiträge zur Physik der Transpiration. Flora, 100, 1910, 451-547.

Richter, A. Etude sur la photosynthèse et sur l'absorption par la feuille verte des rayons de différentes longeurs d'onde. Rev. gén. de Bot., 14, 1902, 151-169, 211-218.

Robertson, K. A., Irvine, J. C., and Dobson, M. E. A Polarimetric Study of the sucroclastic enzymes of *Beta vulgaris*. Bioch. Journ., 4, 1909, 258-273.

Sachs, J. Ueber den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern. Bot. Zeit., 20, 1862, 365-373.

,, ,, Ueber die Auflösung und Wiederbildung des Amylums in den Chlorophyllkörnern bei wechselnder Beleuchtung. Bot. Zeit., 22, 1864, 289-294.

,, ,, (1864b) Wirkung farbigen Lichts auf Pflanzen. Bot. Zeit., 22, 1864, 353-358, 361-367, 369-372.

,, ,, Vorlesungen über Pflanzenphysiologie. Leipzig, 1882-3.

,, ,, Lectures on the Physiology of Plants, English Edition, Oxford, 1887.

,, ,, Ein Beitrag zur Kenntniss der Ernährungsthätigkeit der Blätter. Arbeiten bot. Inst. in Würzburg, 3, 1884, 1-33.

Saussure, T. de. Recherches chimiques sur la végétation. Paris, 1804.

Schimper, A. F. W. Untersuchungen über die Entstehung der Stärkekörner. Bot. Zeit., 38, 1880, 881-902.

Schloessing, T. Sur les échanges d'acide carbonique et d'oxygène entre les plantes et l'atmosphere. Comptes rendus, 115, 1892, 881-883, 1017-1020; 117, 1893, 756-759, 813-816.

Senebier, J. Mémoires physico-chimiques, sur l'influence de la lumière solaire pour modifier les êtres des trois règnes de la nature et surtout ceux du règne végétal. 3 vols., Genève, 1783.

,, ,, Expériences sur l'action de la lumière solaire dans la végétation. Genève, 1788.

,, ,, Physiologie Végétale, contenant une description des Organes des Plantes, et une exposition des phénomènes produits par leur organisation. 5 vols., Genève, 1800.

Senft, E. Über den microchemischen Zuckernachweis durch essigsäures Phenylhydrazin. Sitzungsb. d. k. Akad. der Wiss. in Wien, Abt. 1, 113, 1904, 3-28.

Siegfried, M. Über die Bindung von Kohlensäure durch amphotere Amidokörper. Hoppe-Seyler's Zeitsch. f. physiol. Chemie., 44, 1905, 85-96.

Spoehr, H. A. Photochemische Vorgänge bei der diurnalen Entsäurung der Succulenten. Bioch. Zeitsch., 57, 1913, 95-111.

,, ,, Theories of Photosynthesis in the Light of some new Facts. The Plant World, 19, 1916, 1-16.

Stahl, E. Einige Versuche über Transpiration und Assimilation. Bot. Zeit., 52, 1894, 117-146.

Stahl, E. Zur Biologie des Chlorophylls. Jena, 1909.

Stokes, G. G. On the Change of Refrangibility of Light. Phil. Trans. Roy. Soc. London, 1852, Part 2, 463-562.

,, ,, On the Application of the Optical Properties of Bodies to the Detection and Discrimination of Organic Substances. Journ. Chem. Soc. Trans., 2, 1864, 304-318.

,, ,, On the Supposed Identity of Biliverdin with Chlorophyll, with Remarks on the Constitution of Chlorophyll. Proc. Roy. Soc. London, 13, 1864, 144-145.

Stoklasa, J., Brdlik, V., and Just, J. Ist der Phosphor an dem Aufbau des Chlorophylls beteiligt? Ber. d. deut. bot. Ges., 26a, 1907, 69-78.

,, ,, ,, ,, ,, Ernst, A. Zur Frage des Phosphorgehaltes des Chlorophylls. Ber. d. deut. bot. Ges., 27, 1909, 10-20.

,, ,, Sebor, J., and Senft, E. Beitrag zur Kenntnis der Zusammensetzung des Chlorophylls. Beih. zum bot. Centr., 30, Abt. 1, 1913, 167-235.

Strakosch, S. Ein Beitrag zur Kenntnis des Kohlenhydratstoffwechsels von *Beta vulgaris* (Zuckerrübe). Sitzungsb. d. k. Akad. d. Wiss. in Wien, Abt. 1, 116, 1907, 855-869.

Tammann, G. Die Reactionen der ungeformten Fermente. Hoppe-Seyler's Zeitsch. f. physiol. Chem, 16, 1892, 271-328.

,, ,, Zur Wirkung ungeformter Fermente. Zeitsch. f. physik. Chem., 18, 1895, 426-442.

Thoday, D. Experimental Researches on Vegetable Assimilation and Respiration. V. A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves. Proc. Roy. Soc. London, B, 82, 1909, 1-55.

,, ,, Experimental Researches on Vegetable Assimilation and Respiration. VI. Some Experiments on Assimilation in the Open Air. Proc. Roy. Soc. London, B, 82, 1910, 421-450.

Timiriazeff, C. Recherches sur la décomposition de l'acide carbonique dans le spectre solaire par les parties vertes des végétaux. Ann. de chim. et de Phys. 5e Sér., 12, 1877, 355-396.

,, ,, État actuel de nos connaissances sur la fonction chlorophyllienne. Ann. Sci. Nat., Bot., 7e Sér., 2, 1885, 99-125.

,, ,, Enregistrement photographique de la fonction chlorophyllienne par la plante vivante. Comptes rendus, 110, 1890, 1346-1347.

,, ,, The Cosmical Function of the Green Plant. Croonian Lecture, Proc. Roy. Soc. London, B, 72, 1903, 424-461.

Tollens, B. Kurzes Handbuch der Kohlenhydrate. Dritte Auflage, Leipzig, 1914.

Treboux, O. Einige stoffliche Einflüsse auf die Kohlensäure-Assimilation bei submersen Pflanzen. Flora, 92, 1903, 49-76.

Tsvett, M. S. Recherches sur la constitution physico-chimique du grain de chlorophylle (in Russian). Trd. Obsc. jest., Kazan, 35, 1901, xii + 268.

,, ,, Zur Kenntniss der Phæophyceen Farbstoffe. Ber. d. deut. bot. Ges., 24, 1906, 235-244.

,, ,, Chromophylls in the Plant and Animal Worlds (in Russian). Warsaw, 1910.

Ursprung, A. Die physikalischen Eigenschaften der Laubblätter. Bibl. Bot. Heft 60, 1903.

Usher, F. L., and Priestley, J. H. A Study of the Mechanism of Carbon Assimilation. Proc. Roy. Soc. London, B, 77, 1906, 369-376.

Usher, F. L., and Priestley, J. H. The Mechanism of Carbon Assimilation. II. The Photolytic Decomposition of Carbon Dioxide in vitro. Proc. Roy. Soc. London, B, 78, 1906, 318-327.

,, ,, ,, ,, ,, The Mechanism of Carbon Assimilation. III. Proc. Roy. Soc. London, B, 84, 1911, 101-112.

Van't Hoff, J. H. Über die zunehmende Bedeutung der anorganischen Chemie. Zeitsch. f. anorgan. Chem., 18, 1898, 1-13.

,, ,, ,, Ueber synthetische Fermentwirkung. Sitzungsb. d. k. preuss. Akad. d. Wiss., 1909, 1065-1076.

,, ,, ,, Ueber synthetische Fermentwirkung, II. Sitzungsb. d. k. preuss. Akad. d. Wiss., 1910, 963-970.

Wager, H. The Action of Light on Chlorophyll. Proc. Roy. Soc. London, B, 87, 1914, 386-407.

Weber, C. Ueber specifische Assimilationsenergie. Arbeit. d. bot. Inst. in Würzburg, 2, 1879, 346-352.

Weigert, F. Die chemischen Wirkungen des Lichtes. Stuttgart, 1911.

Wiesner, J. Versuche über den Ausgleich des Gasdruckes in den Geweben der Pflanzen. Sitzungsb. d. k. Akad. d. Wiss in Wien, 79. Abt. 1, 1879, 368-408.

,, ,, and Molisch, H. Untersuchungen über Gasbewegung in der Pflanze. Sitzungsb. d. k. Akad. d. Wiss in Wien, 98, Abt. 1, 1889, 670-713.

,, ,, Der Lichtgenuss der Pflanzen. Leipzig, 1907.

Willstätter, R., and Mieg, W. Über eine Methode der Trennung und Bestimmung von Chlorophyllderivaten. Ann. der Chem., 350, 1906, 1-47.

,, ,, Zur Kenntniss der Zusammensetzung des Chlorophylls. Ann. der Chem., 350, 1906, 48-83.

,, ,, and Stoll, A., Spaltung und Bildung von Chlorophyll. Ann. der Chem., 380, 1911, 148-153.

,, ,, and Stoll, A. Untersuchungen über Chlorophyll. Berlin, 1913.

,, ,, and Page, H. J. Über die Pigmente der Braunalgen, Ann. d. Chem., 404, 1914, 237-271.

,, ,, (1915a) Chlorophyll. Journ. Amer. Chem. Soc., 37, 1915, 323-345.

,, ,, and Stoll, A. (1915b) Über die chemischen Einrichtungen des Assimilationsapparates. Sitzungsb. d. k. Preuss. Akad. der Wiss., 1915, 322-346.

,, ,, ,, ,, (1915c) Über die Assimilation ergrünender Blätter. Sitzungsb. d. k. Preuss. Akad. der Wiss., 1915, 524-532.

,, ,, ,, ,, (1915d) Untersuchungen über die Assimilation der Kohlensäure (Erste vorläufige Mitteilung). Über die Beziehungen zwischen Chlorophyllgehalt und assimilatorischer Leistung der Blätter, Ber. d. deut. chem. Ges., 48, 1915, 1540-1564.

Wilson, E. G., and Atkins, W.R.G. Methods for the estimation of mixtures of four or more Carbohydrates, involving oxidation with Bromine. Bioch. Journ., 10, 1916, 504-521.

R. MADLEY, PRINTER, 151, WHITFIELD STREET, LONDON, W.1.

CARBON ASSIMILATION:
A REVIEW OF RECENT WORK ON THE PIGMENTS OF THE GREEN LEAF AND THE PROCESSES CONNECTED WITH THEM.

New Phytologist Reprints.

No. 1 (Out of print). LECTURES ON THE PHYSIOLOGY OF MOVEMENT IN PLANTS (1907). By Sir F. DARWIN.

No. 2 (Out of print). LECTURES ON THE EVOLUTION OF THE FILICINEAN VASCULAR SYSTEM (1908). By A. G. TANSLEY.

No. 3 (Out of print). THE PHYLOGENY AND INTER-RELATIONSHIPS OF THE PTERIDOPHYTA (1909). By Lady ISABEL BROWNE.

No. 4 (Out of print). THE INTER-RELATIONSHIPS OF THE BRYOPHYTA (1911). By F. CAVERS.

No. 5. FLORAL EVOLUTION: WITH PARTICULAR REFERENCE TO THE SYMPETALOUS DICOTYLEDONS. By H. F. WERNHAM. Pp. viii and 152, with Phylogenetic and other Tables, and an Index. Price 3*s.*, post free. Five copies for 13*s.* 6*d.*, post free.

No. 6. THE VEGETATION OF JAPAN. By H. TAKEDA. Pp. 21. Price 1*s.*, post free.

No. 7. THE INTER-RELATIONSHIPS OF THE FLAGELLATA AND PRIMITIVE ALGÆ. By F. CAVERS. Pp. vi and 52, with Literature List and Phylogenetic Tables, and numerous Figures in the Text. Price 2*s.* post free. Five Copies for 9*s.*, post free.

No. 8. THE CLASSIFICATION OF CONIFERS. By W. T. SAXTON. Pp. 21, with Bibliography and a Phylogenetic Table. Price 1*s.*, post free. Five copies for 4*s.* 6*d.*, post free.

No. 9. A REVIEW OF LITERATURE CONCERNING THE EVOLUTION OF MONOCOTYLEDONS. By N. BANCROFT. Pp. 24, with Bibliography and 4 Diagrams. Price 1*s.*, post free. Five copies for 4*s.* 6*d.*, post free.

WILLIAM WESLEY & SON, 28, Essex Street, Strand, London

THE NEW PHYTOLOGIST.

A BRITISH BOTANICAL JOURNAL,

EDITED BY A. G. TANSLEY, M.A., F.R.S.,

UNIVERSITY LECTURER IN BOTANY, CAMBRIDGE.

This Journal was founded in 1902 to afford a medium for the publication of original papers, critical articles and reviews, summaries of recent advances in botanical knowledge, occasional notes and correspondence on botanical topics. The preference is given to those contributions dealing with matters of general interest to botanists and those branches of the science undergoing rapid current development. THE NEW PHYTOLOGIST has published much of the work of British ecologists and plant-geographers during the last few years, and still continues to do so. The Journal is supported by all the leading British botanists.

The NEW PHYTOLOGIST is well illustrated, and is issued once a month, except August and September; the minimum size of the annual volume is 360 pages. The subscription price is 15/- post free. Single numbers, 2/- each.

Vols. 1, 6 and 7 out of print. Vols. 2—5, 8—15, 15/- each.

Published by MESSRS. WM. WESLEY & SON, *28, Essex Street London, W.C., to whom all subscriptions and business communications are to be addressed.*

All communications for the Editor are to be sent to A. G. TANSLEY, *Grantchester, Cambridge.*