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# THE PHYSIOLOGY OF PHOTOSYNTHESIS

BY

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# PREFACE

PHOTOSYNTHESIS is the name now generally attached to one of the fundamental cosmic processes, the one that underlies the great primitive industry of Agriculture: it is therefore a process which should be completely understood. An idea of the present state of knowledge concerning it will, perhaps, be most satisfactorily conveyed by means of a brief historical sketch of the progress of its discovery. As has been so often the case in scientific research, the starting-point was an accidental observation. In the account of his investigations on the air (1772), Priestley remarks: 'I have been so happy as by accident to have hit upon a method of restoring air which has been injured by the burning of candles, and to have discovered at least one of the restoratives which nature employs for this purpose—it is *Vegetation*. The restoration of vitiated air, I conjecture, is effected by plants imbibing the phlogistic matter with which it is overloaded by the burning of inflammable bodies.' Whilst Priestley was satisfied that the significance of the process was the purification of the air for animal respiration, his friend Percival reached the further conclusion that 'fixed air is far from being *destructive* to vegetation, it is the proper *pabulum* of vegetables, making them to flourish much more than they could do in other circumstances,' which put the matter on the right basis.

Research on the subject was continued by Ingenhousz and by Sénébier, with considerable success. Ingenhousz established the fact that the purifying action of plants on

the air can only take place when they are exposed to sunshine, and especially emphasised the importance of the process as one of nutrition : ' It seems that plants absorb the phlogistic from the atmosphere which constitutes the principal part of their nutriment ; the absorbed air is elaborated by the organs of the plant, with the result that the phlogistic is retained as nutriment, whilst the remainder is restored to the atmosphere.' Sénébier confirmed the importance of exposure to light, and made it clear that the process is mainly carried on in those parts of the plant that are green in colour. This was the stage reached by the end of the eighteenth century.

Important progress was marked by the publication, in 1804, of Th. de Saussure's *Recherches Chimiques*. The advances in chemistry, both theoretical and practical, enabled him to make the experiments which proved, (1) that a green plant exposed to light absorbs carbon dioxide and evolves a rather smaller volume of oxygen ; (2) that the plant at the same time assimilates the elements of water ; and (3) that the gaseous interchange is accompanied by an increase in the dry weight of the plant. The bearing of the gaseous interchange upon the nutrition of the plant was thus clearly defined, and a beginning was made in the direction of the quantitative estimation of the process.

The main factors were now recognised : light ; presence of the green colouring matter, chlorophyll ; supply of a limited quantity of carbon dioxide ; but much yet remained to be explained. What, for instance, is the part played by light in the process ? What is the function of the chlorophyll ? What is the nature of the organic substance formed ? Answers to these questions were slowly forthcoming during the nineteenth century. Light, it was ascertained, is the source of the energy required for the chemical work of reducing and assimilating carbon dioxide and water. The study of the spectrum of chlorophyll showed it to be the means by wkich the light is absorbed

and made available. From the quantitative relation between the volume of the carbon dioxide absorbed and that of the oxygen evolved, it was concluded that the first organic product formed in the plant is a carbohydrate : a conclusion confirmed by the observation that, in most plants, the carbohydrate formed is stored in the chloroplasts as starch-grains. Further knowledge has been accumulated as to the relation of the assimilatory process to temperature ; as to the relative efficiency of light of different colours and degrees of intensity ; and as to the most suitable proportion of carbon dioxide to be supplied by the medium surrounding the plant.

It would appear, therefore, that almost everything that can be known about photosynthesis has now been ascertained. It may be admitted that this is approximately true in the *qualitative* sense, but certainly not in the *quantitative* sense. In spite of many laborious researches, it is not yet possible to attach definite numerical values to the efficiency of light of various wave-length and energy ; nor to the effect of a rise of temperature, or of a variation in the amount of available carbon dioxide, upon the activity of photosynthesis.

The present volume is essentially a record of quantitative research in these various directions. The experiments have been carried out by means of a variety of sensitive apparatus specially devised for the different objects in view ; and the results, having been recorded automatically, are at least free from the error of the personal equation.

The author takes this opportunity of expressing his high appreciation of the valuable assistance given to him, in carrying out the numerous and often prolonged experiments, by Professor N. C. Nag, M.A., the Assistant Director, and by the Scholars of the Bose Institute.

BOSE INSTITUTE, CALCUTTA,
*October*, 1923.

J. C. BOSE.

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# PHOTOSYNTHESIS

## CHAPTER I

### INTRODUCTION

Method of chemical analysis in photosynthetic investigation—Difficulties of the problem arising from complicating factors—The method of evolution of oxygen by water-plants—Errors of the Bubble-counting Method—New method for securing high accuracy in quantitative determinations—Different lines of investigation on photosynthesis by the new method.

ALL vital activity, whether of animal or of plant, is ultimately traceable to the energy of solar radiation. The animal derives its energy from vegetable food. The plant, by virtue of its chlorophyll, absorbs both solar energy and carbon dioxide and builds up organic matter charged with latent energy. This is, in fact, the prime source of all organic matter, as well as of the energy that is set free on its combustion. To stand before a coal-fire is to bask in the sunshine of the Carboniferous Period.

It is to this constructive or synthetic activity of the plant that the term Photosynthesis has been applied. Like other physiological processes, it cannot be fully understood until it has been carefully analysed. The investigations described in the present work are a contribution to the attainment of this end.

In photosynthesis a given quantity of carbon dioxide is absorbed, a corresponding quantity of oxygen evolved, and the end-product is a certain amount of carbohydrate. Thus there are theoretically three possible means for the estimation of photosynthetic activity—namely the measure-

ment (1) of the absorption or intake of carbon dioxide; (2) of the evolution of oxygen; and (3) of the increase of dry weight due to the formation of organic substance. The sources of error in the estimation of $CO_2$-assimilation from either the evolution of oxygen or the increase in dry weight have hitherto been so great that the method generally adopted in photosynthetic measurement has been that of the absorption of carbon dioxide. This is by no means a simple method but requires very complicated chemical analysis. In spite of many difficulties, the ingenuity and persistence of numerous investigators have rendered it as accurate as its various sources of error would permit A leaf or a number of leaves are, according to this method, enclosed within a receiver through which a definite volume of air containing carbon dioxide is passed. Analysis of the inflowing and outflowing air, and its volume, give the quantity of $CO_2$ decomposed by the leaves when in light.

The most accurate means for the determination was devised by Kreussler (1885–90); Bonnier and Mangin (1886–91) introduced numerous improvements in the analysis of the gas. F. F. Blackman (1895) analysed the intake or evolution of carbon dioxide by the leaf, the gas being passed through a standard baryta solution subsequently titrated against standard solution of hydrochloric acid. The various analyses and necessary corrections for estimation of the $CO_2$ absorbed are so numerous, and the labour entailed so great, that the duration of even a single observation is excessively prolonged. This introduces difficulties in the maintenance of constancy of external conditions, such as light and temperature. Variation in the intensity of light during prolonged experiments causes change in the aperture of the stomata through which the gaseous interchange of the leaves takes place. The temperature also undergoes indefinite variation, for the green leaves enclosed in the receiver are surrounded by non-conducting air which is transparent to the thermal rays. The temperature of the leaves is raised by the absorption

of strong light, thus modifying their photosynthetic activity. A thermometer enclosed in the plant-chamber gives no correct indication of the temperature of the leaf, a difficulty which Blackman attempted to overcome by embedding one junction of a thermo-electric couple in the midrib of the leaf. There are other drawbacks also which render the method of the absorption of carbon dioxide unreliable for the accurate estimation of photosynthesis.

We are thus confronted with the necessity of devising means for the determination of photosynthetic activity without recourse to the laborious chemical analysis and the numerous corrections which complicate existing methods. In physiological investigations we are chiefly concerned with the study of the changes induced in the functional activity of the plant under variations of external conditions. We have to start with the constancy of the physiological factor of an individual plant; it is obvious that the statistical method of obtaining average effects over a large number of plants would not be suitable for our purpose. Again, for the study of the effect of variation of any particular factor, all the others should be maintained constant. This can be assured only for a short time; hence the period of the experiment must be reduced to a minimum. There thus arises the necessity for a sensitive method which would indicate the normal rate of photosynthesis and its induced variations in the course of less than an hour, preferably within a few minutes. For the elimination of personal error, it is also desirable to devise means for the automatic record of the normal rate and its induced variations.

A very sensitive means of observing and measuring photosynthesis is afforded by the evolution of oxygen by water-plants; it is a direct method which avoids the necessity of laborious processes of chemical analysis. The photosynthetic action is immediately observable, and any change induced in the normal rate is visually demonstrated by the quickening or slowing down of the evolution of bubbles of gas.

The other advantages of experimenting with water-plants are: (1) that the plant may be maintained under normal conditions in a vessel of water free from unnatural restraint; (2) that there is no transpiration to modify the normal activity; (3) the absence of complicating stomata, the carbonic acid in water diffusing readily through cell-walls saturated with water; and (4) the facility of escape of the excreted oxygen from the intercellular spaces.

The well-known bubble-counting method, so promising at first sight, has many serious defects which are as follows:

(1) The photosynthetic activity is measured by the rate of evolution of gas which is assumed to be pure oxygen. But the evolved gas is seldom pure. The water in which the plant grows contains oxygen, nitrogen and carbon dioxide in solution. These gases penetrate into the intercellular spaces of the plant and may be given out along with the evolved oxygen.

(2) An unknown proportion of the evolved oxygen is liable to be absorbed and retained by the water.

(3) A portion of the oxygen is also lost through respiration by the plant; and, finally—

(4) A very serious defect is that the volume of successive bubbles given out is liable to irregular variation.

These defects, though formidable, are however not insurmountable; I describe various means and appliances by which they have been eliminated. The results obtained show that no other method can rival that of the time-record of the evolution of *equal* volumes of oxygen. The quantitative results obtained possess such a high degree of accuracy that it becomes possible to attack with success many new and important problems of photosynthesis. The new method secures (1) the certainty that the gas evolved is actually pure oxygen; (2) the determination of the times required for the evolution of equal volumes of oxygen; and (3) the elimination of personal error by automatic record.

Photosynthesis is admitted to be a vital phenomenon; we have, however, to enquire if it belongs to the same

category as other phenomena of irritability, which manifest themselves by mechanical response to stimulus or by autonomous response. The important factors which modify photosynthesis are the $CO_2$-concentration, the temperature, and the intensity of light. We have to determine the effect of increasing $CO_2$-concentration on photosynthesis: the effect of rise of temperature has also to be observed, and the minimum and the optimum points determined.

The most important condition for the maintenance of uniform photosynthesis is the constancy of the light intensity; but there is at present no means available for quick determination of the intensity of light, or of the hourly variation of daylight. An Automatic Radiograph and an Electric Photometer had therefore to be devised to meet these special requirements.

In investigating the action of light in photosynthesis we have to determine the effect of varying intensity from the minimum to the maximum, so as to obtain a complete photosynthetic curve. It is also necessary to determine the relation between the *quantity* of light and the *amount* of photosynthetic product.

The investigation of certain other features of photosynthetic action are of very great theoretical importance; for example, the period of photosynthetic induction and its characteristic variations under definite conditions. Another interesting investigation is that of the relative photosynthetic effectiveness of intermittent and of continuous light.

Photosynthesis is found to be affected in a characteristic manner by various anæsthetics and poisons. One of the most unexpected results was the discovery of the influence of infinitesimal traces of certain chemical substances in inducing an extraordinary enhancement of $CO_2$-assimilation.

The photosynthetic products are carbohydrates, and there is no means at present available of direct measurement of the rate of their formation under light. An account will be given of a sensitive method by which this can be determined in the living plant during exposure to light.

There is also the important question of the relative photosynthetic effectiveness of the different rays of the spectrum ; the problem to be solved is whether the differing effects are solely due to the quality of the light, or if they are also due to the differing energy of the various rays of the spectrum. In this latter investigation the energy of the rays has been measured directly by a new Magnetic Radiometer. Evidence has been adduced that some of the energy absorbed by the plant is utilised in doing work other than photosynthesis.

The steam-engine converts the potential energy of coal into kinetic energy of movement, and we can determine the efficiency of this method of transformation. The efficiency of the reverse process of conversion of the kinetic energy of solar radiation into the potential energy stored in plants is of very great interest. The results of the investigation which I have carried out on the subject show that the efficiency is much higher than has been generally supposed.

Photosynthetic activity is changed under the action of not one but many factors. The effects of simultaneous variation of these are unknown ; a general law of photosynthesis has, however, been formulated by which the effect of any combination of different factors can be found.

These and other questions are treated in detail in the succeeding chapters.

## CHAPTER II

### THE EVOLUTION OF PURE OXYGEN UNDER LIGHT

The aquatic plant *Hydrilla verticillata*—Anatomical characteristics—Analysis of pond-water—Proportions of different gases absorbed by water—Method of obtaining evolution of unmixed oxygen—Absorption of oxygen by water.

In experimenting with various water-plants, I found *Hydrilla verticillata* to be the most suitable for the investigation of photosynthesis. It is a flowering plant, having a length of about 80 cm., bearing a number of leaves in whorls at the nodes of the stem. The average area of the leaf is about 25 sq. mm. ; the internodes are short towards the apex, where the young leaves are crowded together. There are two varieties met with :—the stem in the one is relatively thin, about 1 mm. in diameter, and bears from four to five leaves at each node : the diameter of the stem of the thicker variety is about 3 mm., and there are about nine leaves at each node. A transverse section of the leaf and the stem of the plant is given (fig. 1) ; the lamina consists of only two layers of cells, except at the midrib. The upper layer, which faces the sky, consists of relatively large-sized cells containing chloroplasts, the diameter of each cell being about 0.08 mm. The cells of the lower layer are smaller in size. The chloroplasts in the cells are shown in the left half of the figure ; those in the cells of the right half have been decolorised and stained with iodine. This treatment shows about forty starch-grains in each cell, the leaves having been previously exposed to light ; the starch-grains disappear from the cells during prolonged darkness.

A section of the stem is also given in the figure ; it shows about three layers of chlorophyll-containing cells at the

outside ; the cells in the interior also contain chlorophyll but in less quantities. The noticeable characteristic is the presence of large intercellular spaces, which become filled

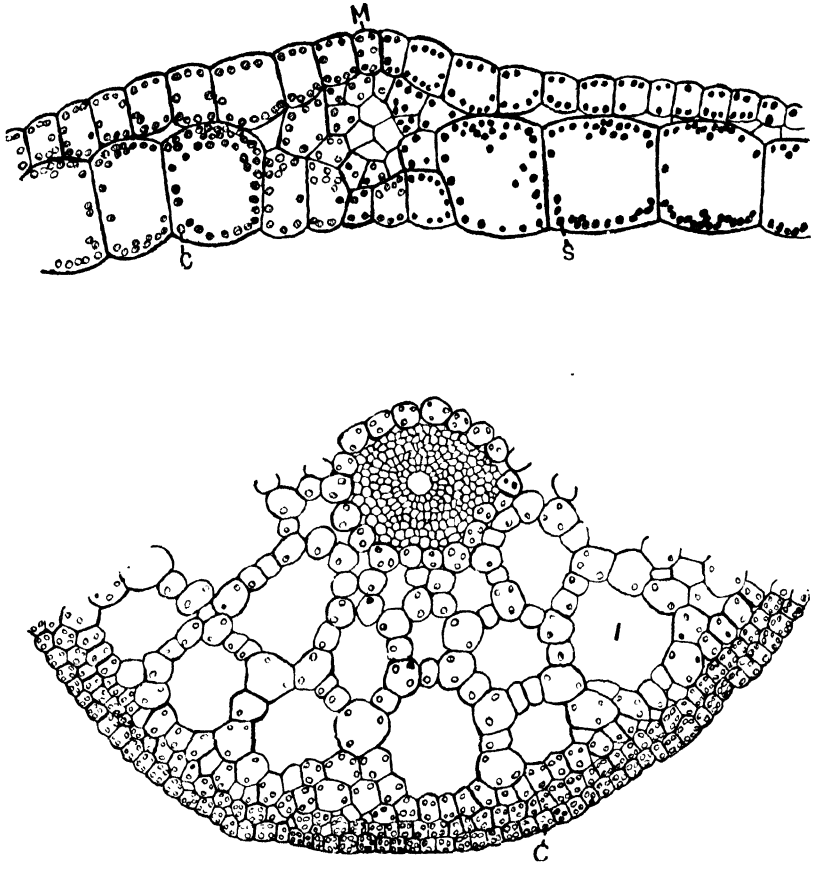



FIG. 1. Transverse Section of the Leaf (upper figure), and of the Stem (lower figure) of *Hydrilla verticillata*

The layer of larger cells in the leaf is in nature exposed to the sky. Note intercellular spaces in the section of the stem.

M, midrib; S, starch-grains; C, chloroplasts.

with excreted oxygen during photosynthesis ; from these the gas escapes in a succession of bubblets at the cut end of the stem. The *Hydrilla* plant grows in lakes, ponds and in watercourses. An economic use was formerly found for it in bleaching the brown Date Palm sugar : layers of the

plant were spread over the sugar and exposed to the sun. It is probable that the oxygen evolved by the plant under sunlight caused the bleaching action. The quantity of oxygen given out by the plant is considerable; the water of the pond in which the plant grows is relatively pure, due to the oxidation of organic impurities. I have introduced a number of these plants into an aquarium in which a large number of aquatic animals are living in comfort. The oxygen given out by the *Hydrilla* is quite sufficient, there being no necessity for further aeration of the water.

The plants employed for the experiments were growing very vigorously in the three ponds in the grounds of the Institute. One of these tanks is exposed to full sunlight; the second is in a relatively dark place, and the third is exposed to bright light from the northern sky but not to direct sunlight. The plants grown exposed to the sun were found to be less sensitive than those exposed to the northern sky; those grown in the shade of trees also exhibited a relative depression of physiological activity. When the plants were brought to the laboratory, the precaution had to be taken to place them in glass or earthen jars. For traces of metal act most injuriously; experiments carried out on the subject showed that ten parts of copper salts in a million are enough to abolish the photosynthetic activity of the plant.

The water of the ponds in which the plants grow was analysed and found to give the following composition.

TABLE I.—ANALYSIS OF 1000 C.C. OF POND WATER

| | |
|---|---|
| Organic solids | 0·090 grm. |
| Inorganic solids consisting of alkali carbonates with traces of chlorides and sulphates | 0·129 ,, |
| Alkaline earth carbonate | 0·055 ,, |
| Insoluble oxides and phosphates | 0·011 ,, |
| Total solids in 1000 c.c. of water | 0·285 grm. |

The following table gives the proportions of dissolved nitrogen, oxygen and carbonic acid, the latter consisting of free and combined $CO_2$. The proportion of nitrogen to oxygen in the atmosphere is as 4 : 1; but, on account of

the lower solubility of nitrogen, the nitrogen and oxygen absorbed by water are as 2 : 1.

TABLE II.—PROPORTIONS OF GASES PRESENT IN 1000 C.C. OF POND WATER

| Specimen of water | Nitrogen | Oxygen | Free and loosely held $CO_2$ | Combined $CO_2$ |
|---|---|---|---|---|
| Specimen I. . . | 12·88 c.c. | 6·35 c.c. | 3·85 c.c. | 40·65 c.c. |
| ,, II. . . | 12·90 ,, | 6·13 ,, | 4·19 ,, | 40·65 ,, |
| ,, III. . . | 12·50 ,, | 5·47 ,, | 4·60 ,, | 41·40 ,, |
| Average . . . | 12·79 c.c. | 5·98 c.c. | 4·00 c.c. | 41·00 c.c. |

The intercellular spaces of the plant are filled with nitrogen and oxygen dissolved in water, which are carried away with the oxygen evolved in photosynthesis. The gas given out in two different experiments was analysed and found to contain the following proportions of oxygen and nitrogen in 10 c.c. of evolved gas.

First experiment . . . Oxygen, 4·29 c.c. ; Nitrogen, 5·71 c.c.
Second experiment . . . Oxygen, 4·2 ,, ; Nitrogen, 5·8 ,,

The proportion of oxygen to nitrogen was thus about 4·2 to 5·8.

No carbon dioxide was found in the evolved gas ; the escape of $CO_2$ from its solution in water takes place only when the quantity of the gas in solution is excessive.

## Evolution of Pure Oxygen

The first problem is to secure that the evolved gas shall be pure oxygen without any admixture of nitrogen, since the photosynthetic activity can only be accurately estimated by the rate of evolution of the pure gas. The difficulty has been met by placing the plant in a beaker filled with pond-water under the receiver of an air-pump. A partial vacuum is produced which removes the air contained in the plant and in the water ; this should be done slowly, for too sudden

a production of vacuum causes injury to the plant. The water is next charged with the $CO_2$, the best proportion of which I find to be from 7 to 9 mg. per 100 c.c. of water. A small quantity of pure oxygen is also bubbled through the water, as its presence is necessary for the normal life-activity of the plant. Before collecting the gas for photosynthetic measurements, it is advisable to expose the plant for a short time for the disappearance of the last trace of nitrogen. Analysis of the gas subsequently evolved under light shows that it is pure oxygen.

The special plant-vessel—to be described in the next chapter—is filled up with the nitrogen-free water charged with a proper quantity of carbon dioxide and oxygen. A special valve prevents the introduction of nitrogen from the outside air.

## Absorption of the Evolved Oxygen by Water

There is a possibility of loss of oxygen through absorption before it is given out of the water in the form of bubbles. As already stated, the water contains a small proportion of oxygen; if the channel through which the oxygen arises were saturated with oxygen there could then be no further absorption. The absorptive power of water for different gases has been carefully determined; in the case of oxygen, 100 c.c. of water absorb about 2·8 c.c. at 22° C. The cut end of the plant is fixed in the plant-vessel (see fig. 2) so that it is about 2 mm. below the surface of the water; hence a continuous stream of oxygen rises through a definite and short length of water which must be soon saturated with it, and the loss of oxygen by absorption therefore becomes quite negligible.

There may be some misgiving that the intercellular spaces might divert a proportion of the oxygen produced. It is obvious that this cannot affect the rate of evolution; for under the action of light the intercellular spaces become completely filled, and on the attainment of the steady

condition the quantity of gas forced out must be equal to the quantity which is formed in the plant under light. The quantitative results, to be given later, establishing a strict proportionality between the oxygen and the other photosynthetic products (Chap. XXIV), afford conclusive proof that photosynthetic activity can be accurately determined from the volume of oxygen given out by the plant.

A few words may be said here about the loss of oxygen by respiration. I shall give later a detailed account of two independent methods (Chap. XVII) by which it has been ascertained that the loss by respiration is relatively small, being about 4 per cent. at a temperature of 22° C. This small correction has to be added in obtaining the true rate of photosynthesis. Most of the experiments on the effect of variation of a single factor were carried out at a constant temperature; hence it is only necessary to determine the *relative* variation of the rate, the small loss due to respiration being the same in all. The loss by respiration has, however, to be taken fully into account in certain other quantitative determinations.

Having secured the evolution of oxygen gas unmixed with other gases, we have to determine the activity of photosynthesis by measuring the volume of oxygen given out in a unit of time. For this the counting of bubblets, as will be presently explained, gives very fallacious results. It is therefore necessary to determine the periods not of successive bubblets, but of successive *equal volumes of gas.* I have used the term ' bubblets ' advisedly, to distinguish them from the ' bubbles ' of equal volume given out by the special Bubbler which will be described in the next chapter.

## Summary

In the determination of the photosynthetic activity by the volume of oxygen evolved, errors are introduced from the admixture of nitrogen with the oxygen and the partial absorption of the oxygen by the water.

At the beginning of the experiment the nitrogen must be removed from the water and the plant by means of an air-pump. After this the gas evolved is found to be pure oxygen.

The cut end of the plant is placed in the plant-vessel so that it is only 2 mm. below the surface of the water. The short length of water soon becomes saturated with oxygen, after which there is no loss by absorption.

# CHAPTER III

## DETERMINATION OF RATE OF EVOLUTION OF EQUAL VOLUMES OF OXYGEN

Frequency and volume of the bubblets—Effect of depth of immersion—Effect of choking of the cut end by mucilage—Spontaneous variation in the frequency of the bubblets—Determination of successive evolution of equal volumes of oxygen—The plant-vessel—The Bubbler—Measurement of photosynthetic activity—Determination of the constant of the Bubbler.

THE most important factor in the accurate estimation of photosynthetic activity is the measurement of the rate of evolution of the oxygen. The usual method of estimation by counting the bubblets given out by the plant is, however, not trustworthy for the following reasons :

(1) The bubblets from an intact water-plant force their way out from the margin of the leaves in a very irregular manner ; this difficulty may be removed by making a large opening by a transverse cut, when the gas-bubbles come out of the section instead of the side of the leaves. But the bubblets are often given off so rapidly that accurate counting of them becomes impossible. In less active specimens, when the successive intervals are longer, the counting of the bubblets gives no accurate indication of the photosynthetic activity, since their volume does not remain constant.

(2) The frequency and volume of the bubblets depend on the depth of the immersion of the cut end below the level of the water. If the depth be in any way increased the frequency becomes decreased while the volume undergoes an increase.

(3) Even the maintenance of a constant depth of immersion does not ensure uniformity in the rate of bubbling, for

a deposit of mucilage at the cut end causes a slowing down. This is specially evident in *Vallisneria*; the vigorous bubbling from a fresh cut leaf becomes slowed down and is completely arrested in the course of an hour. This is, however, an extreme case; other water-plants exhibit this retarding effect, though to a less extent.

(4) Again, the normal rate of bubbling is liable to undergo spontaneous variation; this will be understood from the account of the following experiment, where the rate of bubbling after exposure to constant light was measured at intervals of intervening darkness. The bubbling at first took place at intervals of nineteen seconds; after a period of darkness the plant was again subjected to the same intensity of light as before. On the attainment of an uniform rate, the frequency of bubbling was now found to have undergone a sudden increase, the successive intervals being now reduced to five seconds. It thus appeared as if previous exposure to darkness had enhanced the activity $\frac{19}{5}$ times or nearly four-fold. But independent measurement of the volumes of gas given out during equal periods in the two cases showed that they were equal.

Wilmott[1] has attempted to obviate the difficulty of the changing size of the bubblets by fitting a glass tube at the cut end of the water-plant, the other end of the tube being drawn out into a capillary from which the gas is delivered into an isolated bubbling-cup of distilled water.

For the determination of the photosynthetic activity it is, however, necessary to measure the absolute rate of evolution of oxygen and its modification under definite external variations. In order to eliminate personal error a device has, moreover, to be adopted for the automatic record of the rate of evolution of oxygen. I shall presently describe the Bubbler and the Electromagnetic Recorder by which these special requirements have been fulfilled.

[1] Wilmott—Assimilation of submerged plants in dilute solutions of bicarbonates and of acids; an improved bubble-counting technique—*Proc. Roy. Soc., B.*, vol. 92, 1921.

The experimental water-plant found most suitable is, as already stated, *Hydrilla verticillata* ; the advantages offered by the plant are : (1) That it survives the winter months and can therefore be used throughout the year ; the sensitiveness of this plant is, however, greater in spring than in winter. (2) It yields a considerable amount of oxygen even under a moderate intensity of light. (3) The slightly cuticularised epidermis allows ready access of water containing carbonic acid to the interior of the plant. (4) The large intercellular spaces allow easy escape of the bubbles of oxygen. (5) The cut end of the stem is not to any appreciable extent choked by mucilage ; and, finally, (6) there are no complicating stomata to modify the actual activity.

## The Plant-Vessel

This is a rectangular glass vessel having a capacity of about 200 c.c. (fig. 2). It is filled with water free from nitrogen, but containing the proper quantity of $CO_2$ in solution, which is about 8 mg. per 100 c.c. Inasmuch as the physiological condition of different plants can hardly be the same, it is necessary that the complete investigation be carried out with one and the same specimen of *Hydrilla* in normal condition. A length of 10 cm. is in general taken from the upper part of the plant bearing the most vigorously assimilating leaves. The inverted specimen is placed in the centre of the vessel and held in its place by the uppermost pair of leaves which press slightly against the neck. The vessel is placed inside a rectangular wooden box, not shown in the figure. This box has an internal lining of non-conducting felt ; the plant is thus protected from any sudden variation of temperature. There is a hinged door in front, the opening of which exposes the plant to light. For intermittent illumination a photographic shutter is substituted for the hinged door. Some advantage is secured by fixing a mirror behind the glass vessel, so that the direct and the reflected light produce a more or less uniform illumination of the plant.

## The Bubbler

I next describe the most important part of the apparatus, the Bubbler, by which successive quantities of gas of equal volume are given off. The Bubbler attached to the plant-vessel consists of a thick-walled tube with a relatively small bore; there is a stop-cock, S, by the manipulation of which the tube is put in communication with the atmosphere, or cut off from it. The thick tube has a lateral branch, B, which is shaped as shown in fig. 2. The end of this branch is blown into the form of a hollow cone. The junction between the cone and the B tube is closed with a drop of non-adhesive oil, O, which acts as a valve.

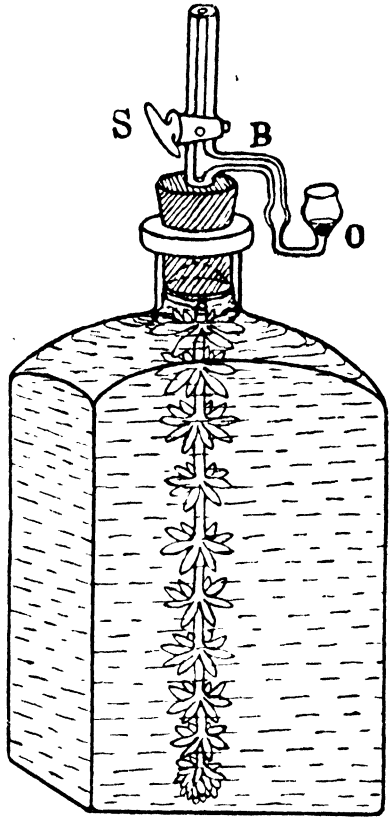



FIG. 2. The Plant-Vessel and the Bubbler

S, stop-cock; B, Bubbler; O, oil-valve.

One end of the Bubbler is thrust through the india-rubber cork closing the plant-vessel which is nearly filled with water. For final adjustment, the stop-cock, S, is opened and the cork pushed in till the level of the water reaches a definite mark in the Bubbler. During this process air is expelled into the atmosphere. The stop-cock is now closed, and we have a definite volume of air enclosed in the Bubbler under atmospheric pressure. On exposure to light, oxygen is evolved and an increase of pressure is produced; this lifts the oil-valve and the volume in excess, V,

escapes into the air; the pressure inside the Bubbler is then restored to the normal. *The successive bubbles in the Bubbler thus represent equal volumes of gas. The occurrence of the bubble is independent of any change in the frequency and the size of the bubblets given out at the cut end of the stem.* Each bubble given out under light is a definite volume of oxygen, indicating a corresponding amount of photosynthetic action. The rate of photosynthesis and its induced variations may therefore be measured by determining the successive periods of evolution of equal volumes of gas.

## Measurement of Photosynthetic Activity

Photosynthesis may be measured by the total volume of gas, NV, given out in a unit of time, where N represents the number of bubbles and V the constant volume of each bubble. In order to avoid fractions we take an hour as the unit of time. Since the volume of the bubbles is constant, *the photosynthetic activity is measured by the number of bubbles* N *given out in an hour.* We need not, however, wait an hour for this determination; for if $t$ be the interval in seconds between the successive bubbles, $N, = \frac{60 \times 60}{t}$, represents the activity.

## Determination of the Constant of the Bubbler

In carrying out investigations on the effect of change in any particular factor, all that is necessary is to determine the induced variation in the period between successive bubbles. For ordinary purposes it is not necessary to know the absolute volume of each bubble, though the constant of each Bubbler can be determined without any difficulty by forcing in a definite volume of air into the plant-vessel and counting the number of bubbles given out by the Bubbler. The sensitiveness of the Bubbler can be varied within wide limits, by an appropriate change in its capacity.

By reducing the capacity, I have been able to obtain record of the successive evolution of such small quantities of gas as 0·2 cubic mm. Bubblers for different requirements can, however, be constructed in which each bubble represents 0·2 to 5 cubic mm. I may give data of a particular experiment in which the successive bubbles under bright sky light were found to take place at intervals of twenty seconds; there were thus $3 \times 60 = 180$ bubbles per hour. In a parallel experiment the total quantity of oxygen collected in six hours from 10 A.M. to 4 P.M. was 1100 cubic mm. or 183 cub. mm. per hour. This corresponded to about the 180 bubbles of the first experiment. The volume of each bubble in the particular Bubbler was therefore 1 cubic mm., which was also found to be the calibrated value. The only precaution that need be taken to ensure accurate working of the Bubbler is to cover the bent tube with a thick piece of silk so as to maintain it at a uniform temperature, the silk having two small slits for the observation of the movement of the oil-valve. The precaution just mentioned is, however, unnecessary when the experiment is carried out in a room protected from draughts of air.

The evolution of oxygen in photosynthesis is continuous, but the formation of the bubblets under water is discontinuous; the intervals are longer the greater the depth of immersion. The gas-evolution could be made continuous by placing the cut end in air at the lower end of the Bubbler; but in that case the cut end would become dried up, thus obstructing the passage of the gas. The cut end is therefore placed about 2 mm. below the surface of water, so that the process of bubbling becomes almost continuous.

In the next chapter is described the special device by which the successive evolution of equal volumes of gas is automatically recorded.

## SUMMARY

The errors introduced into the method of bubble-counting by the spontaneous variation in the frequency and

volume of the bubblets are entirely removed by the employment of the Bubbler. This consists of a small U-tube closed at one end by an oil-valve. The oxygen evolved by the plant, entering the U-tube, produces an increase of pressure, and a definite volume of gas escapes by the lifting of the oil-valve.

The successive bubbles given out by the Bubbler indicate the photosynthetic production of equal volumes of gas, and are independent of any irregularity in the evolution of bubblets at the cut end of the plant.

The capacity of the Bubbler may be determined by forcing in a known volume of gas into the plant-vessel and counting the number of bubbles. The sensitiveness of the Bubbler may be varied within wide limits from 0·2 to 5 cubic mm. per bubble.

The absolute rate of photosynthetic evolution is found by multiplying the constant of the Bubbler by the number of bubbles per unit time.

## CHAPTER IV

### THE AUTOMATIC RECORD OF THE RATE OF EVOLUTION OF OXYGEN

The Automatic Recorder—Electric Pencil with double contact—The Electromagnetic Writer—Condenser for elimination of sparks—Recording Drum—The Governor for variation of speed within wide limits—The Ink-Recorder—Maintenance of constancy of external conditions—Radiant point as a source of light—The experimental procedure—Constancy of results—Advantages of the Automatic Method.

In order to eliminate the error due to the personal equation, which necessarily attends the eye-observation of the Bubbler in action, it is essential to devise a method by which the periods of evolution of equal volumes of oxygen may be automatically recorded. This I have been able to secure by the following electromagnetic apparatus and accessories, which consist of:

(1) A Pencil with double electric contacts which complete an electric circuit for each bubble.

(2) An Electromagnetic Writer for inscription of the record of successive periods on smoked or white paper.

(3) A Condenser for elimination of sparks at break of the circuit.

(4) A Recording Drum.

(5) A Governor by which the speed of the Drum may be adjusted within wide limits.

*The Electric Pencil.*—The successive bubbles of oxygen move the oil-valve up and down through a short length. A drop of mercury is placed on the oil-valve, and the constriction at the end of the Bubbler is made so narrow that the mercury-drop always remains above the bend and does not sink into the oil forming the valve. The evolved

gas pushes up the oil and the drop of mercury with it through a short length at each bubbling. The movement of the mercury, though slight, is sufficient to complete an electric circuit through the pencil provided with double contact. The pencil consists of a conical thin rod of ebonite, on opposite sides of which are sunk two platinum wires which project slightly beyond the pointed end. The pencil is carried by a clamp, which is fixed to the neck of the plant-vessel. Before exposure to light, the platinum points are so adjusted by the micrometer screw, A, that the circuit is just completed through the drop of mercury, and then the electromagnetic writer strikes against the drum. A slight turn of the micrometer screw now lifts the pencil, breaking the circuit, and the writer is released. By this means a very sensitive adjustment is made, such that the movement of the mercury-drop at bubbling completes the circuit with the utmost certainty and the record of successive bubbles takes place without any possibility of failure (fig. 3).

*The Electromagnetic Writer.*—A small horseshoe electromagnet attracts the armature to which the writer is attached, the stroke of which makes a mark on the moving piece of smoked paper. The armature is polarised, so that the feeble current given by a dry cell is sufficient to ensure record.

*The Condenser.*—A serious difficulty arises in prolonged records from the oxidation of the mercury caused by the spark occurring at the break of the electromagnetic circuit. The scum thus formed causes an uncertainty in the contact. This difficulty is, however, obviated by the use of a condenser, C, connected in parallel with the two platinum wires by which the circuit is alternately made or broken. Sparking is thus eliminated and no uncertainty in the record can exist.

*The Recording Drum.*—An experiment by this method may, in a large number of cases, be completed in the course of about ten minutes ; in special cases, such as in researches

on the diurnal variation of photosynthesis, the experiment has to be continued for about ten hours. We have thus to make provision for records of either short or long duration. For immediate detection of the effect of physiological variation it is desirable that the drum should revolve at a quick speed. The distance between the two successive dots representing the period of bubbling is then comparatively

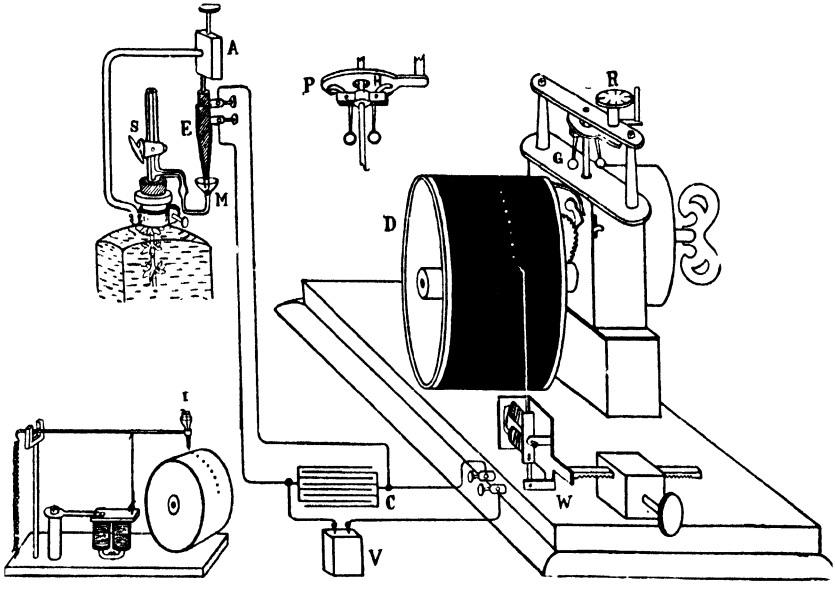



FIG. 3. The Automatic Recorder for Photosynthesis

S, bubbler with stop-cock; E, the electric pencil for completing electric contact through drop of mercury, M; A, adjusting screw; V, voltaic cell; C, condenser; D, revolving drum; W, electromagnetic writer; G, governor, shown separately at P with pair of hinged levers, H; I, ink-recorder.

large, and any induced change in the rate of bubbling is strikingly manifested by the shortening or lengthening of the distance between the successive dots. For long-continued experiments, on the other hand, the speed of the drum has to be correspondingly reduced. The drums ordinarily employed have the following drawbacks:

(1) Each particular drum has a speed of revolution which can only be varied within narrow limits. A number

of different drums have therefore to be available for records of various duration.

(2) The speed is liable to be slowed down by the unwinding of the coiled spring of the clock-work.

For our present purpose we require a single drum, the speed of which can be varied so as to enable us to obtain record for as short a time as ten minutes, or as long a period as ten hours. It is also necessary that the speed should be maintained constant for a long time. These requirements have been secured by the particular governor which will now be described.

*The Governor.*—I have been able to devise a perfect type of frictional governor which consists of a pair of hinged levers, carrying small spherical weights at the lower ends; the upper end of each lever is hook-shaped. By a contrivance to be presently described, the speed of the drum is regulated by causing increasing divergence between the levers which carry the weights. The speed is at its highest when the hinged arms are spread out nearly horizontally; it is slowest when the arms are parallel and vertical. The angle of divergence can be gradually increased or decreased by means of a plate of metal coated with plumbago, which presses against the hooked inner arms of the levers, H. The revolving contact of the governor against the adjusting plate takes place at practically two points. The plate itself can be moved up or down by a micrometer screw: hence the angle of divergence of the arms of the governor may be adjusted by turning the micrometer screw in one direction or the opposite. For every reading of the micrometer screw (which has a circular scale) the speed of rotation of the drum has a definite value, which remains unchanged. It is thus easy to obtain a variation in the speed of from one to ten times, which is *continuous*.

Readings were taken of the successive strokes of a light hammer actuated by one of the revolving wheels in the clock-work. The constancy of the speed of rotation for long periods is shown by the fact that at a certain adjustment

the successive strokes occurred at intervals of thirty-five seconds; at the end of twelve hours the frequency was found to be unchanged. After twenty-four hours the coiled spring was nearly unwound; even in this extreme case the interval between successive strokes was found lengthened from 35 to 35·5 seconds, *i.e.* a variation of only 1·1 per cent.

The maximum variation of the speed of the drum is, as already stated, about ten times. The circumference of the drum is 30 cm.; when the drum revolves once in six minutes, the rate of movement of the recording surface is 50 mm. per minute; the slowest speed is one revolution in sixty minutes, the movement of the recording paper being now 5 mm. per minute. We can easily obtain any intermediate speed between these two extremes.

The Electromagnetic Writer is mounted on a sliding tube, and after the completion of the record, during one complete revolution of the drum, it can be moved through one cm. to make a new record. It is thus possible to obtain a continuous record for ten hours, as in the case of an investigation on the diurnal variation of photosynthesis.

*Ink-Record.*—The record on a drum is usually obtained by inscription on a piece of smoked paper. But coating the paper with smoke and the subsequent fixation of the record entail much trouble. I have simplified the process by arranging for a direct record with ink on white paper. A lever carries at one end a small spherical vessel, with a narrow tube filled with aniline ink; the tube is nearly closed by a thin wire which projects slightly beyond the tube, so that the ink is prevented from dripping: this is the pen (fig. 3, 1). The armature of the electromagnet is attached to the arm of the lever which carries the pen; the other arm is kept down by a light spring, so adjusted that the point of the pen is held 2 mm. above the recording-surface. The completion of the electric circuit on the escape of each bubble causes the pen to come into contact with and to make an ink-mark on the recording surface.

A full description has now been given of the method which enables us to obtain automatic record of the rate of evolution of equal volumes of oxygen. This rate affords a direct measure of the photosynthetic activity. In the study of induced variation of photosynthesis in a particular plant (the physiological condition of which is constant) by change of one factor at a time, it is not necessary, as already stated, to determine the absolute but the relative variation. The experiments may be completed in the course of a few minutes, during which all the factors but one can be kept constant. The intensity of light can be maintained uniform by devices which will be presently described; maintenance of constant temperature offers no special difficulty; the $CO_2$-content of the water is also constant at the concentration of about 8 mg. per 100 c.c.

Among these factors, the securing of a constant intensity of light is a problem of considerable difficulty. The ideal requirements are: (1) that the radiant should be a point; and (2) that the light should be cold, not containing a large proportion of heat-rays.

When the luminous source is a point, it is easy, by means of a lens, to obtain either a parallel or a divergent beam of light. This is impossible when the source of light is a luminous surface, such as a gas-mantle or the large spiral of an incandescent electric bulb. The condition of the radiant being a point is specially necessary in investigations on the effects of different intensities of light; for the varying intensities can in this case be accurately determined by measuring the distance from the radiant, the intensity varying inversely as the square of the distance from the source of light. The arc-lamp, though it gives a strong light from a small source, has the drawback that the intensity cannot be maintained absolutely constant; moreover it gives out a large quantity of heat. The production of heat either by an incandescent mantle or by an arc-lamp introduces various complications; for the temperature of the experimental room itself is continuously

raised, and the heat absorbed by the plant raises its temperature to an unknown degree.

The condition of a satisfactory light of uniform intensity is amply fulfilled by the ' Pointolite ' lamp, in which the source may be regarded as practically a point ; the intensity of light under the same voltage is constant day after day. The light is ' cold,' and the small proportion of heat-rays may be completely absorbed by passage through a thick stratum of alum-solution. When the light falls on the water in the plant-vessel, it produces no variation of temperature, as tested by a thermometer placed in the vessel. The only precaution which need be taken in the use of Pointolite is to place the resistance-coil which regulates the current outside the experimental room, so that the heat produced may not cause any variation of temperature of the room.

It is especially necessary to study the effect of light from the sky on photosynthesis, as the plant is subjected in nature to this light. In employing this light, however, we have (1) to determine the period of the day when it is most constant, and (2) to have a sensitive photometer for testing its uniformity during the period of the experiment. An Electric Photometer has been devised for this purpose which will be fully described in a subsequent chapter.

We may next refer briefly to the favourable physiological condition of the plant on which the constancy of all modes of response depends. I have shown in my previous works that a vigorous plant exhibits uniform responses, both mechanical and electrical, for a considerable length of time. Plants in a depressed condition exhibit, on the other hand, a feeble or irregular response. A plant may be in a sluggish condition at the beginning, in which case stimulus renders it more active ; but prolonged and excessive stimulation causes fatigue and depression of response.

I have thought it necessary to enter into some detail as to the preliminary adjustments for securing quantitative accuracy in the results. It takes some little time to master

these essential details ; but the saving of time and labour in the experiment, the elimination of complicating factors for correction, the directness and accuracy of the results, are features which cannot even be approached by any other method. This will be quite evident from a typical experiment described below, which also explains the method of procedure.

A vigorous specimen of *Hydrilla* was mounted in the bubbling apparatus with the Electromagnetic Recorder.

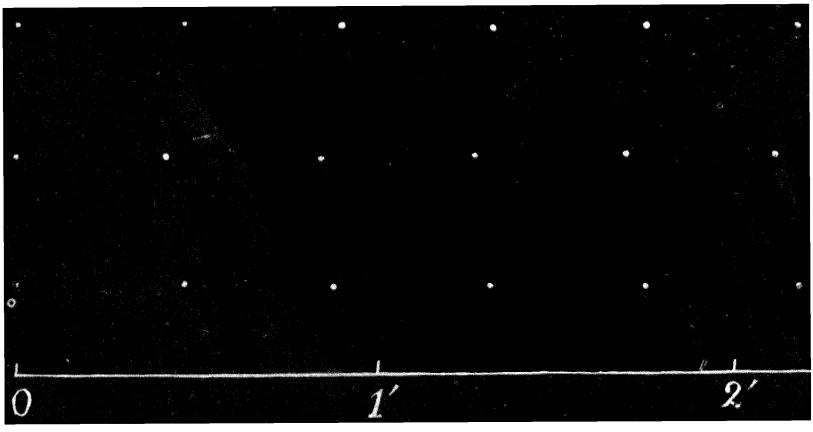



FIG. 4. Records of successive evolution of equal volumes of Oxygen under Constant Light

The three records were obtained at the commencement, after twenty minutes and after forty minutes.

The incident light on the plant was constant, the source being a Pointolite of 100 candle power. The beam was rendered parallel by means of a lens. Records of successive bubbles (of equal volume) were taken continuously for an hour, of which portions at the beginning, after twenty minutes and after forty minutes, are reproduced in fig. 4 ; the responses are seen to be practically uniform throughout the hour. Independent observations were also taken of the periods of successive bubbles, with the help of a metronome beating seconds. Table III. gives the detailed values of the successive periods.

This uniformity in the rate of bubbling for an hour shows how possible it is to maintain the external conditions constant during that period. We find that the interval between any two successive bubbles is practically the same throughout; the mean period of five successive bubbles would, however, be found to be still more accurate. Hence three minutes' observation is enough for determination of the normal period. Should we wish to determine the

TABLE III.—GIVING BUBBLING-PERIODS AT THE BEGINNING, AFTER TWENTY MINUTES AND AFTER FORTY MINUTES

| At commencement | After 20 minutes | After 40 minutes |
|---|---|---|
| 27 seconds | 26 seconds | 27 seconds |
| 25 ,, | 25 ,, | 27 ,, |
| 25 ,, | 25 ,, | 26 ,, |
| 25 ,, | 26 ,, | 27 ,, |
| 27 ,, | 25 ,, | 27 ,, |
| 26 ,, | 25 ,, | 26 ,, |
| 26 ,, | 26 ,, | 26 ,, |
| 26 ,, | 26 ,, | 26 ,, |
| 27 ,, | 26 ,, | 26 ,, |
| 27 ,, | 26 ,, | 26 ,, |
| 27 ,, | 26 ,, | 27 ,, |
| 27 ,, | 26 ,, | 27 ,, |
| Mean 26·5 seconds | Mean 25·7 seconds | Mean 26·5 seconds |

effect of an external change, such as that of intensity of light, we allow two minutes for adjustment to the new condition and then determine the new rate that has been induced. The first observation takes three minutes, the second and subsequent observations five minutes each. About seven different observations would be sufficient to afford data for a complete photosynthetic curve. The whole investigation can be completed in the course of about forty minutes, during which the other factors are maintained quite uniform. The time for obtaining a complete curve for a single plant is so short that more than half a dozen determinations with different plants can be easily made in the course of a single day.

The accuracy of the results thus obtained is of a high order, as will be found in the various determinations given in the following chapters.

## SUMMARY

The record of successive bubbles of oxygen is obtained by an electric device which actuates the Electromagnetic Writer to inscribe successive dots on a revolving drum, the speed of which may be adjusted within wide limits.

The advantages secured by the new method are:

(1) Numerous corrections are rendered unnecessary, since the external conditions are maintained constant during the relatively short period of the experiment.

(2) Being automatic, all personal errors are eliminated.

(3) Shortening the period of the experiment also eliminates the effect of physiological depression brought on by prolonged stimulation under light.

(4) Finally, the photosynthetic activity is measured *directly* from the rate of evolution of oxygen, thus discarding the lengthy and laborious process of chemical analysis.

# CHAPTER V

## PHOTOSYNTHESIS UNDER INCREASING INTENSITY OF LIGHT

The unit intensity of light—Photosynthetic curve under increasing intensity of light from Pointolite—Period for physiological adjustment to changed intensity of light—Experiments with spring- and winter-specimens—Formula for determination of photosynthetic activity under changed intensity of light—Determination of the coefficient by the Differential Method—Ratio of activities in spring and winter.

As a test of the simplicity and high accuracy of the new method, I will explain its working in the determination of the effect of increasing intensity of light on photosynthesis. We have to determine, among other things, the characteristics of the photosynthetic curve, the coefficient for light, the comparative effectiveness of artificial light and sunlight, and the relation between the quantity of light and the amount of photosynthesis.

The essential conditions which must be secured for this investigation are : (1) that the intensity of light should remain constant during the period of the experiment, and (2) that it should be capable of definite and known variation. The light from the sky does not remain constant, nor can it be varied in a definite manner. The choice of light is therefore restricted to artificial light or to sunlight. I have already explained how the light given out by gas-mantles is most unsuitable for quantitative measurements. I therefore make use of Pointolite, the advantage of which has already been referred to. It does not produce any variation of temperature, and the intensity of light can be easily varied in a quantitative manner. The effect of sunlight will be described in a subsequent chapter.

*The unit intensity of light.*—In photometry there is,

unfortunately, no universally accepted standard of illumination. The Geneva Conference, 1911, recommended *lux* as the standard, which is the illumination produced by a standard candle at a distance of 1 metre. In the following experiments I generally employed light increasing from about 100 to 5000 lux. The unit degree of variation of light may be conveniently taken as 100 lux.

*Adjustment for variation of intensity.*—A lens placed in

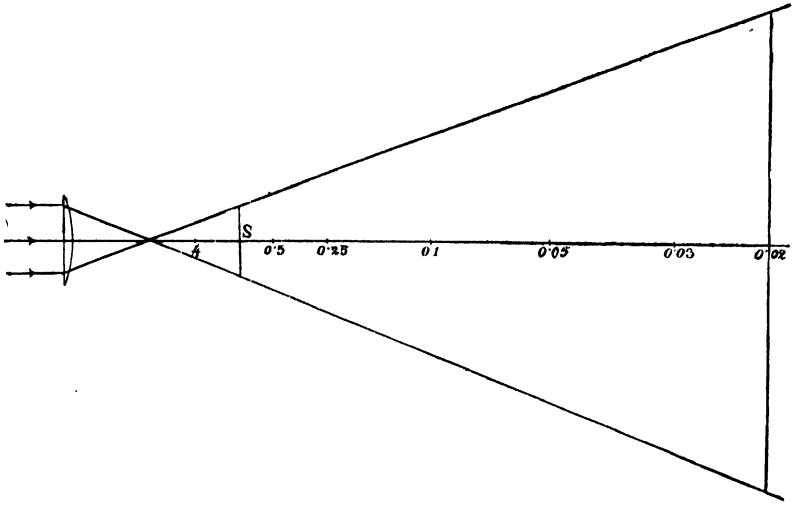



FIG. 5. Diagrammatic Representation of Arrangement for securing different intensities of Light

Parallel beam of sunlight rendered divergent by a convex lens seen to the left. Different intensities in terms of sunlight marked on the table. Light from Pointolite lamp is similarly made divergent by a lens.

front of a 100 c.p. Pointolite produces a slightly divergent beam. Since the radiant is a point, the intensity of light varies inversely as the square of the distance. The positions for intensities from 100 to 5000 lux are marked on a scale fixed on the table. These values were independently verified by a grease-spot photometer and a standard candle.

The sunlight reflected by a heliostat was similarly rendered divergent by means of a lens (fig. 5). At S, the area of the beam is the same as that of the circular opening

in the shutter ; the intensity at S is therefore taken as that of normal sunlight. The various intensities at different positions of the scale are marked in terms of S, which is the intensity of sunlight in the town during unclouded days from November to March. Sunlight increases from morning and attains its maximum at noon : in the afternoon it undergoes rapid diminution. It is necessary to state that the intensity of sunlight in a town is not the same as that in the open country or in the hills. The atmosphere, on account of floating dust particles and slight haze due to diffuse smoke, is not absolutely clear. The sunlight becomes relatively more intense immediately following a rainy day. The sunlight employed will be described in general terms as bright, moderate or dull.

For investigations on the effect of variation of intensity of light, Pointolite offers special advantages. The light emitted by this lamp is perfectly constant ; the value of intensity in lux at any distance from the source of light can, moreover, be easily determined. The effect of varying intensity of sunlight will be described in the next chapter.

In the experiments with Pointolite, the plant-vessel with the Bubbler was moved gradually nearer the light, and the activities at different intensities, say from 100 to 3000 lux, were measured.

*The period for physiological adjustment to changed intensity of light.*—When the plant is subjected to increasing intensity of light, a certain period must elapse before photosynthesis attains a steady value under the increased intensity. The following experiment was undertaken to determine the minimum period for the physiological readjustment, the successive evolution of equal volumes of gas being automatically inscribed by the Electromagnetic Recorder. The plant was first subjected to an intensity of 750 lux (7·5 units), and the bubbling-period was found to be practically uniform at intervals of 60″, 61″, 62″ and 60″. The plant-vessel was then quickly brought nearer the source of light, the intensity being 1000 lux ; the automatic record

being uninterrupted, the successive bubbles then occurred at intervals of 53″, 47″, 47″, 47″, physiological adjustment to steady condition being attained in the course of 53 seconds. When the intensity was increased to 1500 lux, the successive bubbles appeared at intervals of 30″, 30″, 30″, 31″. Here the readjustment was attained very quickly (fig. 6). It is seen that the period of adjustment does not take more than 53 seconds, and an allowance of 1 minute is therefore more than sufficient. As the steady rate subsequently attained is practically uniform, it is only necessary to count the number of bubbles, N, for, say, four minutes. The activity in c.mm. per hour is then :

$$N \times \frac{60}{4} \times \text{the constant of the Bubbler.}$$

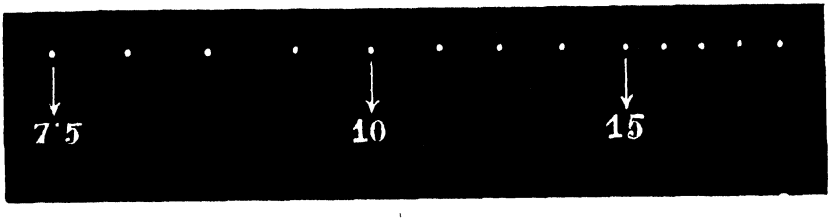



FIG. 6. Records showing the periods of physiological adjustm( under rapid change from 750 to 1000, and then to 1500 lux (7·5, 10 and 15 units)

The rate became uniform at 1000 lux in the course of 53″; at 1500 lux the adjustment was practically immediate.

The period for each individual observation is therefore five minutes only. Eight different observations at different intensities of light are sufficient for the construction of the photosynthetic curve, the total duration of the experiment being thus less than an hour.

## Photosynthetic Curve under Increasing Intensity of Light

A very important element affecting the photosynthetic activity is the tonic factor depending on the season. I will describe the relative photosynthetic activities of the spring- and winter-specimens obtained from the same pond, the

spring-specimens at the middle of February and the winter-specimens at various times from December to January. The results given by a batch of spring-specimens showed remarkable agreement with each other; the same was the case with the winter-specimens. The average activity of *Hydrilla* in spring was higher than in winter, the ratio between the two being more or less definite. About twenty-four different investigations were carried out, half of them in winter and the other half in spring.

## Experiments with Spring-Specimens

Observations were made of the rates of evolution of

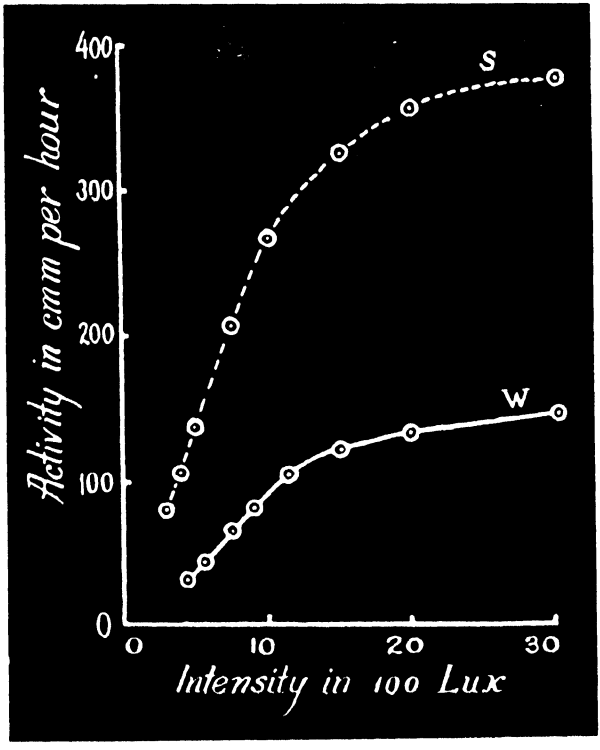



FIG. 7. Photosynthetic Curves under Increasing Intensity of Light, for Spring-specimen s, and for Winter-specimen w

oxygen at various intensities of light from 300 to 3000 lux. Gas began to be evolved at a much lower intensity, but the

rate was very slow. At 300 lux the rate was 77·1 cubic mm. per hour. This increased continuously with increased intensity of light till at 1000 lux the rate was 265·6. The curve is straight from 300 to 1200 lux, after which it tends to become horizontal, the turning-point being slightly above 1200 lux (fig. 7). In summer, when the temperature is relatively high, the curve exhibits a reversal above an intensity of 1500 lux or so. Very strong light thus induces an actual depression in photosynthesis. The general formula for the upper curve in fig. 7 is :

$$y = 3 \cdot 13x - 0 \cdot 08x^2 - 0 \cdot 0002x^3.$$

I give below detailed results of three typical experiments carried out in spring.

TABLE V.—VARIATION OF ACTIVITY UNDER INCREASING INTENSITY OF LIGHT FROM POINTOLITE. (SPRING-SPECIMENS)

| Specimens | Intensity in lux | Activity in c.mm. O per hour |
|---|---|---|
| I. | 300 | 77·1 |
| | 400 | 102·8 |
| | 500 | 131·4 |
| | 750 | 200·2 |
| | 1000 | 265·6 |
| | 1500 | 324·9 |
| | 2000 | 355·5 |
| | 3000 | 375·7 |
| II. | 100 | 16·0 |
| | 150 | 23·5 |
| | 200 | 37·4 |
| | 300 | 62·4 |
| | 500 | 110·2 |
| | 750 | 173·4 |
| | 1000 | 234·8 |
| | 1500 | 300·0 |
| | 2000 | 337·5 |
| III. | 300 | 76·5 |
| | 400 | 102·8 |
| | 500 | 125·6 |
| | 750 | 184·0 |
| | 1000 | 240·0 |
| | 2000 | 313·0 |
| | 3000 | 372·4 |

## Experiments with Winter-Specimens

The results obtained with winter-specimens are given below in Table VI.

TABLE VI.—PHOTOSYNTHETIC ACTIVITY UNDER INCREASING INTENSITY OF POINTOLITE. (WINTER-SPECIMENS)

| Specimens | Intensity of light in lux | Activity in c.mm. O per hour |
|---|---|---|
| IV. | 450 | 29·5 |
| | 562 | 39·9 |
| | 750 | 64·5 |
| | 900 | 78·7 |
| | 1125 | 102·0 |
| | 1500 | 120·6 |
| | 2000 | 133·0 |
| | 3000 | 142·5 |
| V. | 500 | 41 |
| | 750 | 74 |
| | 1000 | 109 |
| | 1500 | 143 |
| | 2000 | 151 |
| | 2500 | 154 |

For purposes of comparison, two curves are given in the same figure (fig. 7), one for a spring-specimen (I.) and the other for a winter-specimen (IV.). The curve for the spring-specimen, s, is the more erect, indicating greater sensitiveness. The effect of season in modifying activity is further shown by the different minimal effective intensities of light. In spring, photosynthesis occurred at an intensity lower than 300 lux, but in winter the minimally effective light was 450 lux or even much higher. The two curves otherwise bear a general resemblance with each other. In both we notice a turning-point, beyond which the curves tend to become horizontal. The middle part of the curve, from about 100 to 1200 lux, is practically straight ; and a method will be presently described for the determination of the coefficient in this middle range.

*Formula for determination of activity for a given intensity*

*of light.*—The photosynthetic activity increases, up to the turning-point, with the intensity of light. The formula given below gives the approximate value of activity $A_L$ at a higher intensity of light L, from the activity $A_l$ at the lower intensity *l*. A more exact formula will be given later.

$$A = A_l \times \frac{L}{l} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

*Examples :*

Specimen I.

Activity at 500 lux = 131·4 c.mm. per hour.

Calculated value for 1000 ,, $= 131{\cdot}4 \times \frac{1000}{500} = 262{\cdot}8$

Observed activity . . . . = 265·6

Specimen III.

Activity at 750 lux = 184·0

Calculated value for 1000 ,, $= 184{\cdot}0 \times \frac{1000}{750} = 245{\cdot}3$

Observed activity . . . . = 240·0

Specimen IV.

Activity at 750 lux = 64·5

Calculated value for 1125 ,, $= 64{\cdot}5 \times \frac{1125}{750} = 96{\cdot}5$

Observed activity . . . . = 102·0

For the determination of the coefficient for light I employ two independent methods—the *Direct,* in which the measurements are made on the physiological scale (Chap. XXVI.), and, secondly, the *Differential Method.* It may be stated here that the coefficients obtained by these two independent methods show a very close agreement with each other.

## Determination of the Coefficient by the Differential Method

We found that the photosynthetic curve is practically straight in the middle range. The coefficient for light is

the ratio of the increase in photosynthetic activity to the increment of light which induces it. If $A_L$ be the activity for intensity of light L, and $A_l$ that for intensity $l$; then the coefficient

$$K = \frac{A_L - A_l}{L - l} \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The unit of light, it should be remembered, is 100 lux.

The following are the determinations made for the spring- and winter-specimens :

*Spring-specimens :*

I. Activity for 300 lux = 77·1 c.mm. per hour
,, 1000 ,, = 265·6 ,, ,,
Increase in activity = 188·5
Increase in intensity of light = 700 lux

$$K = \frac{188 \cdot 5}{10 - 3} = 26 \cdot 9$$

II. (*a*) Activity for 300 lux = 62·4
,, 750 ,, = 173·4

$$K = \frac{173 \cdot 4 - 62 \cdot 4}{7 \cdot 5 - 3} = 24 \cdot 7$$

(*b*) Activity for 500 lux = 110·2
,, 1000 ,, = 234·8

$$K = \frac{234 \cdot 8 - 110 \cdot 2}{10 - 5} = 24 \cdot 9$$

III. Activity for 500 lux = 125·6
,, 1000 ,, = 240·0

$$K = \frac{240 \cdot 0 - 125 \cdot 6}{10 - 5} = 22 \cdot 9$$

*Winter-specimens :*

IV. Activity for 450 lux = 29·5
,, 1125 ,, = 102·0

$$K = \frac{102 \cdot 0 - 29 \cdot 5}{11 \cdot 25 - 4 \cdot 5} = 10 \cdot 8$$

V. Activity at 500 lux = 41·0
„ 750 „ = 74·0

$$K = \frac{74 - 41}{7{\cdot}5 - 5} = 13{\cdot}2$$

The data given above show the accuracy with which the coefficient may be determined from experimental results. Another remarkable feature is that the values of the coefficient obtained from different specimens in the same season are nearly the same.

*The ratio of activities in spring and winter.*—The coefficient for spring-specimens is about 24·9; that for winter in the majority of specimens is 13·2. Occasionally it may be as low as 10·8. The ratio of activities in spring and winter may therefore be taken as 24·9 to 13·2, or as 1·8 : 1.

## Summary

Photosynthesis is slight under feeble intensity of light. The activity then increases at a uniform rate under increasing intensity, the curve being straight with a uniform slope. The turning-point occurs at about 1200 lux, after which the curve tends to become horizontal.

Photosynthetic activity is found to vary with the seasons. The minimal intensity effective in spring may be as low as 100 lux, whereas in winter it is about 500 lux or more. The photosynthetic curve in spring is relatively the more erect.

The approximate formula for determination of enhanced activity under stronger light is

$$A_L = A_l \times \frac{L}{l}$$

The coefficient for light, $K = \frac{A_L - A_l}{L - l}$

and $$A_L = A_l + K\,(L - l)$$

The mean coefficient for spring-specimens is 24·9. In winter-specimens it varies from about 11 to 13.

The ratio of activities in spring and in winter may be taken as about 1·8 : 1.

# CHAPTER VI

## RELATION BETWEEN THE QUANTITY OF LIGHT AND THE AMOUNT OF PHOTOSYNTHESIS

Comparison of photosynthetic effectiveness of sunlight and Pointolite—The limiting maximum, relative and not absolute—Dependence of the maximum on intensity of stimulus—Physiological equivalence of Pointolite and sunlight—The *Hydrilla* plant as a photometer—Effect of duration, intensity and directive angle of light on activity—Relation between the quantity of light and activity.

I WILL in the present chapter deal with the important question of the relation between the quantity of light and the activity of photosynthesis as estimated by the volume of oxygen evolved. Before doing this it is necessary to equate the relative effects induced by different sources of light, Pointolite and sunlight for example. Is a given intensity of sunlight photosynthetically equivalent to a particular intensity (in lux) produced by Pointolite ? The Pointolite is brilliantly white and its spectral components are not very different from those of sunlight ; the latter contains perhaps a larger proportion of the more refrangible rays. But this difference would not produce any great variation, since the more refrangible rays are found to be relatively ineffective in photosynthesis.

### Comparison of Photosynthetic Effectiveness of Sunlight and Pointolite

The experiment was carried out with *one and the same specimen,* placed in water containing 8 mg. $CO_2$ per 100 c.c. Observations of photosynthetic activity were made first with Pointolite at intensities varying from 400 to 3000 lux. After a suitable period of rest, the plant was subjected to

sunlight of increasing intensity, as previously explained, from 0·04 S to 0·40 S. It should be mentioned here that the day was dull and the sunlight comparatively feeble; as far as could be judged by the eye, it was about a quarter the intensity of bright sunlight at midday. I give the results in the following table:

TABLE VII.—PHOTOSYNTHESIS UNDER POINTOLITE AND SUNLIGHT

($CO_2$-concentration, 8 mg. per 100 c.c.)

| Pointolite | | Sunlight | |
|---|---|---|---|
| Intensity in lux | Activity in c.mm. O per hour | Intensity in S | Activity in c.mm. O per hour |
| 400 | 95·7 | 0·04 | 106·0 |
| 500 | 128·0 | 0·06 | 167·0 |
| 750 | 192·0 | 0·12 | 337·5 |
| 1000 | 257·0 | 0·20 | 509·0 |
| 1500 | 318·0 | | |
| 2000 | 348·0 | 0·30 | 606·0 |
| 3000 | 369·1 | 0·40 | 645·0 |

We note from the above data that while under Pointolite of 1500 lux the photosynthesis was 318, under 0·12 S it was

TABLE VIII.—PHOTOSYNTHESIS UNDER INCREASING INTENSITIES OF POINTOLITE AND SUNLIGHT

($CO_2$-concentration, 3 mg. per 100 c.c.)

| Pointolite | | Sunlight | |
|---|---|---|---|
| Intensity in lux | Activity in c.mm. O per hour | Intensity in S | Activity in c.mm. O per hour |
| 500 | 90·6 | 0·07 | 135·75 |
| 750 | 135·0 | 0·09 | 180·1 |
| 1000 | 185·4 | 0·10 | 218·0 |
| 1500 | 263·4 | 0·15 | 315·2 |
| 2000 | 294·3 | 0·20 | 410·1 |
| 3000 | 322·8 | 0·3 | 500·1 |
| 4000 | 341·6 | 0·5 | 611·6 |

337·5. By interpolation we find that 0·12 of sunlight, on this particular occasion, was physiologically equivalent to about 1750 lux.

Again, the maximum photosynthesis of 369·1 was practically attained under 3000 lux, whereas the same specimen gave a higher maximum of 645 under the intensity of 0·40 S.

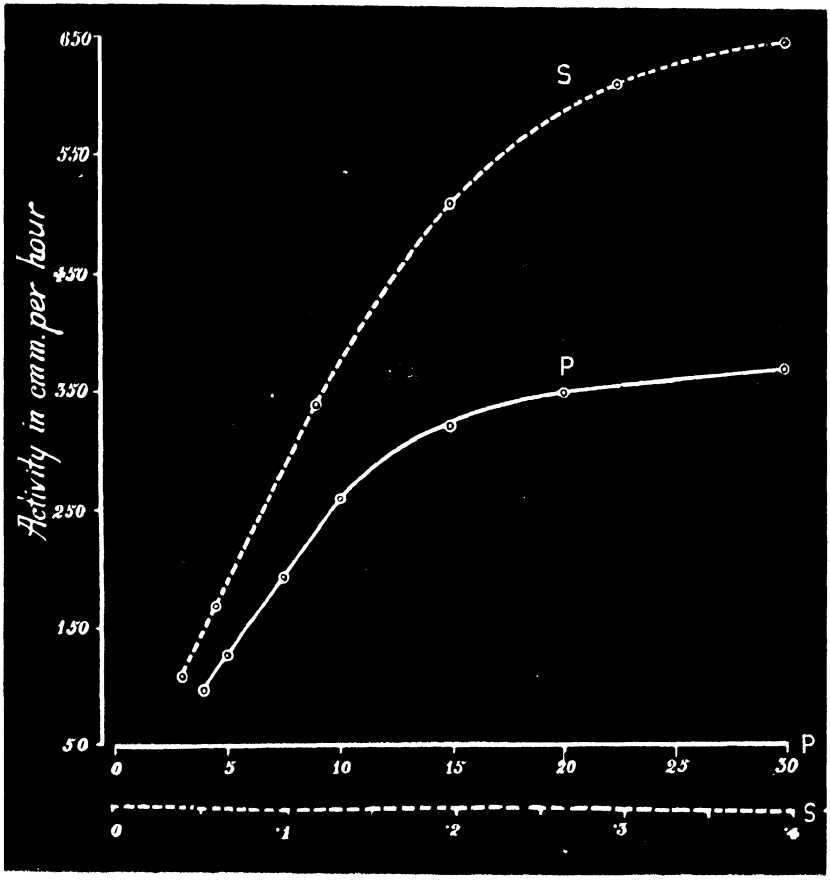



FIG. 8. Photosynthetic Curves for identical *Hydrilla* plant under Sunlight (S) and Pointolite (P)

*The limiting maximum is therefore not absolute but relative, and depends on the intensity of the light.*

It may be urged that the $CO_2$-content was in excess of what could be fully assimilated under the feebler intensity of Pointolite; and that therefore the maximum under the stronger sunlight was greater. The fact that the stronger sunlight produces a relatively higher maximum, independ-

ently of $CO_2$-concentration, was demonstrated in experiments, made with a different plant, in which the $CO_2$-concentration employed was being only 3 mg. per 100 c.c.: the results under increasing intensities of Pointolite and sunlight are given in Table VIII.

These results are practically the same as those with a $CO_2$-concentration of 8 mg. per 100 c.c. Here also, as in that case, the maximum under strong sunlight, 0·5 S, was nearly double that under 4000 lux of Pointolite. This will be seen clearly by comparison of the photosynthetic curves, S and P, under the two lights (fig. 8). The effect of 0·12 S is found by interpolation to be 257, which is about the same as the effect of 1500 lux. On the previous day, with a different specimen (and probably with some difference in the intensity of sunlight), 0·12 S was found to be physiologically equivalent to 1750 lux. The results on the two days are seen to be of the same order.

*Dependence of maximum response on the intensity of stimulus.*—The above results show that the maximum response is relative, and that it increases with the intensity of stimulus. The absolute limit is only reached when the intensity of stimulus is so great as to cause injury and the resulting physiological depression. This will be independently demonstrated by results obtained with other modes of response (*cf.* fig. 10).

## The *Hydrilla* Plant as a Photometer

The results already described show that there is a physiological equivalence between sunlight and Pointolite. An interesting question arises whether the photosynthetic activity of *Hydrilla* could be employed in the measurement of the intensity of the incident sunlight. The following experiment on the subject was carried out at the end of March at 11 A.M., the sunlight being comparatively bright. The light was reflected into the experimental room by a heliostat and rendered divergent by a double convex lens, the various

intensities at different positions on the table being marked in the usual manner. There was a certain amount of loss of light by scattered reflection from the heliostatic mirror, and also by reflection from the surface and absorption in transmission through the lens. For the exact determination of this loss I employed the Electric Photometer, which was at first exposed to light at the point marked S inside the room, when the photometric deflection was found to be 77 divisions. The photometer was next taken outside and pointed directly to the sun, the deflection being now 118 divisions. This showed that nearly one-third of the light was lost in reflection and absorption, the intensity of the light S inside the room being two-thirds of that of direct sunlight.

The physiological and physical measurements of sunlight were carried out as follows. Sunlight entered the room from the right side, while a divergent beam from Pointolite (with the various intensities in lux marked on the table) came from the left. A specimen of *Hydrilla*, mounted in its vessel with the Bubbler, was placed between the divergent beams. The specimen was first exposed to Pointolite, the sunlight being shaded. The experiment was repeated with sunlight, this time shading the Pointolite. The plant was moved in one direction or the other till the two photosynthetic activities under Pointolite and under sunlight were found to be the same, *i.e.* 296 c.mm. per hour. The effect of 1700 lux was found to be the same as that of 0·04 S, the two intensities being physiologically equivalent to each other.

A grease-point photometer was next employed for an independent comparison of the two lights; the intensity of 1700 lux was found to be exactly equal to 0·04 S. The *Hydrilla* is thus found to be a reliable indicator of the intensity of light. For the most sensitive condition of balance, the intensity of light should be chosen to be at or near the turning-point of 1200 lux.

Reference to results obtained in January (Table VII.) shows that while on a dull day in January 0·12 S was physio-

logically equivalent to about 1750 lux, 0·04 S on a bright day in March was both physically and physiologically the same as 1700 lux. The intensity of sunlight on a bright day in March is therefore three times as great as that on a dull day in January.

I now go on to describe experiments on the important problem of the relation between the quantity of incident light and the activity of photosynthesis as determined by the total volume of oxygen evolved. In order to solve this problem we have to find the effect of (1) the duration, (2) the intensity, and (3) the *directive angle*, of the incident light ; by this last is meant the angle between the surface of the organ and the incident ray.

## Effect of Duration, Intensity and Directive Angle of Light on Photosynthesis

It has been shown that—

(1) The amount of photosynthesis under uniform intensity of light increases with the duration of the exposure. If N bubbles of oxygen are given out in a given period, 2N bubbles are produced in twice the time.

(2) Photosynthesis increases (within limits) with the intensity of light. It is shown in Table VII. that while under the intensity of 500 lux the quantity of oxygen evolved was 128 c.mm. per hour, under twice the intensity or 1000 lux the gas evolved per hour became doubled to 257 c.mm. A similar relation also holds good under sunlight : at the intensity of 0·06 S the gas evolved was 167 c.mm. per hour ; at 0·12 S it became 337·5.

It remains to ascertain what is the relation of photosynthetic activity to the angle of the incident light. Special difficulties arose in the measurement of the directive angle ; in *Hydrilla* the small leaves of the plant are disposed in various directions, and it was therefore impossible to use this plant for this purpose. A leaf with a flat surface is required such as that of *Vallisneria*. The leaf was tied

to a thin glass rod; the upper end of the rod carried an index moving against a circular scale. The leaf, with its cut end upwards, was placed vertically in a rectangular trough of water containing the normal proportion of $CO_2$, photosynthesis being measured by counting the number of bubbles given out at the cut end for a given length of time. Sunlight reflected by a mirror was thrown perpendicularly on to the surface of the trough; since the leaf was parallel to the surface of the trough, the directive angle was 90°. The leaf was then rotated alternately through + 45° and − 45° and the evolution of oxygen in these two positions measured, the mean being taken as the value at 45°. The object of the alternate rotation was to make allowance for any asymmetry in the setting of the index. It was also necessary to make allowance for the gradually diminishing evolution of oxygen-bubbles on account of the deposit of the mucilage at the cut end. This was secured by taking observations in the following order: (1) at 90°, (2) at 45°, and (3) once more at 90°. The mean of (1) and (3) gave the average value at 90°.

TABLE IX.—SHOWING THE EFFECT OF DIRECTIVE ANGLE ON PHOTOSYNTHESIS

| Experiment | Directive angle | Number of bubblets |
|---|---|---|
| 1 | 90° | 54 |
| 2 | 45° | 34 |
| 3 | 90° | 48 |
| Mean of 1 and 3 = 51 | | |

$$\frac{\text{Activity at } 90°}{\text{Activity at } 45°} = \frac{51}{34} = 1{\cdot}5$$

$$\frac{\text{Sine of } 90°}{\text{Sine of } 45°} = 1{\cdot}4$$

Hence photosynthetic activity is approximately proportional to the sine of the directive angle.

Taking all the factors into account, we find that *the activity of photosynthesis is proportional to the quantity of incident light.*

## Summary

The limiting maximum in photosynthesis is found to be relative and not absolute. It increases with the intensity of light, this increase being independent of the $CO_2$-concentration of the liquid in which the plant is immersed.

The *Hydrilla* plant is very sensitive to differences in the intensity of light, and may therefore be employed as a photometer.

A definite physiological equivalence was found between sunlight and Pointolite. On a bright day in March, sunlight 0·04 S was found to be physiologically equivalent to 1700 lux Pointolite ; that the two lights were also physically equal was subsequently demonstrated by a grease-spot photometer.

Photosynthesis is shown to be proportional (1) to the duration, (2) to the intensity, and (3) to the sine of the directive angle. The amount of photosynthesis is therefore proportional to the quantity of incident light.

# CHAPTER VII

## THE PHYSIOLOGICAL FACTOR IN PHOTOSYNTHESIS

The anabolic and catabolic processes A and D—External manifestations of A and D effects—Photosynthetic and phototropic curves—Dependence of maximum response on the intensity of stimulus—Effect of variation of temperature—D effect due to shock-stimulus—Phenomenon of alternating response—Dual effects of stimulus of light—Unmasking of the A effect—Effect of chemical agents—Effect of tonic condition on response.

THE process of photosynthesis begins with a photochemical reaction in which $CO_2$ and $H_2O$ are synthetised into some form of organic matter such as formaldehyde. To what extent this is effected, as a purely physico-chemical process, by the chlorophyll proper is still uncertain. Baly and Heilbron have recently shown that 'in the presence of a suitable basic coloured substance, such as malachite green, with which the carbonic acid can combine loosely, the formation of formaldehyde can be demonstrated in visible light, the malachite green acting as a photocatalyst for the reaction.'[1] There can, however, be no doubt that the physiological factor—the reaction of the protoplasm with which the chlorophyll is associated in the chloroplast—plays a very important part in the process of photosynthesis. I hope to establish this in the present and succeeding chapters by showing that photosynthesis is modified by variations of external conditions just as are other modes of physiological response.

The protoplasmic response to external change may be looked at as being the expression either of storage or of depletion of energy. The positive or anabolic response A is connected with the uphill work, increasing the potential

[1] Heilbron, *Nature*, April 14, 1923.

energy of the system, while the negative or catabolic D is associated with run-down of energy. Under external variation there is thus produced either the A or the D effect, or both A and D in different proportions, the resultant being A−D. The different reactions under the incidence of external stimulus may be likened to the effects of the supply of external energy from a dynamo to a storage-cell, and of the expenditure of that energy by a motor. Imagine the storage-cell connected (1) with the dynamo, (2) only with the motor, and (3) with both the dynamo and the motor. In the first case a storage of energy would result (A effect). In the second case there would be expenditure and loss of energy (D effect). In the third case there would be both storage and expenditure, the resultant effect being A−D. As in a storage-cell so also in the plant, the energy-content at any moment is A−D, the algebraical sum of the work done *on* the plant in storage and the work done *by* the plant with consequent expenditure and run-down of energy.

The energy-condition of the storage-cell can be ascertained by an electric indicator, the movement of which in a plus or in a minus direction shows the accession or depletion of energy. The electrical current, though invisible, can be detected by its chemical effect, or by its deflecting action on a suspended magnetic needle, these being but different indications of an identical current. I have shown elsewhere [1] that the invisible internal changes in the plant can also be detected by various physiological indicators. For example, the D effect induced by a stimulus is indicated by a negative response of *fall* of the leaf of *Mimosa*, and by a simultaneous galvanometric negativity of electric response. The sign of the A effect is opposite, a positive or erectile response of *Mimosa*, and an electrical response of galvanometric positivity. The fundamental protoplasmic reactions A or D are not manifested at haphazard, but are responsive changes associated with definite external expression in different modes of response.

[1] *Plant Response* (1906); *Irritability of Plants* (1913).

The positive A effect is externally manifested—

(*a*) By expansion, as manifested by the erectile movement of the leaf of *Mimosa* ;

(*b*) By increase of turgor, and by increase in the normal rate of growth ;

(*c*) By electromotive variation of galvanometric positivity ; and

(*d*) By increase of electric resistance of the tissue.

The negative D reaction, on the other hand, is shown—

(*a*) By contraction and the fall of the leaf of *Mimosa* ;

(*b*) By diminution of turgor, and by retardation of the rate of growth ;

(*c*) By electromotive change of galvanometric negativity ;

(*d*) By diminution of the electric resistance of the tissue.

The widened outlook resulting from the establishment of the correspondence of the characteristic expression of the positive, as also of the negative, in various modes of response has led to the discovery of several important phenomena, notably in photosynthesis, which at first sight would appear inexplicable. Feeble and strong electric stimulus will be shown in the next chapter to induce opposite effects in photosynthesis. Again, in a moderately vigorous specimen of *Hydrilla*, the after-effect of strong intensity of light on photosynthesis is a depression of activity. But in sub-tonic specimens, in which physiological condition is *below par*, the after-effect of strong intensity of light is an enhancement of activity instead of a depression (*cf.* Chapter XVIII.). Still more curious is the effect of rapidly intermittent light, which photosynthetically is even more effective than continuous light. Apparent anomalies like these are frequently met with, and their explanation is to be sought in the general characteristics of protoplasmic irritability.

Some of the most important of these characteristics will be described in the following order : (1) the relation between

stimulus and response; (2) the dependence of maximum response on the intensity of stimulus; (3) the effect of temperature variation; (4) the D effect of shock-stimulus; (5) dual effect of the stimulus of light; (6) phenomenon of alternating response; (7) effect of chemical agents; and (8) effect of tonic condition on response. I will in subsequent chapters show that photosynthetic activity is modified under external variations in a manner parallel to the modification of other physiological activities such as growth and movement.

## Photosynthetic and Phototropic Curves

It has been shown that photosynthesis is feeble when the light-intensity is low; that it increases rapidly in the median range where the rate of increase is uniform; that the curve then reaches a turning-point, after which it tends to become horizontal. Examination of the phototropic curve obtained with the leaf of *Erythrina indica* (fig. 9) shows that its characteristics are very similar to those of the photosynthetic curve (fig. 7).

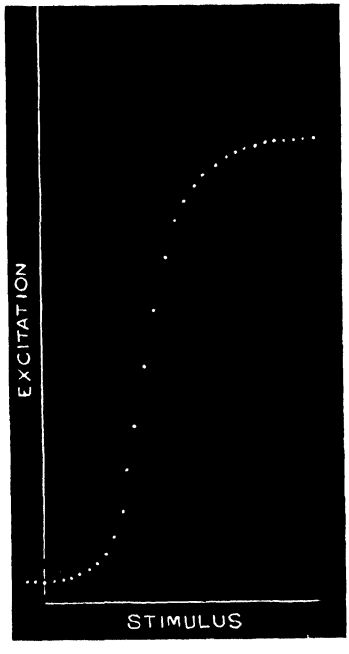



FIG. 9. The Tropic Curve under the Action of Light (*Erythrina indica*)

## Dependence of Maximum Response on the Intensity of Stimulus

I have shown that the maximum photosynthesis is not absolute but relative, and that it depends on the intensity of stimulus. Parallel results are obtained with other modes of response. For instance, we take *Mimosa* and subject it to tetanising electric shocks of increasing intensity. Under moderate intensity of stimulus

the response attains a certain maximum value (fig. 10 (*a*)). After recovery the plant is subjected to an increased

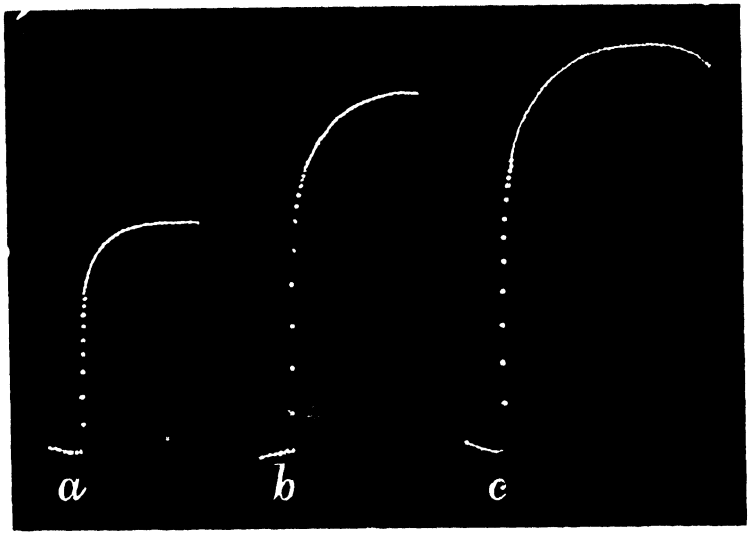



FIG. 10. Maximum Response of *Mimosa* under Increasing Intensity of Electric Stimulus

intensity of stimulus, and a second maximum (*b*) is obtained, larger than (*a*). A still higher intensity of stimulus gives rise to a third maximum (*c*), greater than either (*a*) or (*b*).

## Effect of Variation of Temperature

Up to a certain optimum, rise of temperature causes a general enhancement of physiological activity, as exhibited in different modes of response. It gives rise to an erectile movement of the leaf of *Mimosa*, to an increased rate of the ascent of sap, to quicker frequency of pulsation of the leaflet of *Desmodium gyrans*, and to an enhancement of the rate of growth. In all these modes of response a decline occurs at a temperature above the optimum.

There is also a definite minimum temperature for arrest

of motile response in *Mimosa*, of growth, of autonomous pulsations of the leaflet of *Desmodium gyrans*.

## D Effect due to Shock-Stimulus

A *sudden* variation of external conditions causing a shock gives rise to the predominant D effect. The shock may be due to (*a*) sudden variation of pressure, to friction or to mechanical disturbance of any kind; (*b*) to sudden change of temperature; and (*c*) to sudden variation of electric condition as produced by an induction-current or a condenser discharge. The effect of shock-stimulus is seen in the responsive *fall* of the *Mimosa* leaf, in the diminution of the rate of growth, in the electrical response of galvanometric negativity, and in the diminution of the electrical resistance of the tissue.

## Phenomenon of Alternating Response

After full adjustment to the external conditions, the responses of *Mimosa* under uniform stimulus are found to

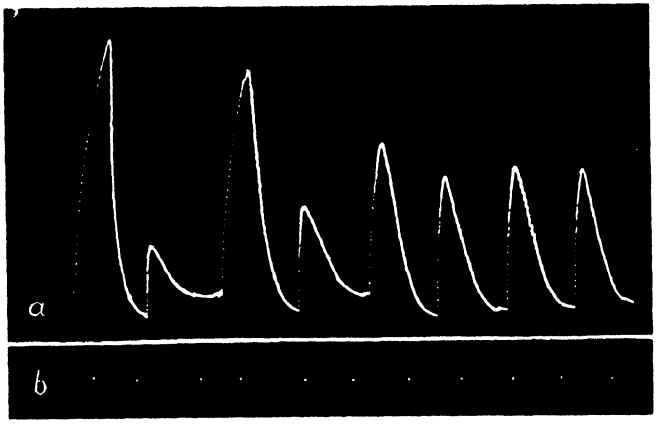



FIG. 11. (*a*) Alternating Response of *Mimosa* (upper record), and (*b*) of Photosynthesis (lower record)

Note that alternating response in both tends to become uniform.

be uniform. But during the process of adjustment to a new condition, the responses are often found to be alter-

nating—that is to say, one response is large and the next small, in regular sequence ; after a time these tend, however, to become uniform. By the employment of the High Magnification Crescograph I find that growth consists of a series of pulses, which are often of an alternating character. The same is true of the autonomous pulsations of the leaflet of *Desmodium gyrans.* By the employment of a sensitive Bubbler which records successive evolutions of 1 c.mm. of oxygen, I find that the photosynthetic response of *Hydrilla* is sometimes of a similar character, a short period alternating with a longer interval. These, as in the alternating responses in *Mimosa,* become uniform after the attainment of a steady condition (fig. 11). The parallelism here observed in the two different modes of response is indeed very remarkable.

## Dual Effect of Stimulus of Light

Light induces both the A and D effects, the resultant being A−D. The $CO_2$-assimilation by the leaf is essentially an anabolic process, and the predominant response of an actively assimilating leaf is A ; in regard to D, it is increased under strong intensity of light, with the resulting diminution in the rate of photosynthesis (p. 36).

In other instances, the negative catabolic D may be predominant and mask the less predominant positive A. It is, however, possible to unmask A (under circumstances when it is more persistent) by the sudden stoppage of light.

*Unmasking of A in mechanical response.*—In excitable growing organs photic stimulus, giving rise to the predominant D reaction, retards the rate of growth ; but I hoped to unmask the A effect on the stoppage of illumination. The investigation was undertaken with the Balanced Crescograph, in which the upward growth is exactly compensated by the subsidence of the plant at the same rate. Hence the tip of the growing organ remains at the same height, and the

record given by the High Magnification Crescograph remains horizontal. Exposure to light upsets the balance, and the predominant D reaction is seen in retardation of growth as an up-curve (fig. 12). If, on the cessation of light, growth returned to the normal, the fact would be demonstrated by the up-curve becoming once more horizontal, which is the balanced record of normal growth. Instead of this we find a down-curve, which represents *an acceleration of growth above the normal,* indicative of the A process. After a time the normal rate of growth becomes restored. The dual effects of stimulus of light are seen in the above, the A effect becoming unmasked on the cessation of the stimulus.

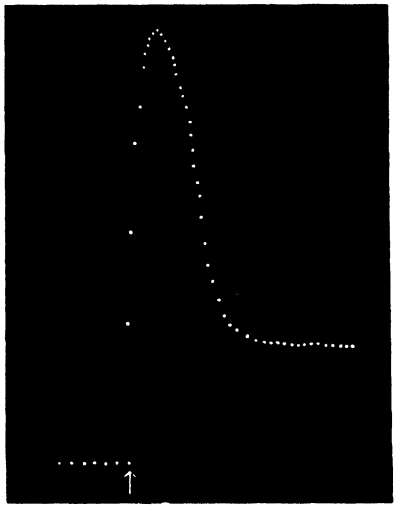


FIG. 12. Direct and After Effect of Light on Growth-elongation of *Kysoor*

First part of the balanced record of growth is horizontal; on application of light of short duration at arrow, there is a retardation of growth (up-curve), followed by acceleration as an after-effect (down-curve). Restoration to normal is seen in the last part of the curve.

The dual effect of the stimulus of light will be further demonstrated by the electric and by the photosynthetic response (Chapter X.).

## Effect of Chemical Agents

The effect of a moderate dose of ether on general irritability is well demonstrated by its effect on the mechanical response of *Mimosa.* In the first part of the record (fig. 13) we observe two uniform responses. Ether-vapour was now introduced into the plant-chamber, with the result of a depression of response. Finally, on substitution of fresh air

in the chamber, the normal irritability was found to be gradually restored.

In regard to the action of poisons, it is often found that while a moderate dose abolishes all response, a minute dose of the same poison causes an enhancement of physiological activity.

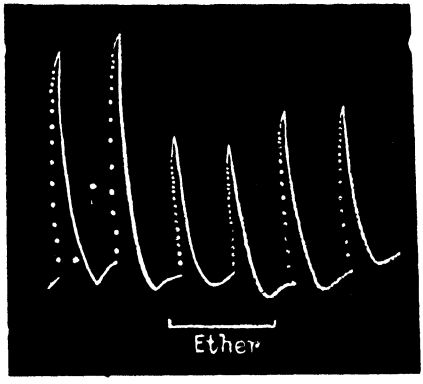



FIG. 13. Depression of Mechanical Response of *Mimosa* under Ether, and Gradual Recovery on Removal of the Anæsthetic

## Effect of Tonic Condition on Response

We come last of all to the very intricate internal factor which will be described as the tonic condition of the plant. Under optimum tonic condition, all modes of response exhibit heightened activity, as shown in the mechanical response of *Mimosa,* in the autonomous pulsations of *Desmodium,* in the movement of growth and in the rate of photosynthesis. In the sub-tonic condition the various responses are found to exhibit a great depression.

In regard to this intricate question of the effect of varying tonic conditions on response, a brief summary of the modifying effects of age, season and unfavourable condition on different modes of response is given below.

(*a*) *Effect of age.*—In *Mimosa* the middle-aged leaf counted from the top is found to be most irritable to mechanical and to geotropic stimulation. The very young leaf near the apex or the old leaves at the lower end of the stem are relatively inexcitable. Similar differences in activity as modified by age are also observed in the pulsating leaflet of *Desmodium.*

(*b*) *Effect after flowering.*—The physiological vigour of the plant is greatly lowered after flowering. The mechanical

response of *Mimosa* and the autonomous response of *Desmodium* undergo marked diminution after the development of the flowers.

(*c*) *Effect of season.*—The spring season is obviously more favourable to physiological activity than winter; and the records which I obtained of the mechanical response of *Mimosa*, of the autonomous pulsation of *Desmodium*, and of the movement of growth exhibit similar differences due to change of season. The greater irritability in spring-specimens of *Mimosa* is exhibited in another way, namely by the low intensity of minimal stimulus which is effective in evoking response. In spring the intensity of minimally effective stimulus is considerably lower than in winter.

In photosynthesis the effect of season has been shown to be in every way similar. Photosynthetic activity is relatively higher in spring than in winter, and the minimal intensity of light which initiates photosynthetic activity is very much lower in spring.

(*d*) *Effect of unfavourable environment.*—If the plant be maintained under unfavourable environment, its tonic condition falls *below par*, with resulting depression of physiological activity. Prolonged maintenance of the plant in darkness, for example, causes a depression in the mechanical response of *Mimosa*, in the pulsations of *Desmodium*, and in the rate of growth in the growing organs. In the sub-tonic specimens, previous stimulation is generally found to confer an enhanced activity to the organism, so that the formerly ineffective stimulus now becomes effective.

The characteristic modifications of response described above will also be found in photosynthesis, proving the importance of the physiological factor in the process.

## SUMMARY

The photosynthetic curve is similar to the phototropic curve; the maximum response is not absolute but increases with the intensity of the stimulus.

The effect of rise of temperature to an optimum is seen in the enhancement of various activities of the plant. These activities decline at a temperature above the optimum. There is also a minimum temperature for arrest of growth, and of autonomous pulsations of the leaflet of *Desmodium gyrans*.

Shock-stimulus induces the D effect, shown in the fall of *Mimosa* leaf, in the diminution of the rate of growth, and in the electric response of galvanometric negativity.

Responses to uniform stimulus are sometimes of an alternating character; this is exhibited by the mechanical response of *Mimosa* and by the photosynthetic response of *Hydrilla*.

Light gives rise to dual effects, anabolic and catabolic, the resulting effect being A–D. The A effect may become masked by the D effect when the latter is relatively predominant. The A effect may, however, be unmasked by the sudden stoppage of light, with resulting ' overshooting ' of the response in the positive direction.

Chemical agents cause characteristic reactions in the plant. The effect of a minute dose of poison is often an enhancement of physiological activity.

Variation of the tonic condition affects all modes of response alike, as shown in the depression of response by age and by unfavourable conditions of the environment. In specimens in a sub-tonic condition, stimulation enhances the general physiological activity of the plant.

# CHAPTER VIII

## CHANGE IN PHOTOSYNTHETIC ACTIVITY UNDER STIMULUS, ANÆSTHETICS AND POISONS

Effect of stimulus, minimal and maximal, on photosynthesis—Effect of chloroform, ether and alcohol—Effect of poison.

I WILL in the present chapter describe in detail the variations of photosynthetic activity induced by varying intensities of stimulus and by the action of different chemical agents.

### Effect of Stimulus

I have already referred to the fact that the effect of stimulus, generally speaking, is modified by its intensity. Thus a feeble stimulus enhances the rate of growth, but strong stimulus retards or inhibits it ; the same is true of transpiration by the leaves.

In investigating the effect of electrical stimulus on photosynthesis, two contacts were made by means of silver wires insulated except at the points where they touched the tip and the base of the plant. These wires were led out of the plant-vessel through the india-rubber cork, care being taken that the cork did not allow any leakage of air into the plant-vessel. The two electrodes were connected with the secondary of an electric induction-coil, the intensity of the shock being adjusted from the minimum to a maximum by the gradual approach of the primary coil towards the secondary.

In the following experiments the temperature was 20° C. ; the source of illumination was sky light, the constancy of which was assured by frequent readings of the Electric Photometer. The normal period of bubbling was found to

be twenty seconds ; application of feeble stimulus enhanced this rate by 17 per cent. The intensity of stimulus was now gradually increased in the manner already described. This caused a diminution of the rate by 20 per cent. ; a slightly stronger stimulus induced a greater retardation of the rate by 35 per cent. Further increase of stimulus brought about a total arrest of evolution of oxygen. After moderate stimulation, the normal rate was found to be gradually restored in the course of about half an hour. Excessive stimulus, however, caused death and permanent abolition of photosynthesis.

## Effect of Anæsthetics

In the study of the effect of anæsthetics and other chemical agents on photosynthesis it is necessary to observe first the normal rate of evolution of oxygen, and then the change in the rate after the introduction of the chemical agent into the plant-vessel. This must be done without causing any mechanical disturbance to the plant, since mechanical or electrical irritation modifies the normal rate of photosynthesis. A special plant-vessel had therefore to be devised for quick introduction or withdrawal of the normal $CO_2$-solution, and of the $CO_2$-solution with a certain percentage of the chemical agent added to it. The plant-vessel is shown in fig. 14. A hollow stopper H with a hole O is fixed on a stand L. The lower end of the plant-vessel with a T-tube is fitted exactly to the stopper. When the vessel is rotated through 90°, the hole O comes opposite to the T-tube, and the liquid is withdrawn from the vessel. A funnel-tube F is then exactly fitted to the T-tube : the solution containing the chemical is poured through the funnel, taking care that the level of the liquid inside the plant-vessel always comes up to the mark M. The vessel is afterwards rotated through 90°, when communication with the funnel-tube F is cut off, and the funnel-tube is then removed.

*Chloroform.*—When the plant is placed in water mixed with chloroform, the rate of bubbling becomes slowed down, culminating in an arrest. This is a qualitative demonstration of the narcotic effect of chloroform. For quantitative determination, ether is preferable, for two reasons: first, because the effect is gradual, and second because it is possible to obtain complete restoration after removal of the anæsthetic.

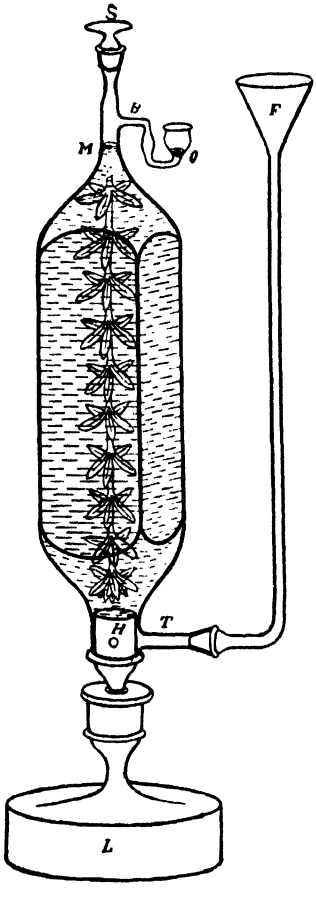



FIG. 14. The Special Plant-vessel for introduction of chemical substances without mechanical disturbance

*Ether.* — Having ascertained the normal rate of bubbling, the usual $CO_2$-solution with 1 per cent. of ether added is introduced into the plant-vessel. The rate of bubbling is now found to undergo a continuous decline with the duration of application. The normal period was 26·5 seconds, or an activity of $\frac{60 \times 60}{26 \cdot 5}$ = 136 bubbles per hour. The bubbling-period under the action of dilute ether became prolonged to 131 seconds in the course of 8·5 minutes, the activity being reduced to 27. The results of the effect of increasing duration of application are given in Table X.

The curve (fig. 15) shows the rate of diminution of activity under the action of ether. It exhibits a rapid decline at the beginning, which tends to reach a maximum.

On the removal of the dilute ether and substitution of normal $CO_2$-solution the rate of photosynthesis was found to undergo recovery. Whereas the depression of activity

TABLE X.—DEPRESSION OF PHOTOSYNTHESIS UNDER DILUTE ETHER

| Duration of application of ether | N bubbles per hour |
|---|---|
| 0 seconds | 136 |
| 34 ,, | 97 |
| 92 ,, | 62 |
| 169 ,, | 47 |
| 260 ,, | 37 |
| 378 ,, | 30 |
| 509 ,, | 27 |

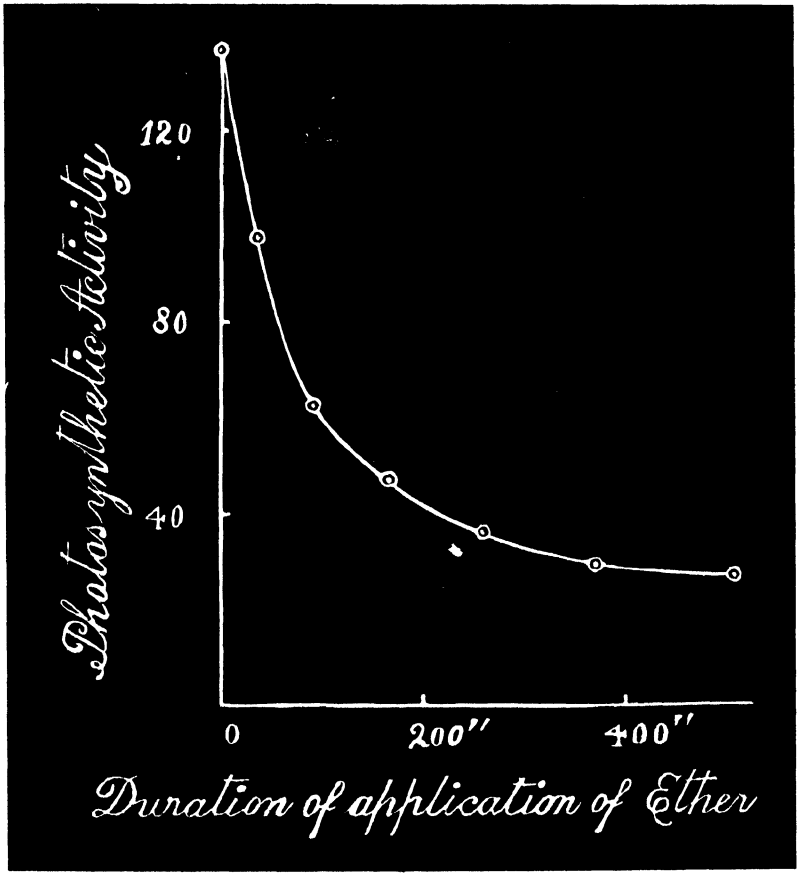



FIG. 15. Curve showing the effect on Photosynthesis of application of 1 per cent. Ether Solution

had been from 136 to 27, on the removal of the ether the activity was restored to 130, which is practically the same as the normal at the beginning.

*Effect of alcohol.*—The effect produced is similar to that of ether. The normal period of bubbling in a vigorous specimen was 13 seconds. One per cent. of alcohol induced a depression which increased with the period of application. The bubbling-period was thus prolonged from 13 to 77 seconds in the course of 5 minutes.

## Effect of Poisons

*Formaldehyde.*—A solution of formaldehyde acts as a very effective poison on various activities of plant-life. It




FIG. 16. Effect of Application of 0·01 per cent. Solution of Formaldehyde on Photosynthesis

Normal period 27 seconds, prolonged to 287 seconds after application for 16 minutes.

arrests the rhythmic pulsation of the *Desmodium* leaflet, and I have recently discovered that the application of this solution to the root or to the cut end of the stem arrests and permanently abolishes the ascent of sap.

On applying 0·1 per cent. solution of formaldehyde to *Hydrilla*, the evolution of oxygen was found to be immediately arrested. I next supplied a very dilute solution of 0·01 per cent. to a fresh specimen. This caused a continuous diminution of activity, the normal period of bubbling of 27 seconds being prolonged in the course of 16 minutes to 287 seconds, culminating afterwards in permanent arrest (fig. 16).

*Copper sulphate solution.*—If a specimen of *Hydrilla* is

kept in a vessel inside the laboratory, its activity is sometimes found to be abolished. This is often due to traces of metallic contamination; for the plants do not suffer on being kept in glass or earthenware vessels, but if kept in brass vessels they lose their activity in a short time. In order to find out what was the minimum dose of metallic poison which produced a toxic effect, I made dilute solutions of copper sulphate. A solution of one part of $CuSO_4$ in 100 millions of water caused a depression of activity from the normal 100 to 88; one part in a million caused a further depression to 70, and ten parts in a million induced abolition of response.

## SUMMARY

The effect of feeble stimulus is an enhancement of photosynthesis, as also of the rate of growth in growing organs, and of the rate of transpiration of leaves. In all, strong stimulus induces the opposite effect of depression.

Anæsthetics such as ether induce a depression in the rate of photosynthetic evolution of oxygen; removal of the anæsthetic is followed by restoration of the original activity.

Formaldehyde acts as a poison and abolishes the activity in a dilution of one part in a thousand.

Copper sulphate solution one part in a million causes a great depression; a solution containing ten parts in a million acts as a poison and abolishes the activity.

# CHAPTER IX

## EFFECT OF INFINITESIMAL TRACES OF CHEMICAL SUBSTANCES ON PHOTOSYNTHESIS

Sudden increase of photosynthetic activity after a thunder-storm—This possibly due to nitrous fumes produced during electric discharge being washed down by the rain—Determination of the effect of infinitesimal traces of $HNO_3$ in enhancement of photosynthetic activity—Effect of traces of extract of thyroid gland, of iodine, and of formaldehyde—Opposite effects of large and small doses on physiological reactions.

WE saw in the last chapter how traces of copper sulphate as minute as ten parts in a million gave rise to a pronounced toxic effect, photosynthesis being thereby completely abolished. The converse of this, namely the possible influence of ultra-measurable traces of certain chemical substances in enhancing the power of assimilation, is a question of much importance in physiology. The $CO_2$-assimilation of water-plants affords an extremely sensitive subject for this investigation.

My attention was drawn to the matter by the extraordinarily great increase in the photosynthetic activity of *Hydrilla* after three days' thunder-storm and rain which lasted from the 10th to the 13th February of this year (1923). The activity of a large number of these plants growing in a particular pond of the Institute had been very carefully determined from 3rd January to 9th February, and was found to be practically the same in all the specimens. Immediately after the thunder-storm, namely on the 14th February, the photosynthetic activity was found to have been increased by more than 100 per cent. This increased activity could not have been due to any variation of temperature, for that remained unchanged; the rain-water could not produce

any change in the plant already submerged in water. Again, this enhancement could not have been produced by any stimulation caused by an electric disturbance during the thunder-storm, for such a disturbance artificially produced did not cause any responsive variation. This is what might have been expected, since the electric disturbance in the air could not affect the plant under water, which is a conductor. The other possible explanation is the production, under electric discharge during the storm, of oxides of nitrogen in the atmosphere, which would be dissolved in the rain, and thus cause an addition of traces of nitrates to the pond-water. But the quantity so added would be inconceivably small, and it is difficult to imagine how such infinitesimal quantities could produce any effect on the activity of the plant.

## Effect of $HNO_3$ on Photosynthetic Activity

I thought, however, that it might be of interest to investigate the effect of small traces of nitric acid on the activity of the plant. I began by adding to the normal $CO_2$-solution one part of $HNO_3$ in 10,000; this, instead of producing any enhancement of activity, caused a depression. One part in 100,000 produced hardly any effect. This led me to conclude that traces of $HNO_3$ could not exert any stimulatory effect. Before giving up the inquiry I, however, tried the effect of one part in a billion (billion in French measure = 1000 millions). With this extraordinarily minute quantity I obtained such a marked stimulation of photosynthetic activity that it surpassed the limits of belief. But repetition of the experiment, an account of which will be presently given, left no possible doubt on the subject. It was found that one part in ten billions produced a very marked enhancement, which continued to increase on further addition till at one part in two billions a maximum increase of nearly 200 per cent. was produced. The activity began to show a decline at a dilution of one part in a billion,

and it was reduced to the normal at a strength of 10,000 parts in a billion. Stronger solutions produced a depression below the normal.

The maximum point and the relative enhancement are modified to a certain extent by the physiological condition of the specimen. The stimulating effect of traces of $HNO_3$ is very marked in inactive specimens, where the change induced is from no evolution of oxygen to a very vigorous bubbling. Very active specimens do not show such a great increase, the enhancement being then about 50 per cent. In moderately active specimens the increase, as already stated, is about 200 per cent. The following table shows the effect of traces of $HNO_3$ on three different specimens which were moderately active.

TABLE XI.—SHOWING EFFECT OF $HNO_3$ ON PHOTOSYNTHETIC ACTIVITY

| Dilution (*n* parts in a billion) | Activity in c.mm. $O_2$ per hour (Normal activity taken as 100) | | |
|---|---|---|---|
| | Specimen I. | Specimen II. | Specimen III. |
| 0·01 | 100 | 100 | 100 |
| 0·1 | 199 | 196 | 198 |
| 0·5 | **278** | **242** | **286** |
| 1·0 | 272 | 233 | 278 |
| 5·0 | 243 | 214 | 242 |
| 10·0 | 225 | 190 | 218 |
| $10^2$ | 206 | 189 | 206 |
| $10^3$ | 198 | 188 | 198 |
| $10^4$ | 108 | 99 | 125 |

*Method of the threshold.*—I discovered another striking method for the demonstration of the great enhancement of activity induced by infinitesimal traces of $HNO_3$. We take a cut specimen of *Hydrilla* fixed in a test-tube in an inverted position with the cut end uppermost. As a moderate intensity of light is required to initiate photosynthesis, it follows that the specimen ceases to give bubbles of oxygen when it is moved away from the window which admits daylight into the room. This usually happens when the plant is at a distance of about 2 metres from the window. If we now add

a minute trace of $HNO_3$ to the water, the bubbling hitherto arrested is renewed with extraordinary vigour.

## Effect of Extract of Thyroid Gland

I investigated the effect of many other substances on photosynthetic activity, of which I will give several instances. The first section of Table XII. gives the results obtained with dilute extract of thyroid gland. In specimen III. a dilution of one part in a billion produced a maximum increase of activity of more than 40 per cent.; in two other cases the maximum was attained with a dilution of ten parts in a billion, the increase being about 100 per cent.

TABLE XII.—EFFECT OF EXTRACT OF THYROID GLAND, OF IODINE, AND OF FORMALDEHYDE

| Chemical agent | Dilution (*n* parts in a billion) | Activity (Normal activity taken as 100) | | |
|---|---|---|---|---|
| | | Specimen I. | Specimen II. | Specimen III. |
| Thyroid | ·01 | 100·0 | 100·0 | 100·0 |
| | 0·1 | 148·0 | 149·0 | 106·0 |
| | 1·0 | 197·0 | 172·3 | *143·7* |
| | 10 | *210·0* | *180·8* | 133·3 |
| | $10^2$ | 187·0 | 174·8 | 127·0 |
| | $10^3$ | 185·0 | 174·0 | 125·0 |
| | $10^4$ | 184·5 | 174·0 | 124·0 |
| | $10^5$ | 183·5 | 117·0 | 115·0 |
| Iodine | ·01 | 100·0 | 100·0 | 100·0 |
| | 0·1 | 133·5 | 130·0 | 120·0 |
| | 1·0 | *149·0* | *143·0* | *162·0* |
| | 10 | 103·0 | 132·0 | 110·0 |
| | $10^2$ | 92·5 | 106·0 | 83·0 |
| | $10^3$ | 92·0 | 104·0 | 82·0 |
| Formaldehyde | ·01 | 100·0 | 100·0 | 100·0 |
| | 0·1 | 137·5 | 154·6 | 124·0 |
| | 1·0 | *185·0* | *176·3* | *140·0* |
| | 10 | 160·0 | 154·8 | 123·0 |
| | $10^2$ | 153·0 | 151·0 | 120·0 |
| | $10^3$ | 152·0 | 150·0 | 110·0 |
| | $10^4$ | 135·0 | 75·5 | 81·0 |

The noticeable fact in the action of thyroid extract is that no reversal or diminution of activity below normal took place for a considerable range in the dilution (fig. 17).

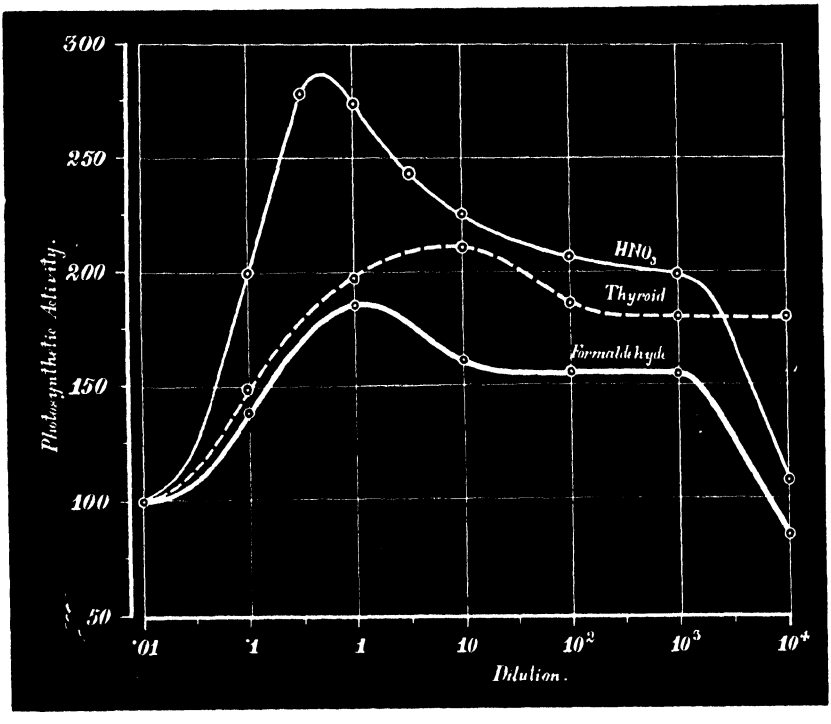



FIG. 17. Curves exhibiting the effect of infinitesimal traces of $HNO_3$, of Thyroid Gland, and of Formaldehyde, on Photosynthetic Activity of *Hydrilla*

The dilutions are *n* parts in a billion.

## Effect of Iodine

The effect of traces of iodine is somewhat similar to the foregoing. One part of iodine in a billion produced the maximum enhancement of about 50 per cent.; but dilution of one part in a million depressed the rate below the normal.

## Effect of Formaldehyde

The effect of traces of the very poisonous agent formaldehyde is of much theoretical interest. A solution of one part

in ten billions caused an increase of activity of about 40 per cent.; one part in a billion induced a further increase to 80 per cent. or so. The stimulating effect of traces of formaldehyde has a special significance in regard to the 'first product' of photosynthesis. There is reason to believe that this first product is formaldehyde, which by polymerisation becomes converted into carbohydrate. The poisonous nature of formaldehyde stood in the way of acceptance of this theory; but the experiments just described show that traces of this substance, instead of being poisonous, have the effect of increasing the photosynthetic activity. We shall have occasion in a later chapter to refer to this subject.

The opposite effects of small and large doses of a substance, as observed with formaldehyde, I find to be of frequent occurrence in physiological reactions. Thus ether, which in large doses kills the plant, in small quantities is a stimulant for growth. The same is true of chloroform, but the range of safety is here more circumscribed. With strong poisons the range is still narrower. Copper sulphate acts as a poison in a solution of one part in a million, but a dilution of one part in 100 millions often acts as a stimulant of photosynthetic activity; the increase is, however, not so great or so unfailing as in the case of formaldehyde.

## SUMMARY

The photosynthetic activity of *Hydrilla* was found to have been increased by more than 100 per cent. immediately after a thunder-storm. This appeared to be probably due to traces of nitrous fumes produced by electric discharge and washed down by the rain.

Investigations on the effect of infinitesimal traces of $HNO_3$ showed that one part of this acid dissolved in two billion parts of water induced an enhancement of nearly 200 per cent. in the rate of photosynthesis.

Minute traces of the extract of thyroid gland, of iodine and of formaldehyde showed similar enhancement of

photosynthetic activity. The effect of traces of formaldehyde in enhancement of activity is of special significance in regard to the possible formation of formaldehyde as the first product of photosynthesis.

At first sight it seems inconceivable that infinitesimal traces of certain chemical substances should have such a potent influence in the life-activity of plants. There is, however, no doubt of the reality of the phenomenon.

# CHAPTER X

## THE ELECTRIC RESPONSE TO LIGHT

Electric response to the stimulus of light—Dual reactions A and D under light—Excitatory D reaction detected by response of galvanometric negativity—Persistence of electric response of motile organs even under physical restraint—The photo-electric cell—The predominant D effect in the electric response of *Musa* to light—The positive after-effect and the phenomenon of ' overshooting '—Positive electric response of *Hydrilla*—Effect of temperature on electric response—Effect of narcotics—Effect of excess of carbonic acid.

IN the two preceding chapters we have discussed the effects of certain physiological variations on photosynthetic activity, and found that they run parallel to those observed in other modes of response. We shall in the present chapter make a special study of the effect of light as determined by the independent method of electric response.

Light, as previously stated, gives rise to dual reactions A and D, which can be detected by electric means. For example, we make suitable electric connections on a *Mimosa* plant, one contact being made with the responding pulvinus and the other with a distant indifferent point. Under any mode of stimulation, the D effect of excitatory fall of the leaf is manifested by an electrical variation of galvanometric *negativity*. This electric concomitant of the D effect is unfailing and is independent of the mechanical response. For, even after the motile response becomes arrested by physical restraint, external or internal, the electrical response persists. Thus, when water is applied on the pulvinus of *Mimosa*, it absorbs it in excess, and in this water-logged condition the response by mechanical movement is inhibited. But the excitatory protoplasmic reaction of the tissue may still be detected by the electric response of galvanometric negativity.

During the period of rest there is a recovery of the normal condition of the tissue from the effect of stimulus: the pulvinus expands, the leaf becomes re-erected, and there is a restoration of the original turgor; the induced electric negativity also disappears.

The A effect is, as previously stated, detected by an electric response of opposite sign, namely of galvanometric *positivity*.

## Electric Detection of A and D Effects under Light

The electric detection of the A and D effects under light will now be described. The difficulty of detection of the

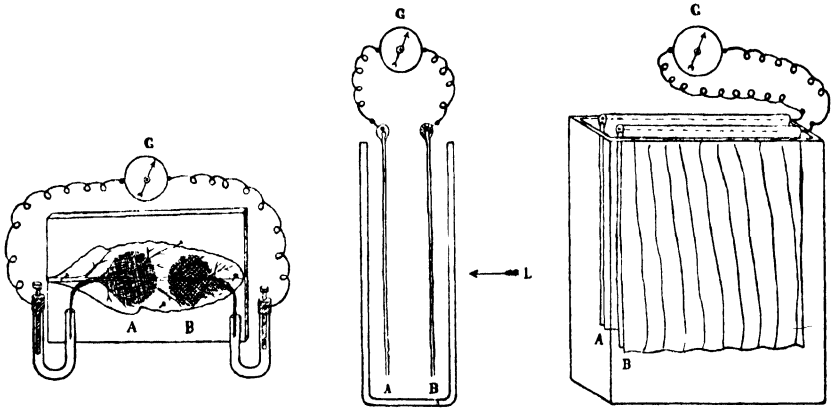



FIG. 18. Arrangement for obtaining Electric Response to Light
The two figures to the right show the photo-electric cell.

A effect arises from the fact that the concomitant positive electric response is relatively feeble, while the negative D is far more pronounced and therefore masks the positive A. It is only under favourable circumstances that the positive electric response indicative of the A effect becomes sufficiently large for external manifestation. This we shall find in an organ which has a special capacity for vigorous photosynthesis.

I will first describe the method for obtaining the electric response of leaves under the action of light. A broad leaf

of *Musa* was taken and two pieces of thin muslin were spread over two areas, A and B (fig. 18, left-hand fig.). When moistened with normal saline solution, the muslin becomes transparent, and therefore does not obstruct the light. The moist pieces of muslin were led to two non-polarisable electrodes, a sensitive galvanometer being interposed in the circuit. The electric response of A and B was obtained by alternately throwing a beam of light on A or B. The response of the leaf of *Musa* was found to be negative, the D effect masking the A. I will presently describe means for unmasking the A reaction.

## The Photo-Electric Cell

The use of non-polarisable electrodes has, however, certain drawbacks: (1) the electrodes offer a very great electric resistance; (2) the zinc sulphate solution in the U-tube may leak into the plant-tissue and exert a poisonous action. These difficulties I have been able to avoid by the device of a photo-electric cell, dispensing with non-polarisable electrodes. The two halves of the leaf are suspended in water in a glass vessel, the leaf having been divided along the midrib, and each half is connected with the galvanometer by means of a gold wire inserted into the midrib. The two halves correspond to the two metallic plates in a galvanic cell, the conducting electrolyte being the water. Stimulation of one half of the leaf by light gives rise to a responsive current flowing in one direction, while stimulation of the other half gives rise to a current in the opposite direction. The electric response is abolished on the death of the leaf.

## Unmasking of the A Effect on Sudden Stoppage of Light

It was stated that the positive A effect under light is often masked by the D effect. I have, however, succeeded in unmasking A by the employment of three independent methods, one of which is mechanical, the other two electrical.

When the light is suddenly stopped, the A effect is often found to be more persistent than the D, the result being a transient overshooting of response in the positive direction.

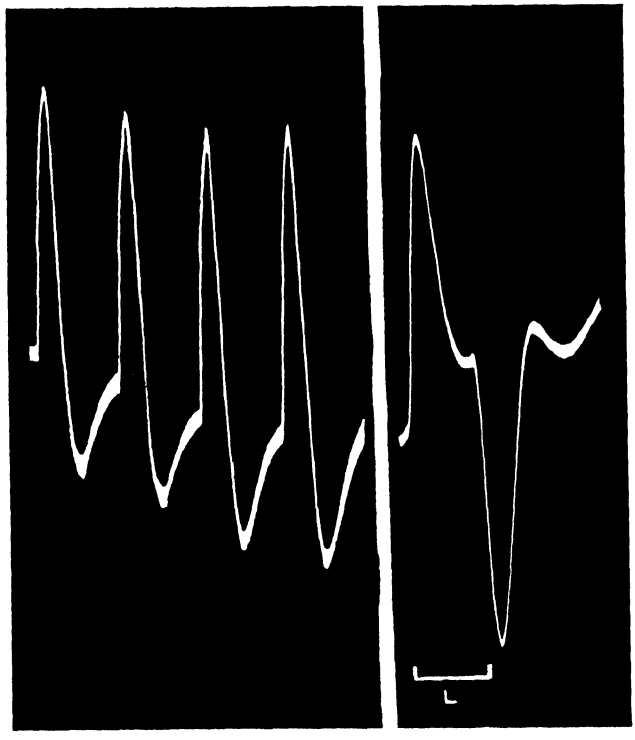



FIG. 19 FIG. 20

FIG. 19. The Negative Electric Response of *Musa* to Light, up-curve exhibiting the predominant D Reaction

Note the unmasking of A in the positive after-effect.

FIG. 20. The ' Overshooting ' of the Response of *Musa* in the positive direction on the cessation of Light

Note neutralisation under continuous light, and the unmasking of the positive A on stoppage of illumination L.

The positive after-effect has already been demonstrated by means of mechanical response (p. 56).

*Electric response : positive after-effect.*—In the record of the electric responses of *Musa* to light, the up-curves indicate galvanometric negativity during the continuance

of light. On the stoppage of light the unmasking of A is seen in the positive after-effect. For the recovery does not stop at the zero base-line, but goes beyond it towards the positive direction and then returns to zero (fig. 19).

*The phenomenon of overshooting after neutralisation.*— I have succeeded in demonstrating the A effect by a different method, where the positive, previously masked, exhibits itself by 'overshooting.' Under the continuous action of light the negative response undergoes a decline almost to neutralisation. This is due to the joint effects of fatigue and of increasing positive reaction which neutralises the negative. On the stoppage of light, the A effect, hitherto masked, exhibits itself by an overshooting of response in the positive direction (fig. 20).

## Positive Electric Response in *Hydrilla*

The *Hydrilla* plant readily absorbs $CO_2$ from water, and the anabolic activity A is quite evident from the rapid rate of evolution of oxygen during photosynthesis. No doubt the excitatory D process is also in operation, but I hoped that in very active specimens the anabolic A would be sufficiently pronounced not to be completely masked by the catabolic D. My anticipations were fully verified, as in the following experiments.

Two middle portions of *Hydrilla* stems bearing leaves were employed as the two plates of the photo-electric cell, electric connections with the galvanometer being made by gold wires thrust through the interior of the stems. After a suitable period of rest the normal activity of the plant was found to be restored. The photo-electric cell was filled with tank-water containing a sufficient amount of $CO_2$. Alternate exposure of the two plants to sunlight (suitably reflected by a mirror) caused photosynthesis, evidenced by the evolution of oxygen by each plant in turn. The photo-electric cell was enclosed in a dark box provided with a photographic shutter for giving the necessary exposure.

I give a record (fig. 21) of the electric response thus obtained; the duration of exposure was one minute, and a very large down-response occurred, indicating galvanometric *positivity* of the exposed leaves. On cessation of light, the electric response disappeared during recovery. The record here given shows the predominant A effect. The responses of *Musa* and of very actively assimilating *Hydrilla* under light are seen to exhibit characteristic differences on account of the relative predominance of the D or A effect. In *Musa* D is predominant, A being exhibited either as a positive after-effect or by the overshooting of response in the positive direction. In an exceptionally active *Hydrilla* plant, on the other hand, A is predominant and the response is positive. In less vigorous *Hydrilla* the positive becomes masked by the negative, when the resultant response appears to be similar to that of *Musa*.

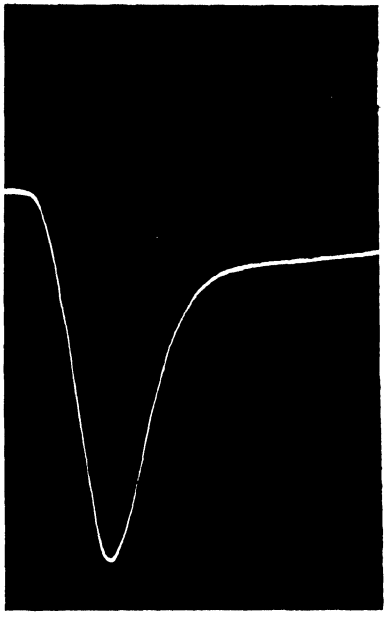


FIG. 21. The Positive Electric Response of *Hydrilla*

We will next observe the characteristic changes in the positive response of *Hydrilla* under external variations.

## Effect of Variation of Temperature

All physiological activity is arrested at a sufficiently low temperature; it is increased with the rise of temperature, till we obtain the strongest action at the optimum; at a still higher temperature the activity undergoes a decline.

These characteristic effects are manifested in the positive electric response of *Hydrilla*. The temperature was first

lowered by the application of water cooled with ice; the response is seen to have been very feeble. The temperature was next raised to the optimum, and then above the

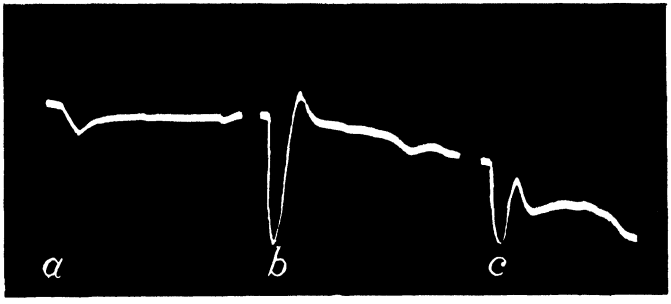



FIG. 22. Effect of Variation of Temperature on Electric Response of *Hydrilla*

(*a*) Feeble response at low temperature, enhanced to (*b*) at optimum and depressed to (*c*) beyond the optimum temperature.

optimum. This caused at first an increase, and afterwards a diminution, of the amplitude of response (fig. 22). The electric response of a photosynthetic organ thus exhibits the characteristic changes under physiological variation of temperature.

## Effect of Narcotics

*Ether.*—In the last chapter we observed the abolition of photosynthetic activity under the continued action of the narcotic ether. The positive electric response of *Hydrilla* is also found to be abolished after etherisation.

*Carbon dioxide.*—Determination of the effect of $CO_2$ is of much theoretical importance. It is true that a certain amount of carbon dioxide is necessary for photosynthesis, but its presence in excess must induce a general physiological depression. I have found growth to be depressed even to the point of arrest by an excess of carbon dioxide The geotropic curvature of stems is not only arrested but even reversed by the action of this gas. The depressing

action of $CO_2$ is clearly seen in the autonomous pulsations of the *Desmodium* leaflet (fig. 23). The normal response is

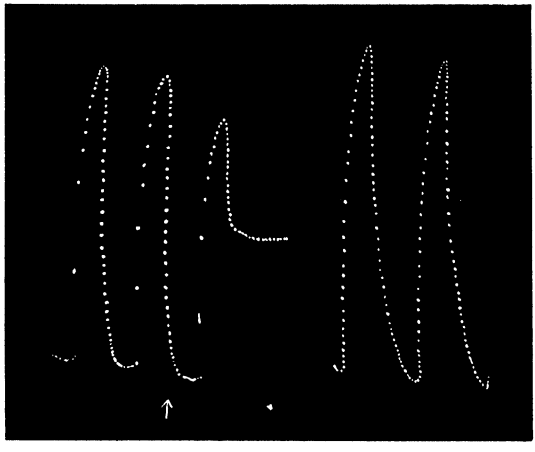


FIG. 23. Arrest of *Desmodium* pulsation under excess of $CO_2$ at arrow, and renewal on removal of the Gas

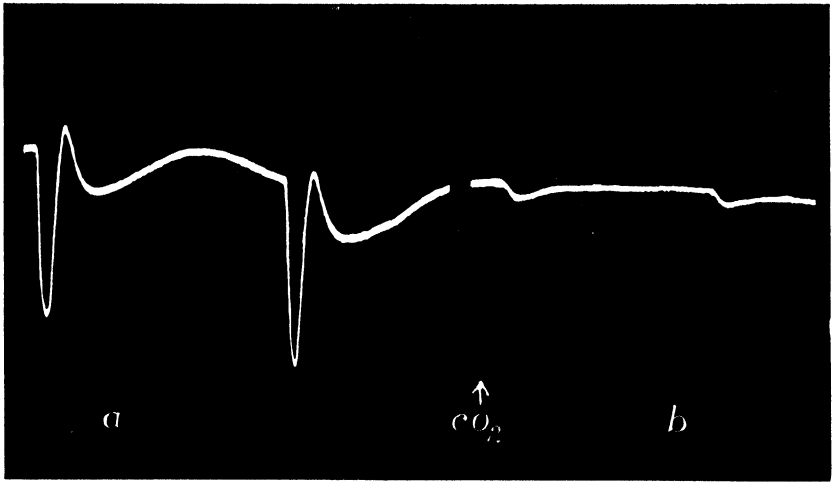



FIG. 24. Depression of Electric Response of *Hydrilla* under excess of Carbon Dioxide

(*a*) Normal response; (*b*) depressed response under application of excess of $CO_2$ at arrow.

arrested by the action of this gas, and its removal is followed by restoration of normal activity.

As regards the effect of $CO_2$ on the anabolic electric response of *Hydrilla*, the following experiment demonstrates the depressing effect of long-continued action of an excessive quantity of this gas. The normal positive electric response of *Hydrilla* in tank-water containing the normal proportion of the gas is shown in the accompanying record (fig. 24, *a*). Water charged with excess of $CO_2$ was next introduced into the photo-electric cell, with the result of the gradual abolition of response.

It will be shown in Chapter XV. that an excess of carbon dioxide also induces a depression of the photosynthetic activity of the *Hydrilla* plant.

## SUMMARY

The leaf of *Mimosa* under excitation undergoes a fall; this excitatory D reaction is detected by a simultaneous electromotive change of galvanometric negativity. The electric response is independent of the mechanical response, and persists even when the motile response is inhibited by external or internal restraint.

The electric response of leaves on exposure to light is most conveniently obtained by the device of the photo-electric cell in which the two plates are leaves or plants.

In the electric response of leaves the predominant negative D often masks the positive A. On the cessation of light, A becomes unmasked and is exhibited as a positive after-effect.

The A effect may also be unmasked by the phenomenon of ' overshooting.'

The electric response of the actively assimilating *Hydrilla* plant is positive, indicative of the predominant anabolism A.

The positive electric response of *Hydrilla* is appropriately modified under physiological variations. Rise of temperature to an optimum enhances response; temperature above the optimum induces a depression.

Anæsthetics such as ether induce depression or abolition of the electric response. Excess of carbon dioxide induces a depression or arrest of growth; it also arrests the pulsatory activity of the leaflet of *Desmodium gyrans*. The electric response of *Hydrilla* is similarly depressed or abolished by an excess of carbon dioxide. Photosynthesis undergoes a parallel depression.

# CHAPTER XI

## PHENOMENON OF PHOTOSYNTHETIC INDUCTION

The relation between the quantity of light and the amount of photosynthesis under continuous light—'Uphill' or positive work—Cessation of photosynthesis on stoppage of light—Molecular strain under photic stress—Partial undoing of positive work on cessation of light—Induction-period and its prolongation under increasing periods of previous darkness—The cyclic curve—Effect of physiological inertia.

THE rate of photosynthetic evolution of oxygen is uniform under constant intensity of light; I have shown in a previous chapter that the amount of photosynthesis is proportional to the quantity of incident light. This law of photosynthetic action is strictly applicable under the action of *continuous* light. The question now arises as to whether this quantitative relation holds good in the case of intermittent light. This would appear to be hardly probable from the theoretical considerations given below.

Photosynthesis is, as we have seen, a process of building up, the work being 'uphill,' the evolution of oxygen coming to a stop after the cessation of light. We may compare this with the rolling of a stone uphill by a continuous push, the stoppage of which is followed by a cessation of movement.

But the stoppage of uphill movement on the cessation of the push is temporary, for the stone may roll 'downhill,' thus undoing the positive work that had been accomplished. In photosynthesis likewise there is a possibility of positive work being partially undone during the cessation of light. The photosynthetic production of carbohydrate is brought about by a series of chemical dissociations and combinations. In the antecedent dissociation the molecules have to be put in a state of strain as a preliminary to rupture. We may visualise the process by observing the

effect of increasing stress on a piece of india-rubber string, which has to be continuously stretched before the breaking-point is reached. A certain amount of preliminary work has thus to be performed in stretching the string, so that the final pull brings about rupture. This preliminary work is partially undone if the stretching force acts intermittently instead of continuously. There is thus a greater efficiency with a continuous than with an intermittent force. In photosynthesis under continuous light a steady condition is attained when the successive bubblings occur at intervals of, say, T, which is the time required for the production of a definite volume of gas. But, if the light be discontinued for a time, the preliminary work may be undone and the molecular strain under light preceding dissociation gradually disappear, the more so the longer the duration of stoppage of light. The result of this would be a prolongation of the period preceding the appearance of the first bubble after re-exposure to light, which I designate as the *Period of Photosynthetic Induction.* From what has already been stated, this induction-period, I, necessary for the evolution of a definite volume of gas, would increase the longer the duration of previous darkness. This increase would not, however, go on indefinitely; for it could not exceed the period of the complete molecular recovery from the photic stress. The induction-period will thus reach a limiting value beyond which there is no further increase.

T may be regarded as the induction-period for continuous light; this will increase to I, I′, I″, with increasing periods of previous darkness. We shall then have $T < I < I' < I'' \ldots$

Having explained the theoretical considerations, the following results are given in confirmation. The experiment was carried out under uniform light from the sky, the temperature being 20° C. Under continuous light the successive bubbles appeared at intervals of 20 seconds. Light was then interrupted for 20 seconds, and the effect of

this period of darkness was that, on re-exposure, instead of the bubble appearing after 20 seconds, it appeared after 25 seconds. One minute's darkness increased the induction-period to 45 seconds, 10 minutes' darkness to 160 seconds, 20 minutes' darkness to 270 seconds, 30 minutes' darkness to 385 seconds, 40 minutes' darkness to 450 seconds, and 60 minutes' darkness to 510 seconds. The results are given in the following tabular form.

TABLE XIII.—PHOTOSYNTHETIC INDUCTION-PERIOD UNDER INCREASING PERIODS OF PREVIOUS DARKNESS

| Duration of previous darkness in minutes | Induction-period in seconds |
|---|---|
| 0 | 20 |
| 1 | 45 |
| 10 | 160 |
| 20 | 270 |
| 30 | 385 |
| 40 | 450 |
| 60 | 510 |

Normal bubbling-period under continuous sky light = 20 seconds

These results show that while an increase of the period of darkness from 0 to 20 minutes increased the induction-

TABLE XIIIA.—EFFECT OF CYCLIC VARIATION OF PERIODS OF DARKNESS ON THE INDUCTION-PERIOD

| Duration of previous darkness in minutes | Induction-period with increasing duration of darkness | Induction-period with decreasing duration of darkness |
|---|---|---|
| | Seconds | Seconds |
| 1 | 60 | 96 |
| 5 | 150 | 200 |
| 10 | 275 | 310 |
| 20 | 435 | 460 |
| 40 | 480 | 495 |
| 60 | 510 | 510 |

period from 20 seconds to 270 seconds, or nearly fourteen-fold, a similar increase in the period of darkness from 40 to

60 minutes increased it from 450 seconds to 510 seconds, *i.e.* only 1·1 times; the induction-period had thus practically reached the limit.

To make allowance for any change that may occur during the experiment, the series of operations were next carried through a complete cycle and the determination

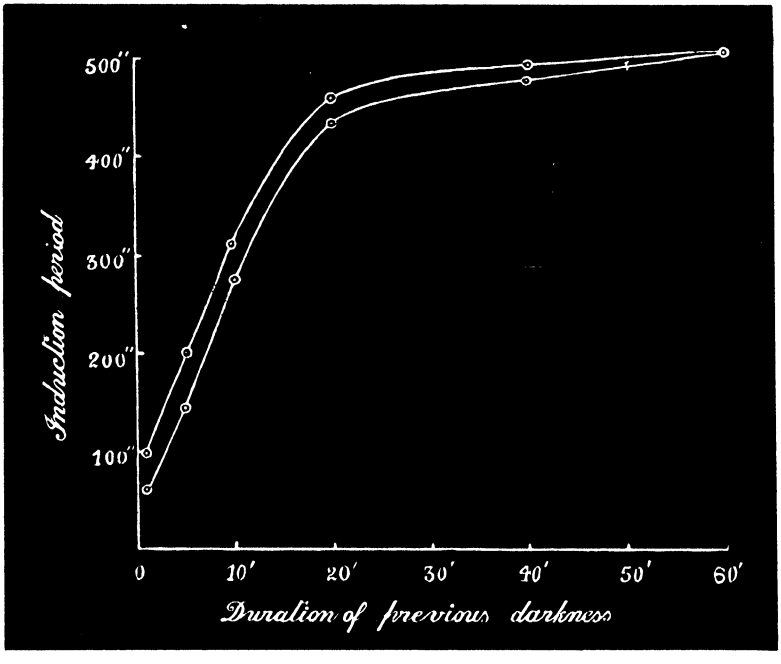



FIG. 25. Cyclic Curve showing effect of Increasing (lower curve) and Decreasing (upper curve) Periods of Darkness on Induction-period

made with periods of darkness increasing as 1, 5, 10, 20, 40, 60 minutes, and decreasing in the reverse order, 40, 20, 10, 5 and 1 minute. The results are given in Table XIIIA (p. 85).

The induction-period is thus seen to increase with increasing duration of previous darkness. The curve is straight up to 20 minutes, but afterwards it tends to become horizontal, the increase of induction-period reaching the limit (fig. 25).

It may be urged that the increase in the induction-

period described above may be due not to chemico-physiological action but to certain other causes; that during the prolonged period of darkness the oxygen which filled the intercellular spaces might have been removed either by absorption by the water or in respiration, and it would then take some time for the empty spaces to be filled up once more with oxygen: that the result is in fact attributable, wholly or in part, to an absorption-respiration factor. This plausible explanation is, however, unsatisfactory, since both the processes of absorption of oxygen by water and loss by respiration are relatively slow. There are, moreover, two independent tests by which the conclusion that the prolongation of the induction-period is in fact a chemico-physiological phenomenon is established. I describe here the first of these tests; the second, namely the effect of successive intermittent illuminations of short duration, will be treated in the next chapter. If the prolongation of the period for the appearance of the first bubble on re-exposure after previous darkness were simply due to the delay in filling the intercellular spaces with oxygen, then, as these spaces are already filled at the evolution of the first bubble, the subsequent second and third bubbles should be at normal intervals. If, on the other hand, the delay were due to physiological inertia in the sense already described, the delay would persist for a short time: the evolution of the second, and probably of the third, bubble would exhibit a gradually diminishing retardation till the normal rate was restored.

The following experiment demonstrates the existence of the physiological factor. The rate of appearance of successive bubbles under continuous light from the sky was 20 seconds. The light was cut off for periods which increased from 1 to 5 and 10 minutes (see table on p. 88).

It will be noted that, while the induction-period was in the three cases increased from 20 seconds to 48, 85 and 120 seconds, the second bubbles also exhibited a retardation, the periods being 21, 30 and 70 seconds respectively. It

was generally at the fourth bubble that the normal rate of 20 seconds was restored.

| Duration of previous darkness in minutes | Successive bubbling-periods in seconds |
|---|---|
| 1 | 48, 21, 20, 20 |
| 5 | 85, 30, 22, 20 |
| 10 | 120, 70, 25, 20 |

In the second experiment of the series the constant source of light was Pointolite. The effect of increasing duration of previous darkness is given in the tabular state-

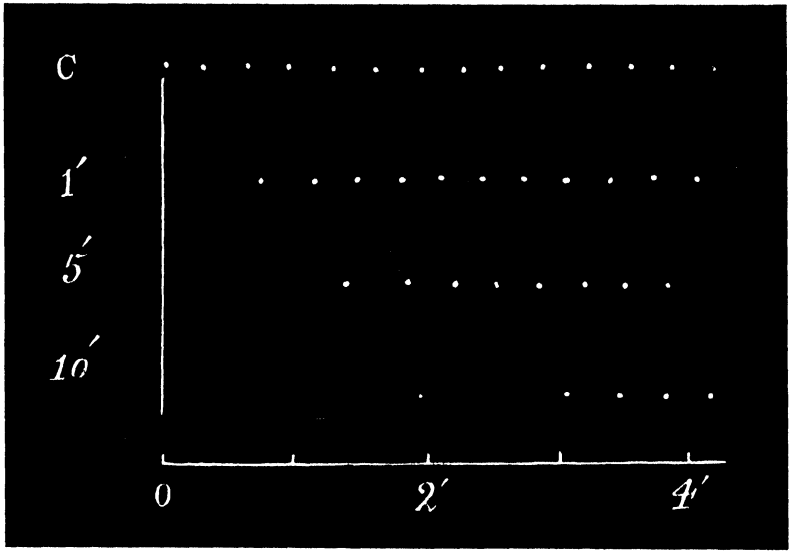



FIG. 26. Successive Records showing Increasing Induction-periods with Increasing Duration of Previous Darkness

Note that not only is the period of the first bubble delayed, but also the second bubble. The record c at the top shows successive bubbles at 20 seconds' interval under continuous light.

ment on p. 89. The normal period under continuous light was 25 seconds.

I also reproduce automatic records, obtained in the first experiment, of the effect of increasing periods of previous

darkness on the induction-periods of the successive bubbles (fig. 26). The results afford strong evidence of the partial

| Period of previous darkness in minutes | Successive bubbling-periods in seconds |
|---|---|
| 1 | 70, 27, 26, 25 |
| 5 | 320, 30, 26, 25 |
| 10 | 520, 30, 29, 25 |

neutralisation of the positive work, brought about by molecular recovery during darkness. In the next chapter I attack the same problem by the independent method of rapid intermittent exposures to light alternating with equal periods of darkness.

## SUMMARY

The total volume of oxygen given out is proportional to the duration of exposure to *continuous* light of uniform intensity.

This quantitative relation does not hold good under interrupted light. The 'uphill' or positive work done in the light is partially undone during the period of darkness.

The induction-period is increased with the period of previous darkness, till it reaches a limit.

# CHAPTER XII

## EFFECT OF INTERMITTENT LIGHT ON PHOTOSYNTHESIS

Further evidence of the intervention of the molecular factor—Effect of intermission on photosynthetic activity under sky light and under Pointolite—Diminution of photosynthetic activity under moderate frequency of intermission—Increased activity under higher frequency —The curve of variation of activity under intermittent light.

CONSIDERATION of the results given in the last chapter leads to the conclusion that the prolongation of the induction-period under interrupted light is principally due to the undoing of the preliminary work of inducing the photic stress which precedes dissociation. Since the absorption-respiration factor acts relatively slowly, its effect would practically vanish on diminishing the period of intervening darkness in the intermittent illumination. But molecular recovery, by which the preliminary work is partially undone, would commence almost immediately after the cessation of light, and the effect of intermittent light would therefore in general be relatively ineffective as compared with that of continuous light.

Crucial evidence of the existence of molecular stress preceding actual photosynthesis would be obtained by the observation of the effect of exposing the plant to rapidly alternating light and darkness of equal periods of, say, 0·5, 1, 2, 3, 5 and 10 seconds. The results would afford an independent demonstration of the relative importance of the absorption-respiration and of the molecular factors.

(1) If the photosynthetic efficiency is to any great extent modified by the absorption-respiration factor, the fact would be exhibited by retardation in the rate of bubbling increasing with the duration of the intermittent periods of darkness; that is to say, the effective rate would be lower

at an intermission of 10 seconds than at one of 5 seconds. As already said, the processes of absorption and respiration are relatively slow, hence short intermission such as that of 3 seconds would produce little or no retardation.

(2) As regards the molecular factor, the physiological momentum may be expected to last for only a very short time, after which the molecular strain will begin to disappear, and the photosynthetic efficiency will decrease with the duration of the intermittent darkness. Thus, to take a concrete example :

If we expose the plant for, say, 160 seconds to (*a*) continuous and (*b*) intermittent light, the total duration of exposure in the latter case will be 80 seconds. If the number of bubbles given out under continuous light (zero intermission) for 80 seconds be N, and under intermittent light of the same total duration be N′, then—

Photosynthetic rate per hour under continuous light will be

$$A_c = \frac{N}{80} \times 60 \times 60$$

Photosynthetic rate per hour under intermittent light will be

$$A_i = \frac{N'}{80} \times 60 \times 60$$

If now it be found that $N' = N$, it follows that photosynthetic efficiency is not affected by intermission ; but if it be found that $N' < N$, then clearly intermission has caused a decline of activity which becomes more marked the longer the periods of intermission, and may therefore be attributed to molecular recovery.

The retardation induced by increasing periods of intermittent darkness will not, however, increase indefinitely. For increase in the period of intermission of darkness and light would also mean exposure to the action of light for longer periods, which would be more effective. Hence we would expect a turning-point in the curve which represents photosynthetic efficiency at various rates of

intermission. The presence of this turning-point would, moreover, prove to be a crucial test for the important factor of photo-molecular strain: for the retardation induced by the absorption-respiration factor would increase continuously without any turning-point.

There is a further modification due to rapid intermission which will be presently explained.

## Effect of Intermittent Sky Light

The following is an account of experiments carried out on the above lines. The plant-vessel was enclosed in a

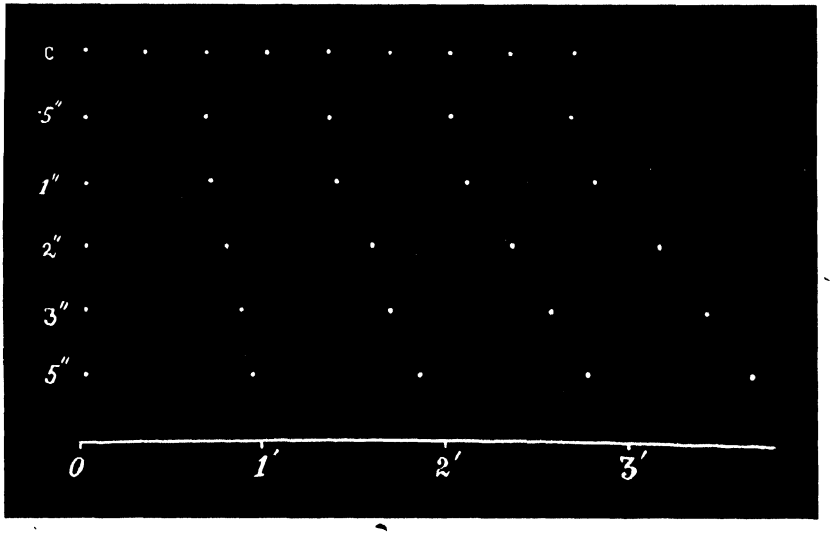



FIG. 27. The Effect of Intermittent Light on Photosynthetic Activity

First line *c* is the record under continuous light. The record in the second line is of intermission of 0·5 second, the last dot being slightly to the left, indicating slightly enhanced efficiency. In the third and succeeding lines note the increase in the bubbling-period as seen in the shifting of the last dot to the right (sky light).

dark box, provided with a photographic shutter worked by a special clockwork by which intermittent exposure consisting of equal alternating periods of light and darkness was produced, the periods being 0·5, 1, 2, 3, 5 and 10 seconds.

The variations of photosynthetic activity under intermittent illumination of different periods of sky light are clearly shown in the series of horizontal records (fig. 27).

(1) The first line is the record under continuous light, there being 8 equal spaces representing 8 bubbles in the course of 160 seconds ; that is, 20 seconds per bubble, or 4 bubbles in 80 seconds, the rate being

$$\frac{4}{80} \times 60 \times 60 = 180 \text{ bubbles per hour.}$$

(2) The second line is the record with an intermission-period of 0·5 second. There are now 4 bubbles in the course of 160 seconds, or 40 seconds per bubble. On account of alternating light and darkness, the total exposure to light was 80 seconds, and the rate of photosynthetic evolution of oxygen was practically the same as before, $\frac{4}{80} \times 60 \times 60 = 180$ bubbles per hour. Careful inspection of the record shows, however, that the fourth bubble occurred earlier, the dotted record being slightly to the left of the last dot in the first line. I will presently refer to this characteristic in greater detail.

(3) At an intermission-period of 1 second, the bubbles appeared at intervals of 41·7 seconds, *i.e.* for actual exposure to light of $\frac{41 \cdot 7}{2} = 20 \cdot 85$ seconds, the rate being $\frac{3600}{20 \cdot 85}$ $= 172 \cdot 8$ bubbles per hour. There is here a distinct diminution in the rate, due to intermission.

(4) The fourth line shows the effect of 2 seconds' intermission, the bubbling-period being increased to 47·6 seconds and the photosynthetic activity reduced to 152·2.

(5) At an intermission-period of 3 seconds, the bubbling-period was increased to 51 seconds and the activity reduced to 141·2.

(6) The sixth line is the record of the effect of 5 seconds' intermission-period. The period of bubbling was increased to 55 seconds and the activity decreased to 130·8. Up to this point there had been a continuous increase in the

retardation, as will be clearly seen by the marked shifting of the last dot to the right in the series of records, the increasing retardation being associated with decreasing photosynthetic efficiency.

I next increased the intermission-period to 10 seconds, the record of which is not given in the illustration. The bubbling-period, which was 55 seconds at 5 seconds' intermission, was now *shortened* to 52 seconds, and the photosynthetic activity increased to 138·4. There is thus a turning-point, which is crucial evidence of the reality of a photo-molecular strain.

The results show that increasing the intermission-period produces diminution of photosynthetic efficiency; but a maximum period is attained after which there is a turning-point, any longer period of intermission giving rise to a partial recovery in photosynthetic effectiveness. These results are in perfect agreement with the theoretical considerations which have been put forward. The turning-point is sometimes found at an intermission-period rather longer than 5 seconds. The effect would obviously be modified to a certain extent by physiological factors such as the condition of the tissue, temperature, and the intensity of light.

## Intermittent Pointolite Illumination

Though intermittent illumination is, generally speaking, less effective than continuous light, yet the second line of the automatic record (fig. 27) shows that for very short intermission the effectiveness may be greater; for the fourth bubble is seen to have occurred in a total period of illumination shorter than 80 seconds. This result was so unexpected that I was at first inclined to believe that it might have been brought about by some accidental variation in the intensity of light from the sky. I therefore employed in the present series of experiments the constant light of Pointolite. The intermission of light was also very accu-

rately adjusted by means of rotating sectors interposed in the path of light, the various periods being 1, 2, 3, 5, 7, 10, 15 and 20 seconds. In order to make the results easily comparable with each other, the activity under continuous light is taken as 100. Under intermission of 1 second, the photosynthesis is found to be 121, *i.e.* an enhancement of 21 per cent. At an intermission of 2 seconds it was 108 ; at an intermission of 3 seconds the activity became lowered to 96. At an intermission of 5 seconds the activity was further reduced to 80 ; at 7 seconds' intermission it was 70, and at 10 seconds' there was a maximum reduction of activity to 63. This was the turning-point, for at an intermission of 15 seconds the activity was partially restored to 79, and more fully to 88 at an intermission of 20 seconds. The results are graphically illustrated (fig. 28), where the ordinate represents the activity, and the abscissa the increasing periods of intermission in seconds. The typical results given above with Pointolite are very definite and exhibit characteristics similar to those under sky light, the slight difference being ascribable to a different physiological condition of the two specimens and to the different intensities of light employed.

The activity above the normal with a short intermission-period is theoretically of great importance, since it gives some indication of a physico-chemical action underlying photosynthesis. The following is a probable explanation of the phenomenon. Light, as previously stated, gives rise to dual reactions A and D, the resultant being due to their difference. We also found that the D effect often disappears immediately on the cessation of light, while the A effect persists for a short time. This was exhibited on the cessation of light (1) by the transient enhancement of the rate of growth above the normal and (2) by the positive after-effect in electric response (pp. 56, 76).

The enhancement of photosynthetic activity under short intermission thus appears to be the immediate after-effect of light, the A effect persisting for a short time. With

longer intermission-periods, the molecular recovery causes increasing retardation till the turning-point is reached. The curve of photosynthetic efficiency thus exhibits three phases: the enhanced efficiency under short intermission

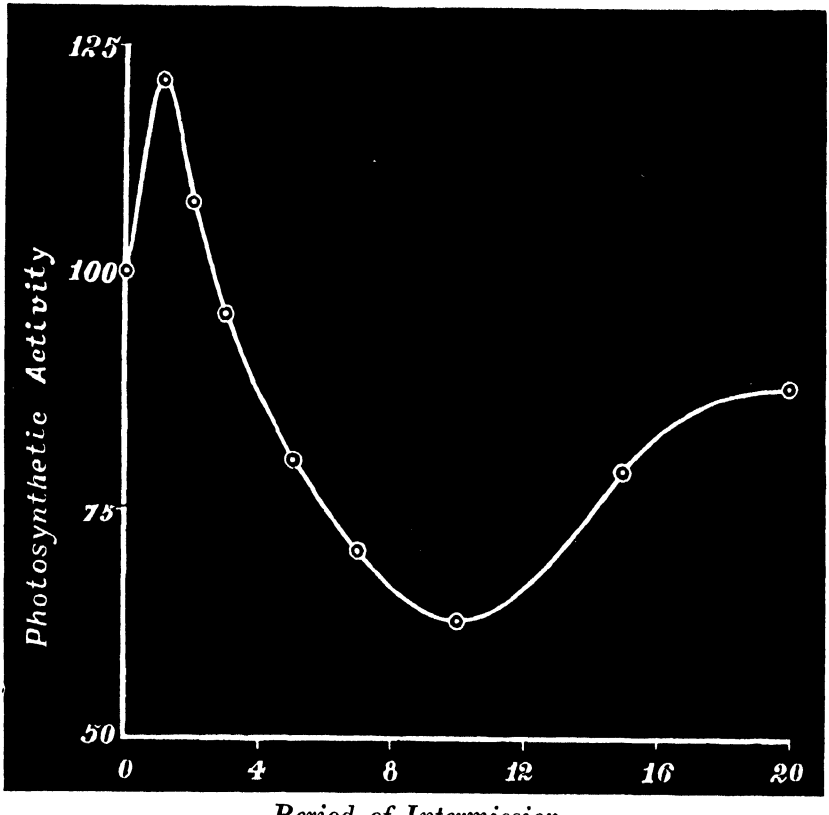



FIG. 28. Curve showing Variation of Photosynthetic Efficiency at various rates of Intermission of Light

Note enhancement of efficiency in the first stage, lowered efficiency in the second stage, and partial recovery in the third stage.

in the first stage, passing through the point of neutralisation to the second stage of decreasing efficiency under increasing periods of intermission, reaching the turning-point; after this at the third stage there is an exhibition of partial recovery.

## SUMMARY

Intermittent illumination of increasing duration lowers photosynthetic efficiency till a turning-point is reached, after which there is a partial recovery of efficiency.

This is a crucial test, the results of which support the theory of photic stress and resulting molecular strain.

With a short intermission-period the efficiency is momentarily above the normal. A probable explanation of this is found in the fact that under continuous illumination the resultant effect is A−D; on the cessation of light A persists longer than D, as demonstrated in the experiment described in Chapter X.

## CHAPTER XIII

### THE AUTOMATIC RADIOGRAPH

The Selenium Cell—Elimination of effect of polarisation—The Wheatstone Bridge for balance—The automatic keys for periodic closure of circuit—The Galvanograph—Automatic record of hourly variation of daylight—Record on a cloudy day—Simultaneous records of variation of daylight and temperature—Light-noon and thermal noon.

THE most important requirements in the quantitative investigation of photosynthesis are, as previously stated, the maintenance of uniform intensity of light, and a means of effecting the quantitative increase of the intensity.

The illumination to which plants are accustomed is that of direct sunlight and of diffused light from the sky; we shall in this chapter confine our attention to these natural sources. Both sunlight and sky light are liable to variation, and there is no means at present available by which we may be assured that the light remains constant during the period of an experiment. This difficulty is specially great when the period of observation is prolonged. Our power of judging variations in the intensity of light is, unfortunately, very defective. In making observations on photosynthesis under the light of the sky it frequently happens that the bubbling-rate undergoes variation though the light appears to be unchanged. By means of the Electric Photometer, which will be presently described, it can, however, be shown that the variation in photosynthesis in such a case is really due to a change in the intensity of light, caused by the passage of impalpable mist undetected by the eye. That a slight variation of light may cause considerable change in photosynthesis is shown by slowly moving the plant through a short distance from a position near the window to the interior of the room. In such an

experiment the variation of light as perceived by the eye is very slight, yet the effect on assimilation is very pronounced, for the vigorous photosynthesis near the window becomes slowed down and arrested.

In utilising sunlight and its dependent light from the sky in experiments on photosynthesis, it is therefore necessary to find the rate at which daylight undergoes variation from hour to hour. In doing this we discover a turning-point in the intensity of light, where the variation is from positive to negative, from an increase to a decrease. At this turning-point the variation of intensity of light is very slight; hence this is the most suitable period for photosynthetic observations.

For the determination of the hourly variations of daylight, and of the turning-point beyond the maximum intensity of light, I have devised a special instrument, the Radiograph.

## The Selenium Cell

Any method for obtaining a record of the intensity of light and its variations depends on utilising the property of some substance sensitive to light. Selenium is well known for the characteristic diminution of its electric resistance under illumination. A definite deflection is produced in a galvanometer when the selenium cell is placed in the dark in series with a battery of voltaic cells. Exposure to light, causing a diminution of resistance, gives rise to an increased deflection. The variation in the deflection of the galvanometer thus indicates the variation in the intensity of light. The selenium cell is relatively more sensitive to the less refrangible rays of the spectrum. I will show in a later chapter that the less refrangible rays are likewise more effective in photosynthesis. Hence the selenium cell is quite suitable for the measurement of the effective rays.

Several difficulties are encountered in practice in obtaining a continuous record of the intensity of light during the day. The resistance of selenium undergoes a change under

the continued action of an electric current ; this is due to polarisation caused by the current, which increases with its strength and duration. But the effect of polarisation is negligible if the current be feeble and of short duration. Another difficulty which might possibly interfere with the accuracy of the readings is the effect of daily variation of temperature on the normal resistance of the selenium cell. This effect may be eliminated by observing, at different hours of the day, the difference in the resistance of the cell (1) in the dark, and (2) after exposure to light. Finally, we have to devise some means for the automatic record of the galvanometric deflection under changing intensities of light.

## The Radiograph

The difficulties enumerated above have been completely overcome by the following devices :

(*a*) A Wheatstone Bridge for balancing the electric resistance of the selenium cell in darkness and its upset on exposure to light ;

(*b*) An arrangement of three electric keys which are automatically put on and off in regular sequence and at predetermined intervals ;

(*c*) A Self-recording Galvanograph.

## The Wheatstone Bridge

This is diagrammatically represented in B (fig. 29). The resistance of the particular selenium cell S is 76,000 ohms in the dark. An approximately equal resistance is placed in the second arm of the bridge. A rheostat, with a large number of turns of fine wire with a sliding contact, is used for the two variable arms of the bridge, diagrammatically represented by a straight line. An approximate balance is obtained when the sliding contact is in the middle ; a slight movement to the right or to the left secures the exact balance, when the galvanometer deflection is reduced to zero. The balance is upset when the selenium cell is

exposed to light, and the resulting deflection gives a measure of the intensity of the light.

## The Automatic Keys

After previous adjustment of the balance in the dark, the electric circuit is completed by the closure of key $K_1$, after

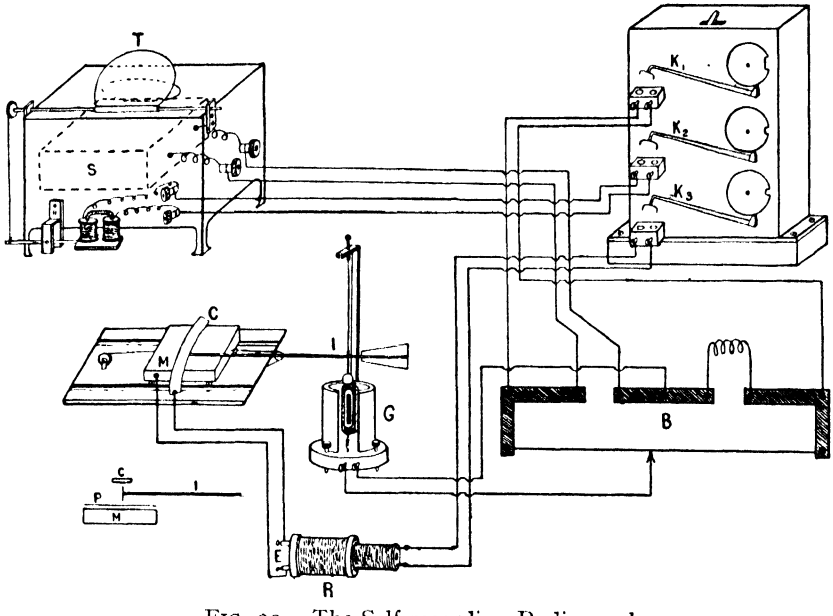



FIG. 29. The Self-recording Radiograph

The selenium cell, S, is periodically exposed to light by the electro-magnetic shutter, T. The selenium cell forms one arm of the Wheatstone Bridge, B. The three keys, $K_1$, $K_2$, $K_3$, are periodically closed and opened by clockwork. G, the recording galvanometer with index, I, carrying a double-pointed platinum wire at its end, which moves between the metal strip, C, and the plate, M. P, paper for record. R, sparking coil with its electrodes connected with C and M. The battery is not shown in the figure (see text).

which the selenium cell is exposed to light by an automatic electro-magnetic shutter. The deflection of the galvanometer is recorded on a moving sheet of paper P by means of electric sparks. These different operations are carried out in proper sequence by the automatic devices described below.

Key $K_1$ completes the battery circuit for about 10 seconds,

by which time the record is completed. The records registering the variations of light are taken at intervals of 15 minutes; the periodic closures of the circuit are thus for 10 seconds at intervals of 15 minutes. In practice this short passage of the current is found to cause no polarisation.

The second key, $K_2$, actuates an electro-magnetic device by which the trap-door T is opened for the definite period of 1 second; the selenium cell S inside the dark box is thus exposed to light for this length of time. The trap-door is seen in the diagram in the roof of the dark box. In reality it is at the upper end of a vertical tube the inside of which is coated with lamp-black to prevent side-reflection. The light that falls on the selenium cell is from a definite area of the sky. The intensity of the light from the sky at different periods of the day causes deflection of the galvanometer which is proportional to that intensity. The maximum deflection of the galvanometer employed is attained in the course of 3 seconds after exposure.

The third key, $K_3$, is for the completion of a spark circuit which records the maximum galvanometric deflection 3 seconds after the exposure of the selenium cell. This key actuates a sparking coil R, the vibrating interrupter of which is not shown in the figure. The sparks, thus produced, puncture the maximum deflection of the galvanometer index on the moving sheet of paper. The details of this process will be presently given.

The successive closing and opening of the keys are made automatically and in proper sequence by means of clockwork, the whole process being repeated at intervals of 15 minutes.

## The Galvanograph

We now come to the difficult problem of the automatic record of the galvanometer deflections. This might be secured without great difficulty by means of photography. A spot of light reflected from the galvanometer mirror might be allowed to fall on a photographic plate which

descends at a uniform rate by clockwork. This, however, would entail the use of a dark room and subsequent development of the plate. The trouble is avoided by the direct record of the galvanometer deflection by means of electric sparks.

A sparking method had been previously employed in which the deflected index of the galvanometer in connection with one electrode of an induction coil leaves a spark record on a moving sheet of paper. Several difficulties were, however, encountered in the employment of this method with a highly sensitive galvanometer. There is a liability to leakage of the high-tension current into the galvano-circuit. Secondly, the discharge of the spark gives a backward kick to the index by which the normal deflection undergoes an unknown variation.

These difficulties were overcome in the following manner. The moving coil of a sensitive D'Arsonval galvanometer bears a long glass index I, at right angles to the plane of the coil. The glass index is coated with shellac varnish to render it highly insulating. The index is prolonged to a short distance on the opposite side, for the attachment of a counterpoise; this takes the form of a vertical vane of mica which acts as a damper. The galvanometer itself is of an aperiodic type, and the addition of the damper makes it perfectly dead-beat. The sensitiveness of the galvanometer is such that a micro-ampere of current produces a deflection of 10 mm. of the index. The recording index has attached to it a short vertical piece of thin platinum wire pointed at its two ends; this end of the index moves between a sheet of metal M and a narrow semicircular strip of metal C. The metal sheet M, bearing the sheet of thin paper P for the record, is mounted on wheels and moves at a uniform rate by clockwork. Record is made by sparks: one electrode of the sparking coil is in connection with C, and the other with M. The sparking thus takes place simultaneously, above and below the vertical and double-pointed platinum wire carried at the end of the index. There

is thus no resultant kick, and the index remains undisturbed. The sparking, as previously stated, takes place 3 seconds after exposure of the selenium cell to light, by which time

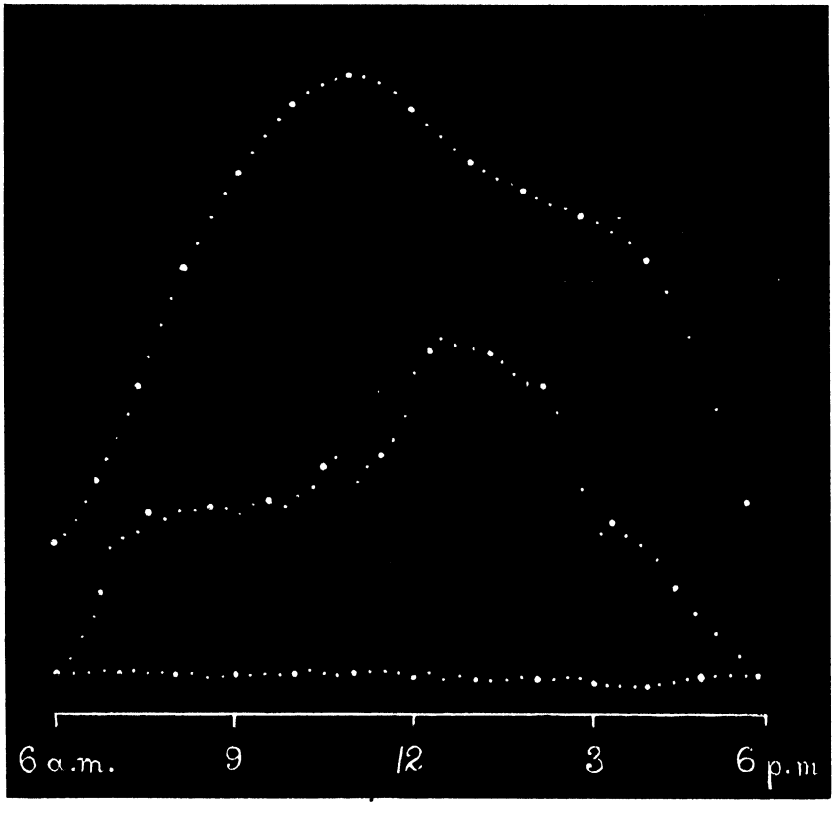



FIG. 30. Radiogram of Variation of Intensity of Light from the sky during 12 hours in winter

The upper record shows the variation on a bright day, the maximum intensity being attained at 12 noon. The lower record exhibits irregular variation on a cloudy day. The horizontal record above the base-line shows that the electric resistance of the selenium cell is but slightly affected by variation of temperature. Successive thin dots at 15 minutes' interval, thick dots at intervals of an hour.

the deflection has reached its maximum. The record thus consists of successive dots at intervals of 15 minutes, the dots representing the maximum deflections of the galvanometer corresponding to the intensity of light.

I have recently been able to perfect another clockwork device by which the bent tip of the galvanometer index is periodically pressed against a moving sheet of smoked paper. This simple arrangement eliminates the complications of the spark-record.

The record given in fig. 30 was taken about the end of January ; the sun rose at about 6.45 A.M. and set at 5.30 P.M.

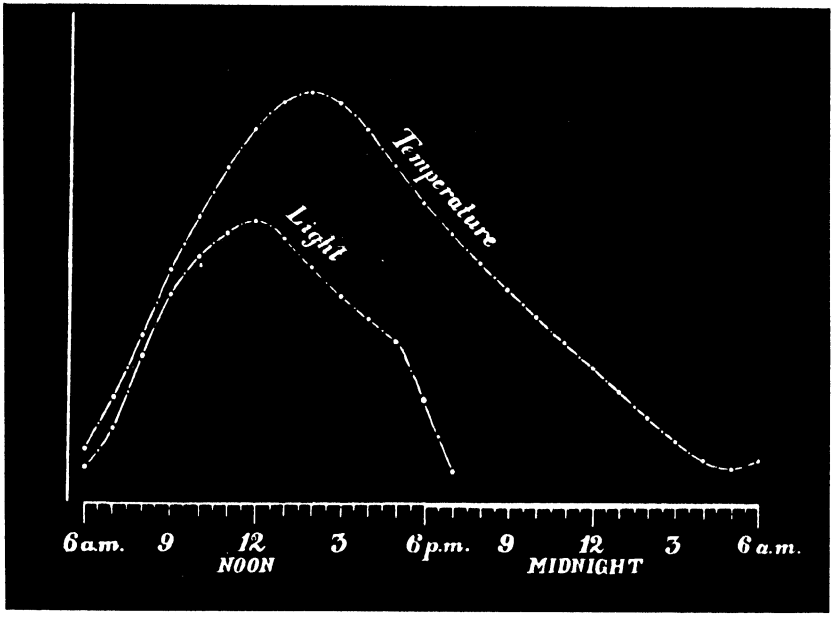



FIG. 31. Record of Diurnal Variation of Light and of Temperature in Summer

The twilight is very short in the tropics : the sky is feebly lighted about 6 A.M. ; it becomes dark about 6 P.M. The record shows the intensity of light to have been exceedingly feeble at 6 A.M. The rise in the intensity was rapid, attaining the maximum at 12 noon. This will be designated as the light-noon. The intensity of light then declined at a rate slower than the rise. But after 5 P.M. the fall of intensity was extremely rapid.

It was stated that there is a possibility of change of

resistance induced by diurnal variation of temperature. In order to determine the extent of this variation, a spark record was also obtained before exposure to light. The dotted record near the base-line shows that the resistance remained practically constant throughout the day, in spite of the variation of the temperature.

In order to determine the diurnal variation of light and of temperature and their periods of maximum and minimum, records were simultaneously taken one day in summer with the Thermograph and the Radiograph. The two curves are given in fig. 31. It will be seen that, while the maximum intensity of light is at 12 noon, the thermal maximum is at about 2 P.M. The thermal noon is thus two hours later than the light-noon. Light disappears at night from 6 P.M. to 6 A.M.; that is to say, the period of minimum is prolonged for twelve hours. But the fall of temperature is gradual, and the minimum is attained at about 5 A.M., which is the thermal dawn. The characteristic variations of these two important factors should be borne in mind, since it is known that the diurnal movements of plants are modified by the algebraical summation of the effects of light and of temperature.

It is sometimes desirable to carry out researches during a period when the intensity of light remains approximately constant; this period is found to be between 11 A.M. and 1 P.M.

The above record of the diurnal variation of light is true of days when the sky is clear. But the passage of a cloud causes change in the intensity which is accurately recorded by the Radiograph. A record of such irregular variation on a stormy day is given in the lower record of fig. 30.

## SUMMARY

The Radiograph gives a record of the diurnal variation of light. On a clear day in January the intensity was found to increase rapidly from 6 A.M. to 12 noon, when it reached

its maximum. Light began to decline slowly up to 5 P.M., the decline being less rapid than the rise in the forenoon. The fall of intensity was very rapid after 5 P.M.

The light-noon and the thermal noon do not coincide: the latter is about two hours later.

## CHAPTER XIV

### THE ELECTRIC PHOTOMETER

The Portable Electric Photometer—Calibration of the Photometer—Measurement of widely varying intensities of light—Best aspect of sky for uniform light—Variation of light from northern sky in the course of the day—Sunlight and artificial light.

HAVING found that the intensity of light is nearly uniform from 11 A.M. to 1 P.M., we have next to choose the most suitable position for utilising the outdoor light for the experiments. For certain special investigations sunlight would be required; but such an intense light, if used for too long a time, is liable to induce physiological depression. The most suitable source of illumination for general purposes is therefore the light from the sky. The plants are at their best after the rainy season, but the sky then becomes clouded in an unexpected manner; one has therefore to take advantage of the short periods during which the sky is clear. In the winter season, from November to January, the days are generally unclouded, but the physiological condition of the plant is not at its best on account of the cold. In investigations carried out at different seasons of the year, the important condition of constancy of light has to be kept in view; the intensity of light during the course of the experiment has therefore to be repeatedly measured.

The apparatus devised for this purpose is a Portable Electric Photometer, which has been rendered highly sensitive and reliable (fig. 32). Selenium is also used here as the sensitive element; it is placed in the fourth arm of a small Wheatstone Bridge of a box pattern. The ratio of the arms of the bridge is 100 : 1000. Balance was secured in the dark when the third arm was 4755 ohms; the resistance

of the particular selenium cell in the dark is therefore 47550 ohms. The galvanometer deflection depends on the intensity of light and also on the electromotive force of the voltaic cell. This latter is a single Edison cell of large capacity, the electromotive force of which is 1·4 volt. As a very small current is utilised for measurement, the voltage remains constant day after day. Hence the deflection of the galvanometer is proportional to the intensity of light.

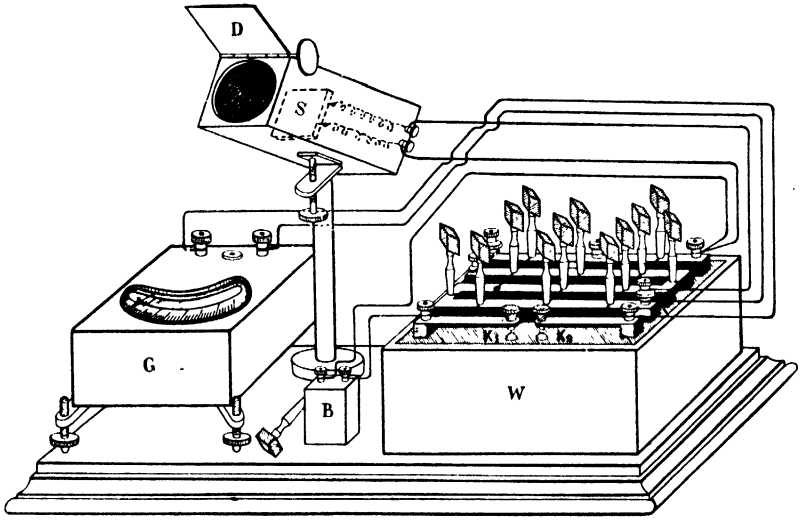



FIG. 32. The Portable Electric Photometer
S, selenium cell; D, shutter; W, Wheatstone Bridge, with tapping keys $K_1$, $K_2$; B, voltaic cell; G, unipivot galvanometer.

The galvanometer employed is of a unipivot type, which is sufficiently sensitive for the purpose.

After the adjustment of the balance, the circuits of the voltaic cell and of the galvanometer are completed by successive pressure on the keys $K_1$ and $K_2$. The selenium cell is then exposed for 5 seconds to the light to be measured, in which time the deflection attains its maximum.

## Calibration of the Photometer

In order to calibrate the instrument, Pointolite is taken as the standard source of light, the small radiant of which

may be regarded as a point. As the intensity of light varies inversely as the square of the distance, the intensity of the light at various distances is known. We place the selenium cell at different distances and measure the corresponding deflections of the galvanometer.

From these results we are able to construct the calibration-curve from which the absolute value of the intensity of light in lux is obtained corresponding to any particular deflection of the galvanometer. The calibration-curve is practically straight for moderate variation in the intensity of light. It is sometimes necessary to measure widely different intensities of light, such as sunlight, sky light, or the light of Pointolite. When the galvanometer is made highly sensitive for the measurement of light of moderate intensity, sunlight sends the index out of the scale. We may get over this difficulty by two different means: (1) by reducing the sensitiveness of the galvanometer to one-tenth or one-hundredth by the employment of suitable shunts; (2) by sufficiently reducing the aperture which admits light to the selenium cell.

Again, through intermediate reduction of sensitiveness of the apparatus it is possible to compare the intensity of a known fraction of sunlight—say, 0·01 S—with a definite intensity in lux given by Pointolite.

## Best Aspect of Sky for Uniform Light

The variation of intensity of the light from the sky vertically above has been given (fig. 30). In the actual experiments, however, the plant received light either from the eastern, western or northern aspects. From November to February the sun rises south-east and sets south-west. The plant cannot be exposed to the south, since sunlight would fall directly on it. When the plant faces the east or west, the light which falls on the plant changes very rapidly. The results given below show that the light from the northern sky remains practically uniform from 11 A.M.

to 1 P.M. Since none of the experiments undertaken with the light from the northern sky lasted for more than an hour, the intensity of light employed may be taken to be constant. As an additional precaution, as already stated, frequent readings of the Photometer were taken. The experiment was rejected if the light did not prove to be constant.

The place chosen for the experiment is the garden of the Institute, open to the northern sky. There is a low building which extends from the east to the west, which protects the place of the experiment from the direct rays of the sun.

TABLE XIV.—READINGS OF THE ELECTRIC PHOTOMETER AT DIFFERENT HOURS OF THE DAY

(*Diffuse Light from Northern Sky*)

| Hours of the day | Photometric deflection |
|---|---|
| 11.30 A.M. | 63 |
| 11.45 ,, | 64 |
| 12 noon | 65 |
| 12.15 P.M. | 64 |
| 12.30 ,, | 62 |
| 12.45 ,, | 62 |
| 1 ,, | 60 |
| 2 P.M. | 48 |
| 2.15 ,, | 48 |
| 2.30 ,, | 47 |
| 2.45 ,, | 46 |
| 3 ,, | 46 |
| 4 P.M. | 44 |
| 4.15 ,, | 43 |
| 4.30 ,, | 40 |
| 4.45 ,, | 31 |
| 5 ,, | 28 |
| 5.15 ,, | 26 |
| 5.30 ,, | 15 |

## Sunlight and Artificial Light

In certain experiments we require strong light the intensity of which may be maintained uniform or increased or decreased in a definite and known manner. The intensity

of sunlight is, as we have seen, practically uniform between 11 A.M. and 1 P.M. I have explained how the intensity of sunlight can be decreased in a known manner by the employment of a double convex lens which gives a divergent beam of light. For moderate intensity of light, Pointolite gives, as we have seen, a range of from 100 to 5000 lux.

Speaking generally, sunlight is most suitable for experiments with strong light. The light from the Pointolite is employed when we wish to use light of moderate intensity which may be increased or decreased in a definite manner. Finally, we have the light from the sky, which may be secured practically constant under proper conditions.

The most serious difficulty in the quantitative determination of the activity of photosynthesis has thus been removed, and the constancy of the various sources of light secured.

## Summary

By means of the Portable Photometer immediate determination of the intensity of light can be made from the galvanometer deflection.

Widely different intensities of light can also be measured with the same apparatus by suitable reduction of sensitiveness by shunting the galvanometer or by reduction of the aperture for the incident light.

By taking readings through intermediate stages, the intensity of sunlight may be compared with that of Pointolite.

The diffuse light from the northern sky remains practically constant about midday, when the light is most intense.

# CHAPTER XV

## RELATION BETWEEN $CO_2$-SUPPLY AND PHOTOSYNTHESIS

Depression of general physiological activity under $CO_2$—Narcotic and poisonous action of excess of $CO_2$ on photosynthesis—Photosynthetic curve for $CO_2$-variation—The turning-point—Determination of the photosynthetic coefficient—Effect of seasonal variation on the coefficient.

We shall study in this chapter the quantitative relation between the $CO_2$-content of the solution and the resulting photosynthetic activity. It is obvious that there should be a lower and an upper limit: no photosynthesis can, in normal conditions, take place in the absence of $CO_2$; excess of $CO_2$, on the other hand, produces a narcotic or even a poisonous effect. We have already seen that excess of $CO_2$ causes an arrest of activity in all physiological processes, partly through deprivation of the oxygen so necessary in all vital activities, and partly through the narcotic action of the gas. Since the decomposition of $CO_2$ produces a certain amount of oxygen, it would take a relatively larger proportion of $CO_2$ to cause an arrest of photosynthesis. An excess of $CO_2$, though not immediately injurious, may yet prove to be so after long-continued action. For example, with a proportion of 10 mg. of $CO_2$ in 100 c.c. there is most vigorous photosynthesis; this activity is, however, found to undergo a slow decline even to permanent arrest. The specimen thus treated does not show any revival of activity even after replacement in a weaker solution of $CO_2$. On the other hand, I may mention instances where the specimen was kept in a solution of 7 mg. of $CO_2$ per 100 c.c., yet the activity was found to remain uniform for at least five days, though the cut specimen was all the time confined within the plant-vessel with the

Bubbler-attachment. For long-continued experiments it is therefore advisable to employ solutions containing about 8 mg. per 100 c.c.

## The Special Plant-Vessel

For obtaining the characteristic curve under variation of $CO_2$, it is necessary that an identical plant should be used for the complete investigation, since the curve is liable to modification by the physiological condition of the specimen. Special care has to be taken that the fresh introduction of solution of a different strength does not cause any mechanical disturbance, for a mechanical shock cannot but affect the normal rate. The apparatus devised for the introduction of different solutions without causing mechanical shock to the plant has already been described (see fig. 14); the different solutions are introduced by the appropriate manipulation of the stop-cocks.

The constant light employed was that of Pointolite at about 1200 lux. Each investigation was completed in less than an hour.

## The Photosynthetic Curve under Variation of $CO_2$-Concentration

For descriptive purposes I reproduce (fig. 33) a characteristic curve in which the ordinate represents the activity and the abscissa the $CO_2$-concentration of the solution in mg. per 100 c.c. This curve may be regarded as typical, the curves given by a dozen different specimens being practically the same.

Referring to the general curve, we find that it is straight from a concentration of 3 to 7·5 mg., and that a turning-point occurs after 8 mg. After this, photosynthesis *tends to reach a limit, and this limiting curve is not abruptly horizontal.* The above description holds good in the generality of cases; but in a few instances a depression was produced

above the concentration of 12 mg. per 100 c.c., when the curve exhibited a reversal.

I give detailed results obtained with three different specimens taken from the same pond during the winter

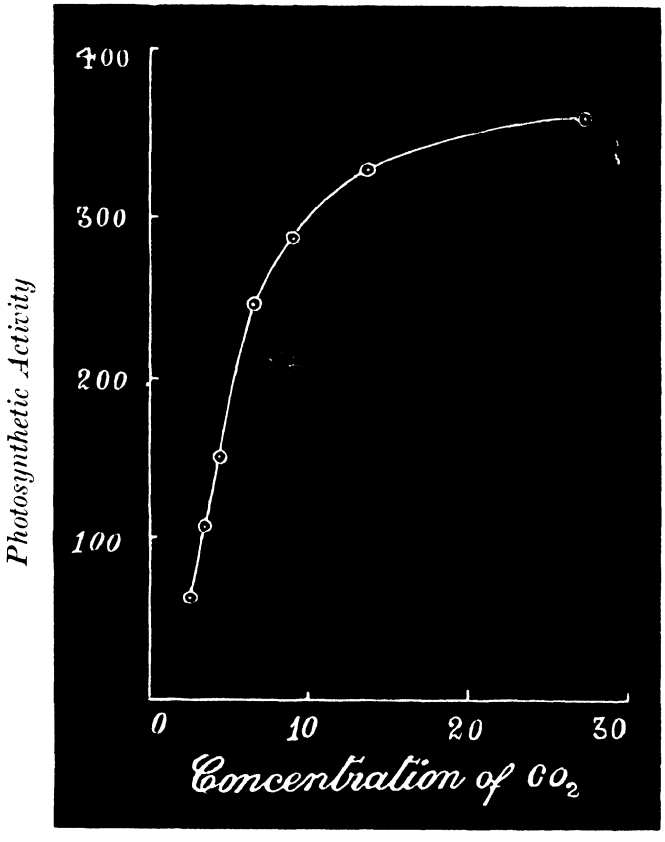



FIG. 33. The Photosynthetic Curve under Variation of $CO_2$-concentration in mg. per 100 c.c.

months from November to January. The plants were less sensitive in winter than in spring, as will be seen from other results which will be given presently.

I compare my results (see Table XIVA, p. 116) with others obtained by Pantanelli and by Blackman in their experiments with *Elodea*. Pantanelli (1903) employed the method of counting the bubbles from the cut end of the plant; the

unreliability of this method must account for his high value of 20 mg. per 100 c.c. for the turning-point. Blackman's results are in agreement with mine; for he (by the method of estimation of $CO_2$ absorbed) finds the maximum to be at or about 10 mg. of $CO_2$ per 100 c.c. of water.

TABLE XIVA.—SHOWING PHOTOSYNTHETIC ACTIVITY AT DIFFERENT CONCENTRATIONS OF $CO_2$ (POINTOLITE)

(*Winter-specimens*)

| Specimens | $CO_2$-content in mg. per 100 c.c. | Activity in c.mm. $O_2$ per hour |
|---|---|---|
| I. | 3·00 | 70·96 |
| | 3·75 | 99·22 |
| | 5·00 | 149·50 |
| | 6·00 | 194·75 |
| | 7·50 | 248·46 |
| | 10·00 | 336·17 |
| | 15·00 | 425·76 |
| | 30·00 | 456·18 |
| II. | 2·70 | 60·50 |
| | 3·37 | 87·27 |
| | 4·50 | 131·73 |
| | 6·75 | 215·88 |
| | 9·00 | 245·96 |
| | 13·50 | 293·10 |
| | 27·00 | 319·32 |
| III. | 2·62 | 70·07 |
| | 3·50 | 110·16 |
| | 5·25 | 183·57 |
| | 7·00 | 249·57 |
| | 10·50 | 293·31 |
| | 15·75 | 314·88 |
| | 21·00 | 328·19 |

I reproduce Blackman's average curve, which was obtained from irregularly distributed points. The causes of these irregularities are explained by Blackman as follows:

'With *Elodea* the assimilation values along the horizontal part of the curve are not as regular as with *Fontinalis*, but their agreement is perhaps as close as might be expected when it is noticed that the experiments range over three

years, and that identity for this value in successive experiments depends on repeating identically intense stimulation of an identical area of leafy tissue. The intensity of illumination varies with the square of the distance of the chamber from the light, and this had to be adjusted for each experiment but could not be measured directly, as the bath window and cooling screen intervened. An error of a few millimetres may have occurred, and the light must have

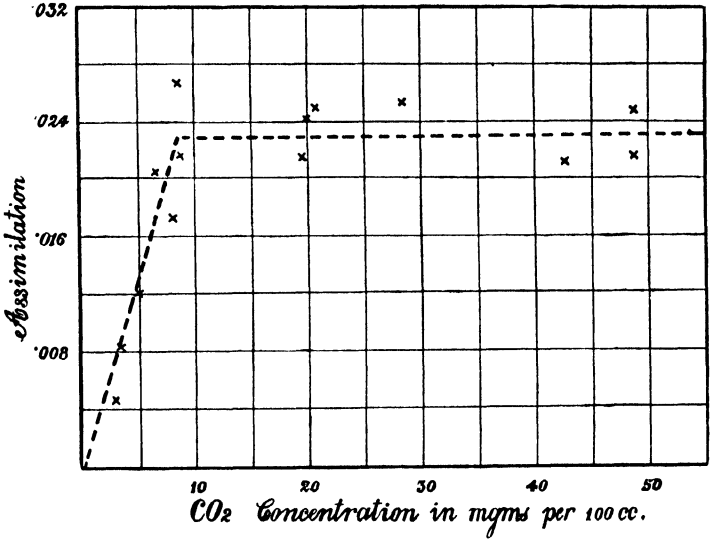



FIG. 34. Curve of Photosynthesis of *Elodea* under Variation of $CO_2$ concentration (after Blackman)

The crosses represent the points in the curve, the dotted line being the mean curve.

varied from changes in the efficiency of the mantles. The uniformity of the area illuminated, 137 sq. cm., depends upon careful packing and distribution of the green shoots upon the silver grid; this was always covered as completely as possible, and the error from this cause is probably not great.'[1]

My results show that the top of the photosynthetic curve rounds off gradually after the turning-point, and is never abruptly horizontal as in the curve obtained by Blackman. No conditions could have been more ideally perfect than in

[1] Blackman and Smith, *Proc. Roy. Soc.*, B, 1910, pp. 392, 410.

my experiments. The solutions were introduced into the plant-vessel without causing any disturbance; light and temperature were maintained absolutely constant; the same plant was used throughout the investigation, so that the physiological condition was uniform; changes due to seasonal variation were excluded; the observations were completed in the course of less than an hour. The remarkable smoothness of the curves which I obtained testifies to the high accuracy which is secured by the new method. With this it is possible to obtain a physiological constant for the plant which is found to be almost as definite as a physical constant of inorganic matter.

## Determination of Coefficient for $CO_2$-Concentration

We found that the photosynthetic curve for variation of $CO_2$-concentration is straight up to 8 to 9 mg. per 100 c.c. We can therefore determine the coefficient by dividing the increment of activity by the increment of $CO_2$-concentration which induces it. For unit variation of concentration I will take an increase of 1 mg. per 100 c.c.

$$K = \frac{A_C - A_c}{C - c} \quad . \quad . \quad . \quad . \quad (1)$$

where $A_C$ is the activity for the higher concentration C, and $A_c$ is the activity for the lower concentration *c*.

We will first determine the coefficient of the specimen (I.) for two pairs of different concentrations: (*a*) 3 mg. and 5 mg., and (*b*) 5 mg. and 7·5 mg.; and then those of other specimens.

SPECIMEN I. (*a*) $CO_2$-concentrations of 3 mg. and 5 mg.:

Activity for 3 mg. = 70·96
,, 5 mg. = 149·50

$$K = \frac{149{\cdot}5 - 70{\cdot}96}{5 - 3} = 39{\cdot}27$$

(*b*) $CO_2$-concentrations of 5 mg. and 7·5 mg.:

Activity for 5 mg. = 149·50

,, 7·5 mg. = 248·46

$$K = \frac{248 \cdot 46 - 149 \cdot 50}{7 \cdot 5 - 5} = 39 \cdot 58$$

SPECIMEN II. $CO_2$-concentrations of 2·7 mg. and 4·5 mg.:

Activity for 2·7 mg. = 60·5

,, 4·5 mg. = 131·73

$$K = \frac{131 \cdot 73 - 60 \cdot 5}{4 \cdot 5 - 2 \cdot 7} = 39 \cdot 57$$

SPECIMEN III. $CO_2$-concentrations of 2·62 mg. and 7·0 mg.:

Activity for 2·62 mg. = 70·07

,, 7·00 mg. = 249·57

$$K = \frac{249 \cdot 57 - 70 \cdot 07}{7 \cdot 0 - 2 \cdot 62} = 40 \cdot 95$$

The practical agreement of the coefficients of the different winter-specimens is indeed remarkable. It shows that under similar conditions the physiological constants of different plants are of the same order. I shall in a later chapter explain a different method of obtaining the coefficient from measurements on the physiological scale.

The following is the formula for the calculation of a photosynthetic activity $A_C$ at a higher concentration, from the activity $A_c$ at a lower concentration:

$$A_C = A_c + K (C - c)$$

## Seasonal Variation of the $CO_2$-Coefficient

The general physiological depression due to winter persisted till the middle of February, after which the activity increased with the return of spring. By the end of February the photosynthetic activity exhibited a general enhancement. In the winter months no photosynthesis occurred at a lower $CO_2$-concentration than 2·5 mg.;

but by the end of February the activity was initiated at as low a value as 1 mg I give the following results of observations made with a spring-specimen, the light being maintained constant at 1200 lux from the Pointolite.

TABLE XV.—PHOTOSYNTHETIC ACTIVITY UNDER VARIATION OF $CO_2$-CONCENTRATION (POINTOLITE)

(*Spring-specimen*)

| Specimen | $CO_2$-concentration | Activity in c.mm. $O_2$ per hour |
|---|---|---|
| IV. | 1·00 | 71·4 |
| | 2·00 | 142·2 |
| | 3·50 | 252·1 |
| | 5·00 | 355·5 |
| | 7·00 | 469·0 |
| | 10·00 | 533·6 |
| | 12·00 | 540·0 |
| | 15·00 | 535·1 |

The curve obtained from the above data is similar to that given in fig. 33. It is straight up to the turning-point at about 9 mg. After this the curve becomes rounded, the maximum being attained gradually and not abruptly. A reversal often occurs above a concentration of 12 mg. per 100 c.c. The curve of spring-specimens is more erect than that of winter-specimens.

Determination of the coefficient of spring-specimen :

SPECIMEN IV. $CO_2$-concentrations of 2 mg. and 5 mg. :

Activity for 2 mg. = 142·2

,, 5 mg. = 355·5

$$K = \frac{355·5 - 142·2}{5 - 2} = 71·1$$

The average coefficient for the winter-specimens is 39·9, that for the spring-specimens is 71·1. The ratio of the coefficients for spring- and winter-specimens is therefore 1·8 or nearly 2. This was the ratio which we also found in the enhancement of the coefficient for light under variation

of season. The effect of a favourable season is therefore to produce a simultaneous increase of the coefficients for various factors.

## SUMMARY

In experiments on the effect of different concentrations of $CO_2$, the introduction of the different solutions into the plant-vessel causes a mechanical disturbance which modifies the normal rate of bubbling. This was avoided by a special device.

The effect of excess of $CO_2$ is to produce a narcotic or a poisonous action.

The general characteristic exhibited by the photosynthetic curve in relation to $CO_2$-supply is that it is straight up to a concentration of about 8 mg. of $CO_2$ per 100 c.c. Above this there is a turning-point, when the curve becomes rounded and gradually approaches the maximum. The upper part of the curve is never abruptly horizontal.

The coefficient alters with the season. The average coefficient in winter is 39·9; in spring it is nearly double that in winter.

# CHAPTER XVI

## PHOTOSYNTHETIC EVOLUTION OF OXYGEN IN THE COMPLETE ABSENCE OF CARBON DIOXIDE

Photosynthetic evolution of oxygen by *Hydrilla* in complete absence of supply of carbon dioxide—Photosynthesis of specimens in acid condition under increasing supply of carbon dioxide—Translocation of the characteristic points in photosynthetic curve—Electric response of acid plants—Unreliability of method of $CO_2$-absorption—Assimilatory and respiratory quotients—Assimilation of organic acids—Effect of increasing strengths of malic acid on photosynthesis—Possible use of product of internal respiration in photosynthesis.

THE experiments on the effect of different $CO_2$-concentrations carried out during winter months showed that the minimum concentration necessary for the initiation of photosynthesis was about 2·5 mg. of $CO_2$ per 100 c.c. of water; that the turning-point occurred at a concentration of about 9 mg., after which the photosynthetic curve became rounded off, exhibiting a slow rise. In spring-specimens the minimum $CO_2$-concentration for photosynthesis was 1 mg. per 100 c.c. of water, and a reversal of curve indicative of depression occurred above the concentration of 12 mg. per 100 c.c. The experiment was repeated in April, which may be regarded as the beginning of summer. Three specimens from the same pond were examined, and determinations made of the minimum $CO_2$-concentration necessary for initiation of photosynthesis. One of these gave the normal result—that is to say, the minimum $CO_2$-concentration was 1 mg. per 100 c.c. The results given by the second and third specimens were, however, found to be extraordinarily different, for they continued *to evolve oxygen in the complete absence of any supply of carbon dioxide.* The specimens had in fact been placed in distilled water, yet the rate of

evolution of oxygen was found to be as high as 60 c.mm. per hour in the one case and 71 c.mm. in the other. This evolution of oxygen in the absence of $CO_2$-supply was by no means transitory, for it persisted at a uniform rate during the whole period of observation, extending over two hours. The bubbles appeared only under exposure of the plant to light, the rate increasing with the intensity of light. The bubbling took place even in freshly boiled distilled water, but the appearance of bubbles in that case was delayed for about half an hour, for the escape of the gas is only possible after saturation of the water with the evolved oxygen. The bubbles appeared quickly in distilled water which had previously been made to absorb a small quantity of pure oxygen.

A few specimens exhibited this peculiarity early in April, but by the end of the month every specimen showed it: the complete transformation occurred in the course of a few weeks.

It is at first difficult to imagine how photosynthesis could take place at all in the absence of a supply of carbon dioxide. It must have been due to some internal change brought about by the change of season. Further investigation showed that, while the juice of the plants was practically neutral in winter and spring, it was very strongly acid in summer. The acid condition of the plant therefore appeared to be connected with its power of photosynthetic evolution of oxygen in the absence of $CO_2$. The acid condition in summer is probably associated with the prevailing high temperature, which, in contrast with the average temperature of 24° C. in spring, rose to 43° C. (110° F.) in summer. High temperature means increased respiration and greater production of organic acid. The acidity of the plants was found to be due to the presence of malic and oxalic acids, the latter in small quantities.

The results given in Table XVI. (p. 124) relate to the reactions of *Hydrilla* in an acid condition.

Comparing the results of non-acid plants in spring with

those of acid plants in summer, we find: (1) that in the former photosynthesis is initiated at a minimum $CO_2$-concentration of 1 mg. per 100 c.c., while in the latter it occurs in the absence of $CO_2$; and (2) that the point of reversal in spring-specimens is at 12 mg., while in acid specimens it is at the lower value of 9 mg.

TABLE XVI.—SHOWING THE EFFECT OF INCREASING $CO_2$-CONCENTRATION ON PHOTOSYNTHESIS OF ACID SPECIMENS OF *Hydrilla*

| $CO_2$-concentration in *n* mg. per 100 c.c. | Activity of evolution of $O_2$ in c.mm. per hour |
|---|---|
| 0 | 60 |
| 3 | 301 |
| 6 | 487 |
| 9 | 566 |
| 12 | 531 |

These facts show that in acid plants the organic acids serve as a partial substitute for $CO_2$; consequently the characteristic points in the photosynthetic curve are transposed backwards.

## Electric Response of Acid Plants

The conclusion arrived at above, that the organic acids present in the plant may, under certain conditions, serve as a substitute for carbonic acid, finds a very interesting confirmation in the results of a different line of investigation on the electric response. It has been shown (p. 78) that the anabolic process of building up which occurs in active photosynthesis has an electrical concomitant in an electric response of galvanometric positivity. As acid plants exhibit photosynthesis even in the absence of an external supply of carbon dioxide, I expected to obtain a marked positive electric response from these plants. This was actually observed in *Cicer arietinum*, the leaves of

which excrete malic and oxalic acids, and have been collected as ' vinegar ' from ancient times in India and used for medicine and diet.

Records of electric responses of the *Cicer* are given below which are seen to exhibit very pronounced electro-positivity. The photosynthetic action depends on the effective intensity of light, and it will be shown in a later chapter

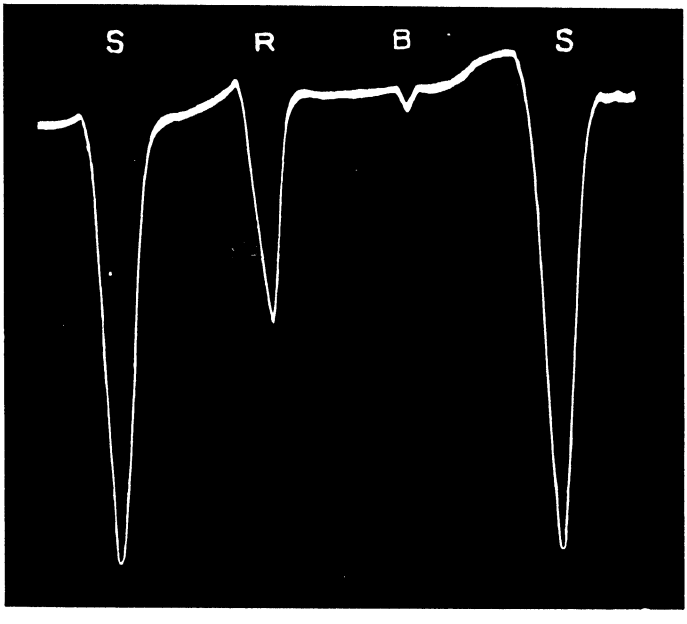



FIG. 35. Electric Response of *Cicer arietinum*

The first record, S, is under direct sunlight; the second, R, under light transmitted through red glass; the third, B, under light transmitted through blue glass; and the fourth, S, once more under direct sunlight.

that red light is more effective in photosynthesis than blue light. The electric response exhibits corresponding variations. The first large response (fig. 35, S) was given on exposure to strong sunlight. Red glass was now interposed in the path of light, and the response (R) was still of considerable amplitude. On the interposition of blue glass the response (B) became extremely feeble. The response taken once more under sunlight exhibited the original

amplitude, proving that the sensitiveness of the plant had remained constant during the whole period of the experiment.

The experiments which have been described show that the photosynthetic evolution of oxygen takes place in acid plants even without absorption of carbon dioxide. *Hence the evolution of oxygen is a surer index of photosynthesis than the intake of carbon dioxide.* Since organic acid serves as a partial substitute for $CO_2$, the quantity of this gas absorbed by acid plants under light will be less than in non-acid plants.

*Assimilatory quotient.*—This introduces certain complications in the ratio of the oxygen evolved to the carbon dioxide absorbed. Taking the following equation for the normal photosynthetic production of carbohydrate:

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

the normal assimilatory quotient $\frac{O_2}{CO_2} = 1$. But in acid plants the denominator of the fraction ($CO_2$) is less than normal; hence $\frac{O_2}{CO_2}$ has a value greater than 1.

*Respiratory quotient.*—In normal cases the respiratory quotient $\frac{CO_2}{O_2} = 1$; but in acid plants the quotient is less than 1, and in extreme cases it is zero.

The above considerations explain the variation from unity of the assimilatory and respiratory quotients observed in succulent plants. The biological feature of these plants is that they are *xerophytes, i.e.* are adapted in their form and structure to conditions (heat, dry soil, etc.) which involve protection of the contained water against excessive transpiration. Their structural adaptations are a thick epidermis with few stomata, and compact internal tissue with relatively small intercellular spaces. These peculiarities of structure affect, however, not only the exhalation of water-vapour in transpiration, but also the gaseous exchange

between the plant and the external air. The plant has difficulty in getting what $CO_2$ and $O_2$ it requires from the air.

In green plants during day-time, photosynthesis produces $O_2$ in the tissue, and so the respiratory needs are more or less provided for. But in darkness the supply of $O_2$ is insufficient to allow the formation of $CO_2$ in the oxidative catabolism. Consequently less highly oxidised organic acids are formed, which accumulate in the cells.

## The Assimilation of Organic Acids

The succulent plant which has become charged with organic acids in darkness becomes less acid on exposure to light, and evolves $O_2$. The question is, How is this effected? Aubert (1912) and Gerber (1897) argue that under the influence of light the organic acid is split into sugar and $CO_2$. Mayer found that malic acid in watery solution gave off $CO_2$ in light. It is contended that the $CO_2$ so produced is the material for the photosynthesis of the plant when exposed to light. According to this view, the organic acids stored during the night provide *indirectly* the material for photosynthesis during the day, in the form of $CO_2$.

It seems unreasonable to suppose that when the plant can assimilate or reduce so highly oxidised an acid as $H_2CO_3$, necessitating a large expenditure of energy, it should not reduce the less oxidised organic acids. The view that the organic acids serve *directly* for photosynthesis has been held by Tréboux and others. The *Hydrilla* plant appeared to be most suitable for further investigation on this subject, and I carried out parallel experiments with two specimens which had been rendered acid by the influence of seasonal variation and the prevailing high temperature. Having first observed the effect of increasing strengths of $CO_2$-solution on photosynthesis (*cf.* Table XVI.), I tried the effect of substituting malic acid for carbon dioxide.

## Effect of Increasing Strengths of Solution of Malic Acid

Preliminary experiments enabled me to find the range within which increased supply of the acid enhanced the rate of photosynthesis, avoiding an excessive dose which proves to be as toxic as an excessive supply of carbon dioxide. The following is an account of a typical experiment on the effect of supplying increasing strengths of malic acid. The particular specimen of *Hydrilla* evolved oxygen at a rate of 56 c.mm. per hour in distilled water; in a solution of malic acid 4·1 parts in 10,000 the activity was enhanced to 290 c.mm. per hour; double the strength of this dose, *i.e.* 8·2 parts in 10,000, increased it to 460 c.mm. per hour; 12·3 parts in 10,000 enhanced the rate of evolution of oxygen still further to 510 c.mm. per hour. This was the climax, for a stronger solution of 16·4 parts in 10,000 produced a depression, the rate being reduced to 455 c.mm. per hour; the results are given in the following tabular form.

TABLE XVII.—PHOTOSYNTHESIS WITH INCREASING STRENGTHS OF MALIC ACID SOLUTION

| Strength of solution in *n* parts per 10,000 of water | Photosynthetic activity in c.mm. $O_2$ per hour |
|---|---|
| 0 | 56 |
| 4·1 | 290 |
| 8·2 | 460 |
| 12·3 | 510 |
| 16·4 | 455 |

I reproduce (fig. 36) the photosynthetic curves of two different specimens of acid *Hydrilla*, the one under increasing $CO_2$-concentrations, and the other under increasing strengths of malic acid. The two curves show a remarkable resemblance throughout the entire range of reaction. The reversal took place just above 9 mg. of $CO_2$ in the one and 12·3 parts of malic acid in 10,000 in the other.

It is clear that malic acid serves as a perfect substitute for $CO_2$ in photosynthesis in acid specimens. I have, in fact, tried the effects of $CO_2$ and of malic acid alternately on the

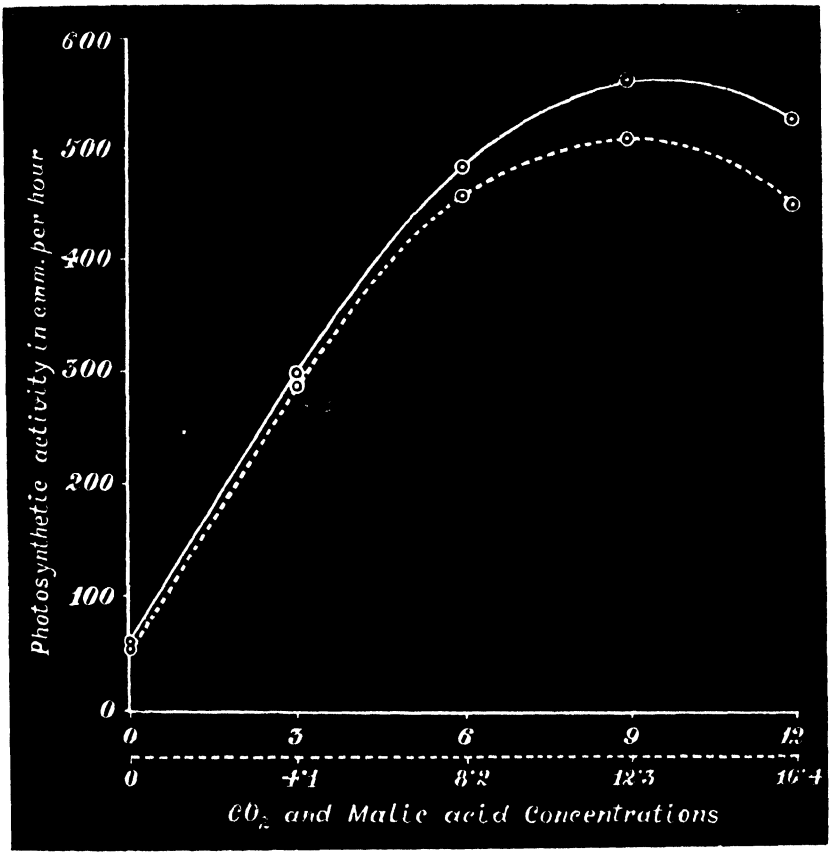



FIG. 36. Photosynthetic Curves under Increasing Concentration of $CO_2$ (upper curve) and of Malic Acid Solution (lower curve)

The upper abscissa represents $CO_2$-concentration in mg. per 100 c.c.; the dotted lower shows $n$ parts of malic acid in 10,000 parts of water.

same plant, and have found that the previous action of malic acid produced no prejudicial effect on the normal photosynthetic action with carbon dioxide. Care should, however, be taken to avoid an excessively strong dose of malic acid, which has a toxic effect.

Similar results were obtained with oxalic acid, the solutions of which had, however, to be made ten times more dilute to guard against any poisonous effect.

I have stated that all the *Hydrilla* plants became strongly acid in the course of a few weeks of April, during transition from spring to summer. The heat was greatly modified by the rainy season, which commenced in July. The result was that, by the third week of July, the *Hydrilla* plants reverted to the normal non-acid condition. It was also most interesting to find that their photosynthetic activity had also returned to the normal—that is to say, that the plant no longer exhibited any photosynthesis in the absence of an external supply of $CO_2$. The relation of the acid condition of the plant to its power of assimilation of organic acid is therefore fully established.

Though land-plants obtain their main supply of carbon dioxide for assimilation from the air, it is not impossible that a portion may also be derived from the carbonic acid dissolved in water in the soil. There is yet another possible source of supply of carbon dioxide for assimilation. In my work on the 'Physiology of the Ascent of Sap' I have shown that the ascent of water is maintained by the pumping action of actively pulsating cells situated in the inner cortex. The work performed in raising large masses of water to the top of the tree is considerable, and necessitates a corresponding consumption of energy. It is obvious that organic substances in the actively pulsating cells will be rapidly oxidised in order to supply the necessary energy. The carbon dioxide thereby produced will be carried in the same direction as the ascending sap along aeriferous systems, reaching the large intercellular spaces in the leaves. Here two streams of carbon dioxide will be available for assimilation, from the exterior as well as from the interior. Since plants can exploit their own organic acids in assimilation, it would be surprising if they did not utilise the carbon dioxide produced in their tissues by internal respiration.

## SUMMARY

*Hydrilla* plants, under seasonal variation of summer with its prevailing high temperature, become acid. Photosynthesis, as evidenced by evolution of oxygen, is then found to take place in the complete absence of supply of carbon dioxide.

The photosynthetic curve of acid *Hydrilla* plants exhibits a variation from the normal, the characteristic points of the curve being transposed towards the origin. This indicates that the organic acids present in the plant were assimilated in lieu of carbon dioxide.

The electric response of the markedly acid plant *Cicer arietinum* is positive, which is indicative of anabolic activity. This characteristic positive response takes place even in the absence of carbon dioxide.

Since, in the photosynthesis of acid plants, the organic acids can replace carbon dioxide, the absorption of $CO_2$ by these plants is less than normal. Hence the assimilatory quotient $\frac{O_2}{CO_2}$ in acid plants is greater than unity, while the respiratory quotient is less.

The photosynthetic curve with increasing strengths of malic acid exhibits a remarkable similarity to that with increasing $CO_2$-concentrations.

The acidity of the plant disappears during the rainy season, when photosynthesis is found to return to the normal.

It is highly probable that plants utilise for assimilation the carbon dioxide produced in their own internal respiration.

# CHAPTER XVII

## EFFECT OF VARIATION OF TEMPERATURE ON PHOTOSYNTHESIS

Determination of the respiratory loss—Importance of accurate adjustment of temperature—Shock-effect caused by sudden variation—Determination of thermometric minimum for photosynthesis—Phenomenon of accommodation—Thermometric minimum for autonomous pulsation—Determination of the optimum point for photosynthesis—Effect of intensity of light and of season—Photosynthetic curve under variation of temperature—Optimum point for various activities of plants—Determination of the temperature-coefficient of photosynthesis by the differential method.

THE investigation of the effect of variation of temperature on photosynthesis involves the determination (1) of the thermometric minimum, (2) of the optimum, and (3) of the general character of the photosynthetic curve.

In order to avoid errors of observation, special precautions have to be taken to produce and maintain constancy of the different temperatures, and an estimation must be made of the loss of oxygen in respiration. The volumes of oxygen evolved at different temperatures are, of course, reduced to standard temperature and pressure.

### Loss of Oxygen in Respiration

*Determination of oxygen by the $CO_2$ respired.*—Ten different plants of standard size were put into a bottle filled with 100 c.c. of oxygenated distilled water, another similar bottle filled with distilled water serving as a control. They were kept in the dark for 12 hours at a definite temperature. After the expiration of this period the water in both the bottles was tested separately by the volumetric method (standard alkali and phenolphthalein). The con-

trol was found free from $CO_2$, thus proving the absence of any accidental contamination during the period of the experiment. The process was repeated at temperatures of 18°, 22°, 27° and 32° C. The estimation of the $CO_2$ evolved in the bottle containing the plants gave the following results.

TABLE XVIII.—RATE OF RESPIRATION AT DIFFERENT TEMPERATURES

| Temperature | Rate of respiration in mg. $CO_2$ per hour | Rate of respiration in c.mm. per hour at N.T.P. |
|---|---|---|
| 18° C. | 1·32 | 6·60 |
| 22° C. | 1·49 | 7·45 |
| 27° C. | 1·70 | 8·50 |
| 32° C. | 1·90 | 9·50 |

The rate of loss by respiration at any intermediate temperature can be found from the curve plotted on these data. Since the average rate of evolution of oxygen under moderate intensity of light at 18° C. is about 150 c.mm. per hour, the loss of oxygen in respiration is about 4·4 per cent.

I have also determined the percentage of respiratory loss by another method. It will be shown in Chapter XXIII. that the production of carbohydrate during photosynthesis causes an increase of weight. In a typical experiment this increase in the weight of the plant after 9 hours' exposure to diffuse light from the sky was 1·4 mg., or 0·155 mg. per hour. After this preliminary experiment, the plant suspended from the balance in the dark lost through respiration 0·1 grm. in weight after 14 hours, or 0·0071 grm. per hour, at 18° C. average temperature. The percentage of loss is therefore :

$$\frac{0{\cdot}0071 \times 100}{0{\cdot}155} = 4{\cdot}6$$

In the absolute determination of photosynthesis, the volume of oxygen is reduced to N.T.P., and a correction added for respiration at the particular temperature.

Example: The volume of gas given out under an intensity of light of 1000 lux was 259·6 c.mm. per hour at 21° C. and at 751 mm. barometric pressure, the tension of aqueous vapour being 18·6 mm.

$$V_0 \text{ (N.T.P.)} = 259{\cdot}6 \times \frac{751 - 18{\cdot}6}{760} \times \frac{273}{294}$$
$$= 232{\cdot}3 \text{ c.mm. per hour.}$$

Respiration at 21° C. = 7·2 c.mm. per hour.

Absolute value after correction = 232·3 + 7·2
= 239·5 c.mm. per hour.

## Adjustment of Temperature

The temperature of the leaves of land-plants surrounded by air, as already stated, cannot be accurately determined; but no such difficulty exists in the case of water-plants, the temperature of which is the same as that of the surrounding water. Difficulty arises, however, in the production of definite variations of temperature. Addition of ice or of warm water is not merely a crude method, but the sudden change is tantamount to a shock which causes a physiological reaction independent of the true effect of change of temperature. The disturbance caused by the shock-effect was completely eliminated by the following device.

A large-sized test-tube is drawn out so that its upper end B serves as the Bubbler (right-hand illustration, fig. 37). A close-fitting india-rubber cork closes the lower end, to which are fixed a sensitive thermometer, and a glass rod to which the plant is tied. There is also a tube with a stop-cock $S_1$, ending in the funnel F. The plant-vessel is filled with the $CO_2$-solution up to a definite mark M in the Bubbler by means of the funnel F, after which the stop-cock $S_1$ is closed.

For adjusting the temperature, the glass vessel is surrounded by an outer rectangular metallic vessel V, one side of which is a sheet of glass for the passage of light.

This water-vessel is fitted water-tight outside the plant-

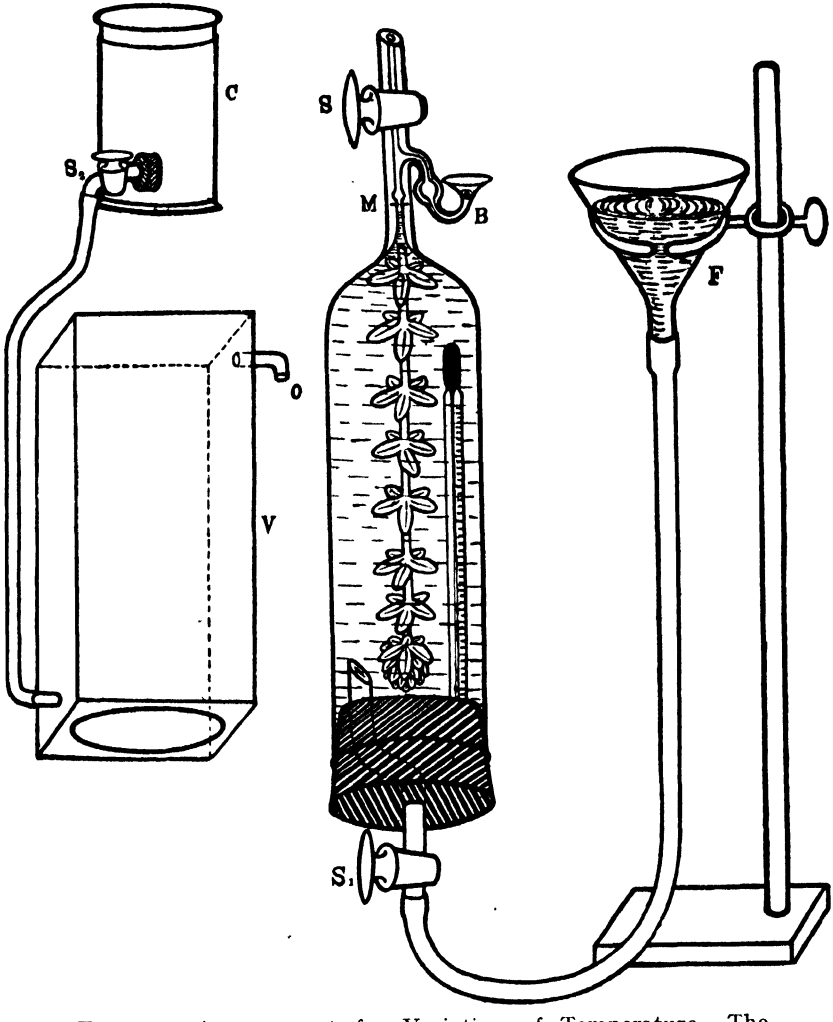



FIG. 37. Arrangement for Variation of Temperature. The Plant-vessel with Bubbler seen to the right; outer enclosing Vessel V seen to the left

Level of water up to definite mark M adjusted by the funnel F. Variation of temperature produced by flow of warm water from the cylinder C; attainment of constant temperature is assured when the thermometer inside the plant-vessel indicates the same as the second thermometer in the outside vessel V.

vessel, and communicates with a cylinder C, with a non-conducting jacket of felt, which contains warm water at

about 35° C. Partial opening of the stop-cock $S_2$, attached to C, allows a stream of water to circulate in the metallic outer vessel, and the temperature of water in the inner plant-vessel is gradually raised. With a little practice it is possible by proper manipulation of the stop-cock $S_2$ to control the rate of rise of temperature. The plant-vessel is at first filled with water at 8° C., at which temperature photosynthesis is completely arrested. The temperature is now gradually raised, by adjusting the flow of water in the outer vessel, at a rate of about 1° C. per minute. This uniform rate of rise causes no shock to the plant. The temperature of the plant is thus gradually raised by, say, 3° C. The temperature has to be maintained absolutely steady for about two minutes for the completion of the observation of the rate of photosynthesis at the given temperature. A thermometer placed in the outer vessel gives the temperature of the water outside ; the steadiness of the internal temperature is assured when the temperature indicated by the two thermometers in the outer and the inner vessels is the same. When the temperature of the inner vessel is lower than the temperature of the room, heat enters it ; when, on the other hand, it is higher, the flow of heat is outwards. By partial opening or closure of the stop-cock $S_2$ the temperature of the water in the plant-vessel can be so adjusted that it remains absolutely constant during the period of two minutes for an observation. The successive bubbling periods are now found to be very uniform. The importance of adjustment of temperature for securing accurate results will appear from the following occurrence in an experiment in which uniformity of the rate of bubbling was being observed. The uniformity was suddenly disturbed : the cause was afterwards traced to the opening of a door which allowed a draught of air to enter the closed room in which the experiment was being conducted. The change of the rate of photosynthesis produced by such a slight variation of temperature is not very great ; it is, however, necessary to avoid anything of the kind in experiments for the accurate

plotting of the photosynthetic curve under variation of temperature.

Another necessary adjustment is the maintenance of constancy of volume of the water in the Bubbler, at the level of the mark M, to which it was adjusted at the beginning of the experiment. The expansion of the water during rise of temperature raises this level to a small extent ; the level can, however, be easily readjusted for each series of observations by the manipulation of the adjusting funnel F.

## The Thermometric Minimum

The critical temperature for arrest of activity is very well defined. Thus, in an experiment with a particular specimen, the critical point of arrest was found to be 9°·5. A rise of temperature of half a degree initiated photosynthesis, while a fall of half a degree arrested it. In spring the critical point is a little higher, about 11° C. or so. The plant becomes accommodated to the lower temperature in winter, and the critical point is lowered by a degree and a half. It is interesting to compare the above with the critical point of arrest of the pulsation of the leaflets of the tropical plant *Desmodium gyrans*. In the summer the point of arrest of pulsation is about 17°, but in winter it is as low as 11° C.

This gradual accommodation to a lower temperature is illustrated in a very interesting manner in the following experiment. A spring-specimen of *Hydrilla* was subjected to repeated lowering of temperature to the point of arrest. On the first occasion the stoppage of activity occurred at 12° C., on the second at 11°·5, on the third at 9°·5, and on the fourth at 9° C. ; the fifth repetition did not produce any further lowering of the point of arrest.

I had found a similar accommodation to lower temperature in the ascent of sap. The power of suction was arrested in tropical specimens of *Impatiens* when the temperature of the water supplied to the cut end of the stem was 15° C.

Repetition of the experiment lowered the critical point from 15° to 14°, and finally to 13° C., which was the lowest value observed.

## Photosynthesis under Variation of Temperature

The following results concerning the activity of photosynthesis at various temperatures may be taken as typical

TABLE XIX.—SHOWING VARIATION OF PHOTOSYNTHESIS AT DIFFERENT TEMPERATURES

| Specimen | Temperature centigrade | Absolute activity in c.mm. O per hour |
|---|---|---|
| I. | 10·5 | 87·1 |
| | 13·0 | 111·5 |
| | 15·0 | 136·6 |
| | 17·5 | 168·1 |
| | 21·0 | 239·5 |
| | 24·5 | 318·2 |
| | 27·8 | 376·8 |
| | 29·0 | 313·2 |
| | 30·0 | 266·8 |
| II. | 15·0 | 118·4 |
| | 17·0 | 147·9 |
| | 20·5 | 209·8 |
| | 24·2 | 288·4 |
| | 28·0 | 360·1 |
| | 29·8 | 400·4 |
| | 31·8 | 430·2 |
| | 33·8 | 381·3 |
| III. | 16·5 | 138·9 |
| | 18·1 | 148·2 |
| | 21·3 | 191·1 |
| | 24·0 | 244·0 |
| | 25·8 | 273·3 |
| | 28·3 | 337·5 |
| | 30·5 | 416·3 |
| | 32·8 | 386·0 |

of about a dozen experiments with different specimens carried out in winter. The light was maintained constant at 1200 lux from Pointolite.

The experiment was commenced at a temperature of

8° C., at which no evolution of oxygen was observed. The temperature was then allowed to rise slowly, and the evolution of oxygen was found to be initiated at 9°·5 C. The activity increased at first slowly, then more rapidly with further rise of temperature. The rate of increase became uniform in the middle portion of the curve. After the optimum point, which was generally above 30° C. and very sharply defined, the decline of photosynthetic activity was

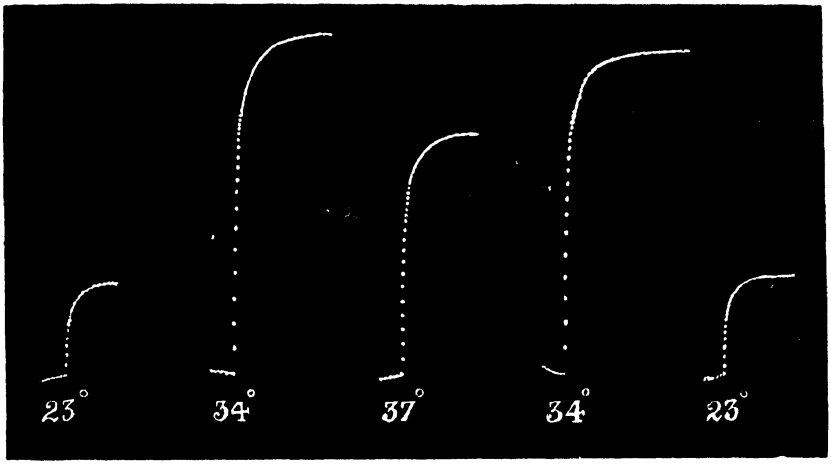



FIG. 38. Responses of *Mimosa* to Testing Stimulus of same intensity through Cyclic Variation of Temperature at 23°, 34°, 37°, and back to 34° and 23°

Note maximum response at 34°, which is therefore the optimum temperature.

very rapid. Thus, in a typical case, an activity of 337·5 c.mm. at 28°·3 was increased to 416·3 c.mm. at 30°·5. Above this it declined to 386·0 c.mm. at 32°·8. In another case the optimum was found to be at or about 32° C.

The optimum point is to some extent modified by the influence of the season. In winter the optimum may be as low as 28° C., while in summer it is about 33° C.

Stronger intensity of light is also found to raise the optimum point.

The table on the opposite page gives the results obtained with three typical specimens.

The photosynthetic curve plotted from the data for Specimen I. is given in fig. 39. It is seen that the curve is practically straight from 17° up to 28° C.; there is a sharp decline. The straight portion of the curve when produced

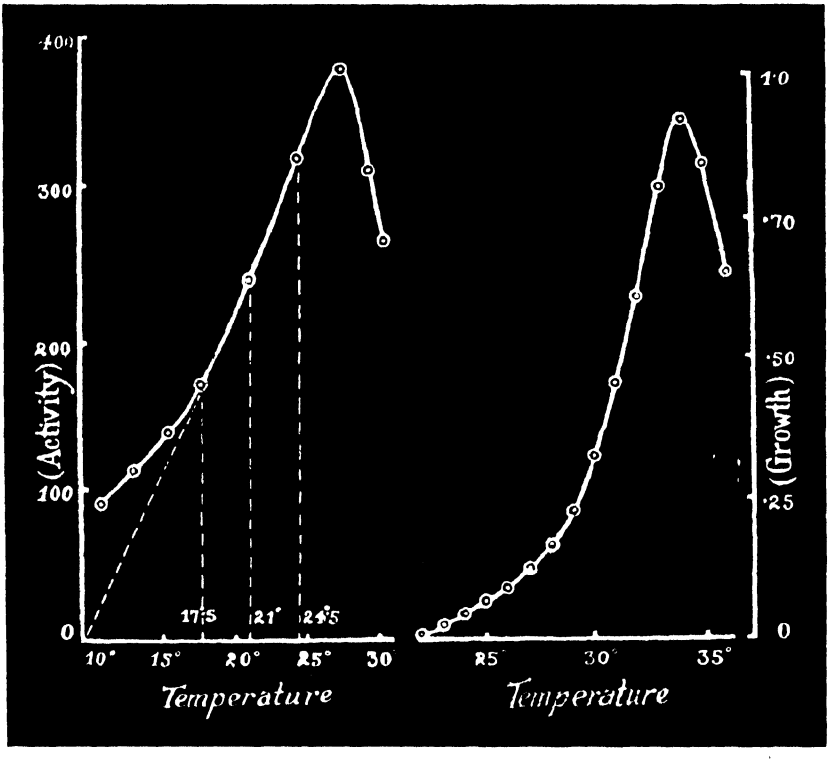



FIG. 39 FIG. 40

FIG. 39. Photosynthetic Curve under Variation of Temperature
The curve is straight from 17° to 28°. Note abrupt fall of curve after the optimum temperature of 28° (under stronger light the optimum is 33°).

FIG. 40. Curve of Growth under Variation of Temperature
Ordinate represents growth in $\mu$ per second.

backwards cuts the abscissa at 9°·2 C., which will be shown in a later chapter to be the physiological zero point. The activities corresponding to the three temperatures of 17°·5, 21°, and 24°·5 are shown in the record. We will presently determine the temperature coefficient.

I have previously referred to the remarkable similarity of the modifications of photosynthetic activity to those of other activities of the plant under external variations. In regard to the effect of temperature, the irritability of *Mimosa* is at its maximum at 34° C., which is therefore the optimum. In fig. 38 (p. 139) are given records of responses of *Mimosa* under a definite testing stimulus, during a complete cycle of temperature variation from 23° to 37° C. and back once more to 23° C. The maximum response is seen to take place at 34° C.

I have also determined [1] the optimum temperature for growth in the tropical plant *Scirpus Kysoor*. The curve of growth of this plant is reproduced side by side with the photosynthetic curve (compare figs. 39, 40). The similarity between the two curves is very striking.

## Determination of the Photosynthetic Coefficient for Temperature

The rise of photosynthetic activity in the middle range of variation of temperature is practically uniform, and the coefficient may be found in two ways: first, from measurements from the physiological zero—discussed in a subsequent chapter—and, second, by dividing the increase of activity by the increase of temperature.

$$K = \frac{A_T - A_t}{T - t} \quad . \quad . \quad . \quad (1)$$

where $A_T$ is the activity in c.mm. per hour for the higher temperature T, and $A_t$ is the activity for the lower temperature $t$.

*Examples:*

Specimen II.

Activity at temperature 21°·3 C. . . 191·13
,, ,, 28°·3 C. . . 337·46

$$K = \frac{337{\cdot}46 - 191{\cdot}13}{28{\cdot}3 - 21{\cdot}3} = 20{\cdot}9$$

[1] *Plant Response* (1906); *Life Movements of Plants* (1917).

Specimen III.

Activity at temperature 24°·2 C. . . 288·4
,, ,, 29°·8 C. . . 400·4

$$K = \frac{400 \cdot 4 - 288 \cdot 4}{29 \cdot 8 - 24 \cdot 2} = 20 \cdot 0$$

It may be said that a rise of 7° C. practically doubles the activity.

The average coefficient in the typical instances given above is 20·4: by the following formula the activity $A_T$ at the higher temperature T is calculable from the activity $A_t$ at the lower temperature $t$:

$$A_T = A_t + K\,(T - t) \quad . \quad . \quad . \quad (2)$$

According to Van't Hoff, the rate of chemical reaction is increased two or three times for a rise of 10° C. According to this the coefficient for a rise of 10°, $K_{10}$, varies from 2 to 3. The values for $K_{10}$ in the above specimens of *Hydrilla* (II. and III.) are the same, *i.e.* 2·2, between the temperatures of 18° and 28° C.

## Summary

In the accurate determination of photosynthesis under variation of temperature, corrections have to be made for loss in respiration. This has been determined by two independent means: (1) by the volumetric method and (2) by the loss of weight by the plant. The two determinations give concordant results. At 18° C. the loss due to respiration is about 4 per cent.

In the determination of the effect of temperature, very careful adjustments are necessary, for any sudden variation produces a shock-effect; the adjustment is made by the special apparatus which has been fully described.

The increase of photosynthetic activity is uniform in the middle range. At the optimum temperature the activity is at its maximum, after which there is a very sharp decline.

The thermometric minima for various activities of tropical plants are more or less definite. In the photosynthesis of *Hydrilla* the critical point for arrest varies in different specimens from 9°·5 to 11° C.

The average optimum temperature for photosynthesis in *Hydrilla*, under moderate intensity of light of about 1200 lux, is 30° C. But under increased intensity of sunlight the optimum point is raised to 33° C. This is of the same order as the optimum point, 34° C., for maximum response in *Mimosa*. The optimum point for growth of tropical plants is also about 33° C.

The temperature coefficient obtained by the differential method is :

$$K = \frac{A_T - A_t}{T - t}$$

The value of the coefficient is found to be about 20·4.

A rise of temperature of 7° C. is found to double the photosynthetic activity of *Hydrilla*. For a rise of 10° C. the increase is 2·2 times.

The activity at a higher temperature may be found from the following formula :

$$A_T = A_t + K\,(T - t)$$

# CHAPTER XVIII

## THE TONIC FACTOR IN PHOTOSYNTHESIS

The effect of season on physiological activity—The tonic level—The Tonometer—Measurement of tonicity from the ratio of response to stimulus—Effect of season on photosynthesis—Induced change of the tonic level under seasonal variation—Effect of minute traces of chemical substances in modifying the tonic condition—Correlation of the number of chloroplasts and photosynthetic activity—Effect of excess of starch-content in the cell—After-effect of stimulus in modifying the tonic condition exhibited (*a*) by mechanical response, (*b*) by autonomous response, and (*c*) by photosynthetic response—Determination of physiological Hysteresis by the Method of Cyclic Curve—Negative, Positive and Zero Hysteresis.

THE diverse activities of the plant are profoundly modified by the internal physiological factor vaguely described as the tonic condition. This is strikingly shown in the effect of season : the mechanical response of *Mimosa* in winter is very feeble, while a few weeks after, in spring, the response becomes greatly enhanced. I also find that the velocity of transmission of excitation in this plant is increased from about 4 mm. in winter to 30 mm. in summer. Again, the minimal intensity of stimulus which is effective in evoking response in summer is quite ineffective in winter.

Similar changes under variation of season are observed in the autonomous activities of growth, which, arrested in winter, become revived in spring. The pulsatory activity of the leaflet of *Desmodium gyrans* is greatly depressed in winter, while in spring or summer it becomes very vigorous. Photosynthesis presents parallel variations, the photosynthetic activity in spring being at least 1·8 times greater than that in winter.

There are other factors, whose influence on the tonic condition has not been fully recognised on account of their

complexity. Among these may be mentioned (1) the composition of the atmosphere surrounding the plant ; (2) traces of chemical substances present in the soil ; and (3) the after-effect of stimulus.

The effect of differences in the composition of the atmosphere is shown in a striking manner by *Biophytum sensitivum*: the plants growing seven miles outside Calcutta exhibit motile sensibility in a high degree, whilst others in my experimental grounds in Calcutta are found to be quite insensitive.

The effect of traces of chemical substances in the soil is shown in the differences of growth in equally vigorous transplanted seedlings: of these some show normal rate of growth, while others exhibit either an exceptionally high or an exceptionally low rate. The difference cannot be accidental, but must be due to traces of substances in the soil which it is impossible to detect by the most refined chemical tests. The effect of infinitesimal quantities of chemical substances in enhancement of photosynthesis has already been described ; there is no doubt that traces of chemical substances affect growth and other activities of the plant.

Special investigations will be described which will show that the tonic condition of the plant is also modified by the action of stimulus.

The difficulties in the present investigation are accentuated by lack of suitable terms for describing the tonic condition, and also by the absence of means for measuring it. I will use the term *tonic level*, of which P is to be the symbol, to indicate the underlying protoplasmic activity whose variation modifies the tonic condition. When the tonic level is raised from P to P′, the diverse physiological activities undergo a corresponding enhancement ; the agents or conditions which modify the activity are therefore to be regarded as so many tonic factors. I have devised the Tonometer for the measurement of the tonic condition and changes induced in that condition. The tonic level of a

plant may be estimated from the amplitude of response to a definite testing stimulus.

## The Tonometer

In my previous work I have explained how the internal condition of the plant may be revealed through the quantitative relation of response to the stimulus which provokes it. The response may be recorded in diverse ways, as a mechanical or an electrical response, or as a variation in the rate of growth. We thus obtain a ratio $\frac{R}{S}$, in which R represents the response and S the stimulus. The response under the same stimulus is increased when the tonic level P is in any way raised; contrariwise, when the internal condition or tone of the organ becomes depressed, the ratio $\frac{R}{S}$ is correspondingly decreased. If we keep the testing stimulus constant, the amplitude of response R affords a measure of the changed tonicity of the plant. In photosynthesis the tonic condition is similarly gauged by the coefficient, which is the ratio of the increment of activity to the increment of stimulus.

I will now describe the effects of external and internal changes on the tonic condition of the plant; of the conditions which modify tonicity, some are under our control, while others are not. The following are the investigations which have been carried out on the subject:

(1) The effect of season has already been considered in previous chapters.

(2) The effect of traces of chemical substances on photosynthesis is fully discussed in Chapter IX.

(3) The effect of the chlorophyll- and starch-content of the cell.

(4) The effect of stimulus in modifying the tonic condition as exhibited (*a*) by mechanical response, (*b*) by autonomous response, and (*c*) by photosynthetic response.

## Effect of the Chlorophyll- and Starch-Content of the Cell

One of the internal factors in photosynthesis is the presence of chlorophyll. The following investigation was undertaken to correlate the number of chloroplasts with the photosynthetic activity of the leaf. For this purpose three different whorls of leaves were taken from the same *Hydrilla* plant, one from the youngest portion of the stem 5 mm. from the top, the second from the still young portion 2 cm. below the top, and the third, a relatively old whorl, from 6 cm. lower down. Their photosynthetic activities were measured by the number of bubbles of oxygen given out. Microscopic examination of the leaves was also made to determine the relative number of chloroplasts. Representing by 100 the activity of the youngest and immature whorl (1) at the top, that of the second whorl (2) was 125, while it was only 86 for the oldest whorl (3) of leaves lower down. The activity was thus at its maximum in the moderately young (2), and at its lowest in the old leaves (3). The activity of the youngest leaves (1) exhibited a decline under the continued action of light ; but it was uniform in the leaves (2). Microscopic examination showed that the youngest leaf (1) contained about 30,000 chloroplasts, while the (2) leaf had as many as 60,000. The number of chloroplasts diminished to 25,000 in the oldest leaf (3). A relation is thus found to exist between the number of chloroplasts and the photosynthetic activity of the leaf. It should, however, be borne in mind that the protoplasmic factor is even more important, for it has been shown that excessive stimulation induces an arrest or permanent abolition of photosynthesis.

*Effect of excess of starch-content.*—The *Hydrilla* plant growing in the sun-exposed pond exhibits, as previously stated, a relatively feeble photosynthesis. This may be partly due to the after-effect of strong and continuous

stimulation, and also to the overloading of the cells with starch-grains formed under strong light, of which there may be as many as forty in each cell. In the analogous case of charging a storage-cell, a fully charged battery exhibits increased counter-electromotive force which opposes a charging current. Similarly the overloading of a vegetable cell with photosynthetic products may to a certain extent inhibit further formation. This inference finds support in the following experiment.

Some of the sun-exposed plants which had exhibited relatively feeble photosynthesis were kept for two days in a semi-dark room. Microscopic examination showed that the starch-grains which were present in such abundance had practically disappeared. The specimens depleted of starch were now found to exhibit considerable increase in the rate of evolution of oxygen.

## Effect of Stimulus in modifying the Tonic Condition

External stimulus is found to modify the physiological tone of the plant in a definite manner. I have shown that external stimulus brings about both the anabolic and catabolic changes A and D, the resultant effect being that of their algebraical summation. The up-change A is associated with an increase of the potential energy of the system, while the down-change D is attended by a run-down of energy. The tonic level is raised when A is greater than D.

The relative intensity of the A and D reactions is also modified by the existing tonic condition of the plant : in a condition *above par,* D is relatively predominant, whereas in a condition *below par,* or of sub-tonicity, A is the more pronounced.

These conclusions are demonstrated by the following typical results.

(*a*) *Effect of stimulus on mechanical response.*—There are three characteristic types of response which I have obtained with *Mimosa.*

(1) The specimen was in an ordinary condition, and the stimulus applied was strong and long-continued. The D change was found to be greater than the A (A < D). At the end of the operation the tonic condition fell below the former level, as evidenced by the responses exhibiting fatigue depression.

(2) The specimen was in a sub-tonic condition, as shown by the feeble character of the response. Stimulus stirred up the relatively inert tissue to activity, and the up-change A was greater than the down-change D (A > D). The tonicity was now found to have been raised by stimulus above the former level, as shown by a marked enhancement of the response. Other instances of this are met with in the so-called 'staircase effect,' where uniform stimuli evoke responses which undergo a continuous increase.

(3) An intermediate type was found in which successive stimulations of moderate intensity applied to vigorous specimens gave rise to uniform responses. The tonic condition remained unchanged, the A and D changes being equal (A = D).

Thus two diametrically opposite effects are produced in responding tissues the tonic condition of which is *above* or *below par*. In highly excitable tissues response undergoes diminution under strong stimulus, while in sub-tonic tissues it exhibits enhancement.

(*b*) Effects parallel to these are found in the autonomous activities of the plant. Strong stimulus inhibits the vigorous pulsation of the leaflets of *Desmodium gyrans*; in relatively inactive specimens, where the pulsation is feeble or at a standstill, stimulus enhances or renews it. In growth also the rate is depressed in active specimens under the action of stimulus; but in specimens with feeble growth, moderate stimulus enhances the rate.

## Detection of After-effect of Stimulus on Photosynthesis by the Method of Cyclic Curve

I have been able to discover parallel effects in photosynthesis by the Method of Cyclic Curve, which enables us at the same time to follow the history of the induced change. The cycle of operations consists in the application of stimulus from a minimum to a maximum and back once more to a minimum. After this, the tonic condition of the plant is generally found to be different from that at the beginning: there is residual physiological change, in virtue of which the original activity is enhanced or depressed in a definite manner. I describe this as physiological *hysteresis*, from certain analogous phenomena in physics.

In the experimental determination of physiological hysteresis in *Hydrilla* we take a series of observations of photosynthesis under increasing intensity of light, followed by a decrease to the original intensity. The Cyclic Curve plotted from the above data consists of an ascending and a descending curve. These do not usually coincide: under certain definite conditions the descending curve is to the right (*negative* hysteresis), and under other conditions it is to the left (*positive* hysteresis). There are occasions, again, when the ascending and the descending curves coincide (*zero* hysteresis). I give illustrative examples of the three cases, and explain the conditions of their genesis.

*Negative hysteresis.*—The particular specimen of *Hydrilla* was moderately sensitive: the photosynthetic activity at 500 lux was 41 c.mm. per hour. At 1500 lux the activity increased to 144, and at 3000 lux to 152·5. During the reverse process of diminishing the intensity from 3000 to 500 lux, the activity at 1500 was reduced from the first value of 144 to 137; at 500 lux it was 22·5 in the place of 41. A depression of activity had thus occurred in consequence of the previous strong and long-continued stimulation (see Table XX., p. 152). The ascending and the

descending curves did not coincide, but *the return-curve was to the right of the ascending curve*, indicative of depression of activity, the physiological hysteresis being negative (fig. 41).

*Positive hysteresis.*—In this experiment the specimen was in sub-tonic condition, and sunlight had to be employed for

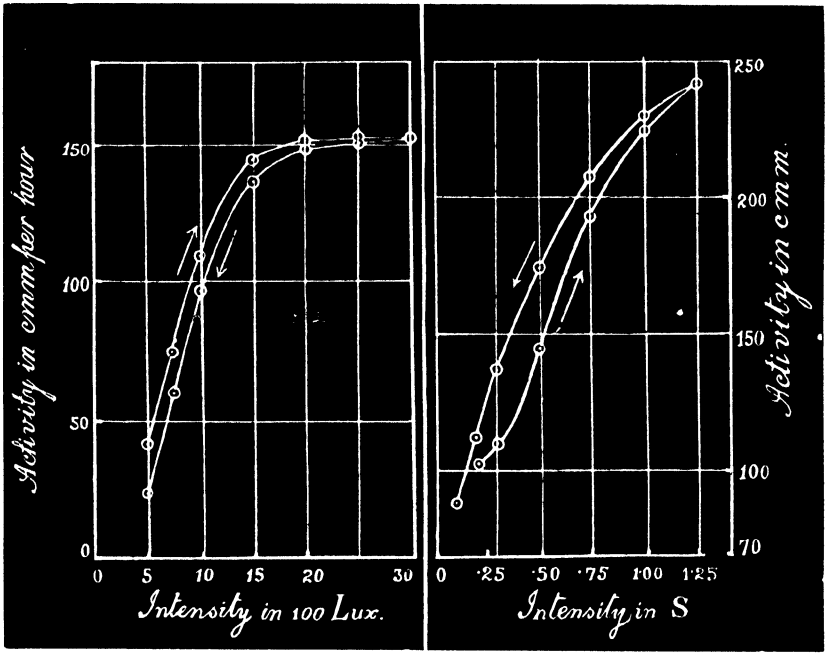



FIG. 41 FIG. 42

FIG. 41. Negative Hysteresis

The return-curve is to the right, photosynthetic activity being depressed after the cycle.

FIG. 42. Positive Hysteresis

The return-curve is to the left, photosynthetic activity being enhanced after the cycle.

initiating photosynthesis. The Cyclic Curve was obtained under an intensity of light increasing by steps from 0·10 S to 1·25 S and back once more to 0·10 S. At the commencement of the experiment the intensity of 0·10 S was below the minimum; hence it did not cause any photosynthesis: at 0·20 S the activity was 104 c.mm. per hour.

Photosynthesis increased with increasing intensity of light: at 0·50 S it was 145, and at 1·25 S it increased to 243. In

TABLE XX.—PHOTOSYNTHESIS UNDER CYCLIC VARIATION OF INTENSITY OF LIGHT

(*Negative Hysteresis*)

| Intensity of light in lux | Activity in c.mm. O per hour | |
|---|---|---|
| | During ascent | During descent |
| 500 | 41 | 22·5 |
| 750 | 74 | 60·6 |
| 1000 | 109 | 97·0 |
| 1500 | 144 | 137·0 |
| 2000 | ↓ 151 | ↑ 148·5 |
| 2500 | ↓ 152 | ↑ 151·0 |
| 3000 | 152·5 | 152·5 |

the return-curve we observe *a relative enhancement of activity* in consequence of the previous stimulation. For, during descent, the activity at 0·75 S was found to be 208 in the

TABLE XXI.—CYCLIC PHOTOSYNTHETIC CURVE EXHIBITING POSITIVE HYSTERESIS

| Intensity of light in terms of sunlight (S) | Activity in c.mm. O per hour | |
|---|---|---|
| | During ascent | During descent |
| 0·10 | 0 | 89 |
| 0·20 | 104 | 113 |
| 0·30 | 111 | 137 |
| 0·50 | 145 | 174 |
| 0·75 | ↓ 194 | ↑ 208 |
| 1·00 | ↓ 225 | ↑ 230 |
| 1·25 | 243 | 243 |

place of 194; at 0·50 S the increase was from 145 to 174; and the enhancement of activity due to previous stimulation was found to persist throughout the series (Table XXI.). The return-curve is thus to the *left*, indicative of enhanced activity and of *positive* hysteresis (fig. 42, p. 151).

The rise of the tonic level as an after-effect of stimulus was also shown in a different and very interesting manner. Stimulus of the intensity 0·10 S did not, as stated before, produce any photosynthesis, that particular stimulus being then ineffective; but after the cycle of operations (which raised the tonic level from P to P′), the former ineffective stimulus became effective, the rate of photosynthesis under 0·10 S being now 89 c.mm. per hour. *The heightened tonicity is thus manifested in the induced lowering of the intensity of the minimally effective stimulus.*

*Zero hysteresis.*—In this experiment a more sensitive spring-specimen was used; care was taken to avoid excessive stimulation by prolonged application of light, which might have otherwise caused fatigue. The observations were taken for the comparatively short period required for application of 400, 600, 800 and 1000 lux, both during the ascent and descent; the photosynthesis during ascent and descent was found to be practically the same (Table XXII.). The two curves coincide, and the hysteresis is zero. The physiological condition (P) of the plant at the end of the operations was the same as at the beginning.

TABLE XXII.—CYCLIC PHOTOSYNTHETIC CURVE EXHIBITING ZERO HYSTERESIS

| Intensity of light in lux | Activity in c.mm. O per hour | |
|---|---|---|
| | During ascent | During descent |
| 400 | 117·1 | 118·3 |
| 600 | 182·5 | 183·4 |
| 800 | 237·3 | 237·7 |
| 1000 | 243·1 | 243·1 |

This is the ideal method of experimentation for obtaining a perfect photosynthetic curve and for the most correct determination of the physiological constant. The entire observation should be completed in as short a time as

possible; this can be done, as previously stated, in the course of less than an hour, during which short period the external conditions can be maintained absolutely constant.

## Summary

The tonic condition of a plant may be gauged by the Tonometer. The ratio of response to stimulus, $\frac{R}{S}$, measures the physiological tone of the plant. In photosynthesis the ratio, K, is found by dividing the increment of activity by the increment of the factor which induces it.

Seasonal variation exerts a marked effect on photosynthetic activity. The tonic level in spring is found to be nearly double that in winter.

Traces of certain chemical substances produce a heightening of the tonic level and enhancement of activity.

A relation was found between the chlorophyll-content of the cell and photosynthetic activity. The number of chloroplasts in a fully developed but still young leaf was considerably larger than in very young or old leaves; the photosynthetic activity exhibited by the still young leaf was found to be relatively the greater.

Excess of starch-content in the cell exerts a partial inhibitory action on photosynthetic activity.

In photosynthesis the after-effect of stimulus in modifying the tonic condition is detectable by the Method of Cyclic Curve. Three types of physiological *hysteresis* are observed under definite conditions: first, the *negative hysteresis*, where physiological activity becomes depressed under strong and long-continued stimulation; second, the *positive hysteresis*, where the feeble activity of a subtonic tissue is enhanced in consequence of stimulation; and third, the *zero hysteresis*, where the activity remains unchanged.

# CHAPTER XIX

## THE DAILY VARIATION IN PHOTOSYNTHETIC ACTIVITY

Effect of environmental changes on rate of photosynthesis—Variation of light and temperature—Extreme sensitiveness of the plant to external change—Method of simultaneous determination of photosynthetic activity, intensity of light and temperature—Complete apparatus for the three determinations—The automatic records of activity throughout the day—The effect of variation of intensity of light—Effect of variation of temperature—The combined effect of light and temperature—Resultant curve of variation.

THE change in the rate of photosynthesis under variation of one factor at a time has been studied in detail in the previous chapters, including the effects of $CO_2$-concentration, of temperature, of light, and of the factor of tonicity. The coefficient and the characteristic curve for each factor have been determined, so that it is now possible to obtain by simple calculation the value of photosynthetic activity under variation of one or the other of these factors.

There now remains the important question as to whether the effect of any one of these factors is independent of those of the others, or whether there is a mutual interaction between the factors ; and further, to ascertain if a law can be formulated which would enable us to calculate the resulting effect which would arise from the simultaneous independent variation of different factors. In the present chapter the inquiry is limited to the question of the effect of changes in the natural environment on the photosynthetic activity of the plant. The experiments described had hitherto been carried out inside the laboratory, all the factors being maintained absolutely constant except the one the effect of whose variation was to be determined. But out in the open two important factors, light and temperature, are changing from moment to moment.

The light from the sky varies with the course of the sun; it is extremely feeble at dawn, increasing rapidly as the sun rises above the horizon. The light attains its maximum at noon; it then wanes gradually up to about 4.30 P.M., after which the fall of the intensity is extremely rapid. This relates to light from a point of the sky vertically overhead; the variation of the intensity of the reflected light from the northern sky, as previously explained, is relatively more uniform, the rise and fall being less abrupt. The temperature also undergoes variation which is not exactly parallel to that of light; the thermal noon, for example, is about two hours later than the light-noon. The fall in temperature is gradual in the afternoon, whereas light falls abruptly to zero in the course of less than two hours after 4 P.M.

These are the two environmental factors which conspicuously affect the photosynthetic activity of the plant growing in the open. The questions naturally arise, (1) How quickly does the plant respond to the external changes? and (2) Does each factor produce the whole or only part of its characteristic effect? A complete answer to these queries can be obtained only by simultaneous determination of the photosynthetic activity and of the intensities of the two factors which affect it. Three determinations have therefore to be made at the same time, for which special appliances had to be devised.

The investigation was undertaken with specimens grown in the pond which was exposed to northern light from the sky, direct sunlight being intercepted by a tall building to the south. The method employed is so sensitive that the plant responded to the slightest variation in the intensity of light caused by reflection from two walls situated to the north of the Institute, one of which was dark and the other white. In the morning there was a feeble and diffuse reflection from the dark wall; later in the day, sunlight was diffusely reflected from the white wall. The changes in the light reflected from the neighbouring buildings were

found to produce marked variation in the rate of the photosynthetic evolution of oxygen.

For securing conditions closely approximating to the natural variation of light from the northern sky, the specimen mounted in the Bubbler was taken to the roof of the Institute, at a height of 40 feet (13 metres) from the ground. No intervening buildings now interrupted the northern light. The complete apparatus was placed in front of the Observatory at the south-east corner, the plant being thus protected from the sun.

The apparatus for the simultaneous determination of the hourly changes in photosynthesis and in the environmental conditions consists of a base-board, 40 × 40 cm., on which three separate instruments are fixed in the closest proximity to each other, namely :

(1) The Photosynthetic Recorder, in which the bubbling apparatus containing the plant is provided with an electromagnetic arrangement for inscribing the record ; the drum was placed in the Observatory a short distance from the Bubbler, the two being in electric connection by a pair of leading wires.

(2) The Electric Photometer, previously described, was placed by the side of the Bubbler, the line of sight of the two being the same. The wires from the Photometer led to the galvanometer, which was inside the Observatory ; the intensity of light at any period was measured by the galvanometric deflection.

(3) The Thermograph consisted of a compound strip of metal attached to a recording lever. The excursions of the lever were recorded on a smoked glass plate allowed to fall at a definite rate by clockwork. A sensitive thermometer was suspended near the Thermograph to give independent readings of the temperature.

A glass cover was placed over the base-board bearing the three instruments, the object being to protect the instruments from disturbance caused by currents of air. The complete apparatus thus permits the record of the

change which is continuously taking place in the assimilatory activity under the combined influence of changes of light and temperature which are registered by the Electric Photometer and the Thermograph (fig. 43). For our present purpose it is sufficient to take simultaneous observations

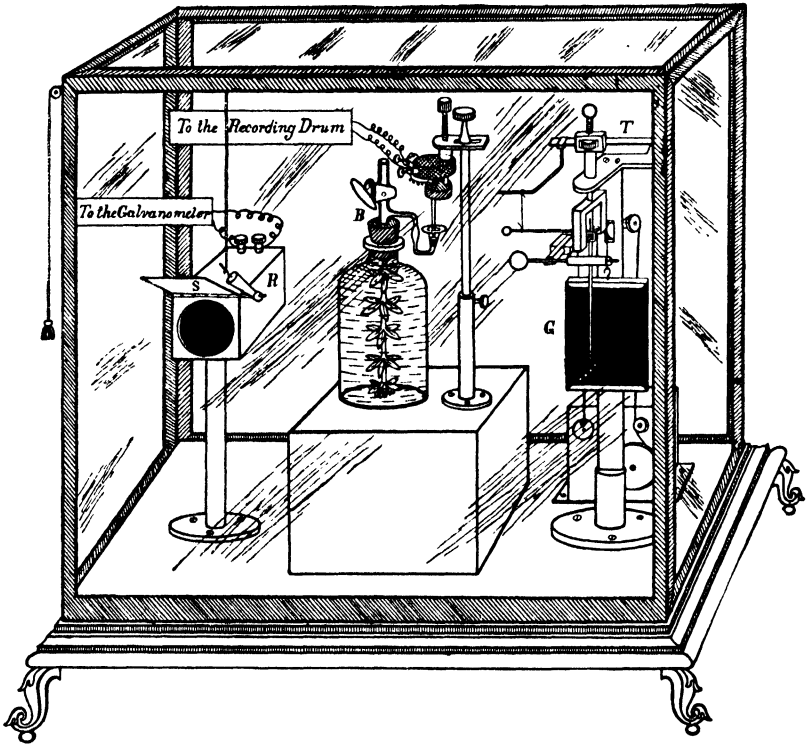



FIG. 43. The Complete Apparatus
B, the Bubbler; R, the Electric Photometer; and T, the Thermograph.

once every half-hour during periods of rapid change, and once in an hour when the variation is less rapid.

The method of procedure in taking the observations is as follows. The activity of photosynthesis at different periods is given by an automatic record lasting for five minutes; the record commenced two and a half minutes before the hour and continued for two and a half minutes after the hour. The mean of these gives the average

value of photosynthesis at the particular hour. The plant was exposed to light two minutes earlier for preliminary accommodation to light.

The plant might have been continuously exposed to light and the records taken throughout the day; but it was exposed to light for only seven minutes at a time, in order to guard against the possibility of depletion of $CO_2$ by uninterrupted photosynthesis for twelve hours. As the quantity of solution contained in the apparatus was more than 200 c.c., the depletion would have been slight even after such continuous exposure to light; it was nevertheless thought desirable to adopt the safer course of giving an exposure only long enough to make the observations at definite periods. There was a simple contrivance (not shown in the figure) for giving the necessary exposure. An opaque cylindrical cover cut off the light from the plant-vessel; the pressure of a lever projecting outside the glass cover enabled the observer to give the exposure for the definite duration of seven minutes.

The Electric Photometer was exposed to light by manipulating a string which actuated the shutter; the intensity of light at the hour was found by observing the maximum deflection of the galvanometer which was reached in the course of five seconds. The temperature at the hour was recorded by the Thermograph; an independent reading of the thermometer inside the case was also taken at the same time.

## The Automatic Record

The variation in the rate of photosynthesis is shown in a very striking manner by the automatic records given in fig. 44, for successive periods of five minutes. The sun rose at 6.46 A.M., but the light was too feeble to be effective. At 7.30 A.M. photosynthesis was initiated at a rate of 135·8 c.mm., the intensity of light indicated by the Electric Photometer being 61, and the temperature 19° C.: there

were now four bubbles, represented by as many spacings, in the course of five minutes. The activity increased

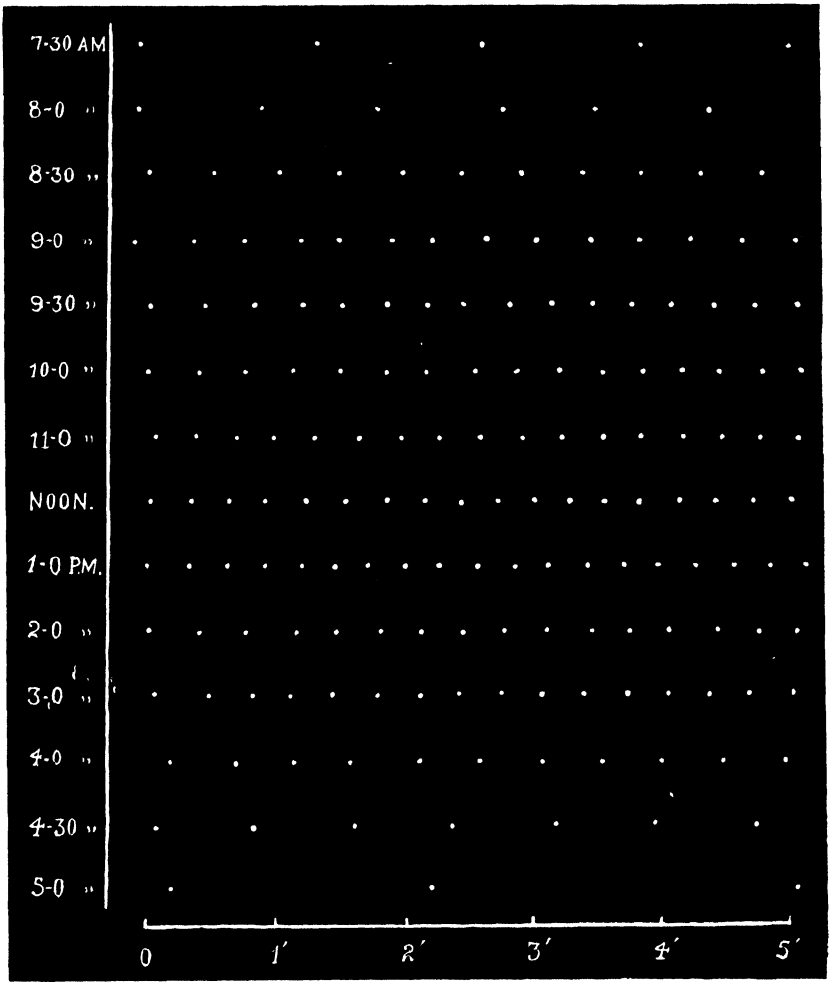



FIG. 44. Automatic Records of successive Bubblings for five minutes during different periods of the Day

Note the slow rate at 7.30 A.M. and 4.30 P.M., and the quick rate at midday.

rapidly: by 8 A.M. the rate had risen one and a half times, there being now 6 spacings in the record. At 8.30 A.M. the activity was enhanced to about three times

the original value, with 12 spacings. This enhancement was due to the increase in the intensity of light, which was now 67, and to the rise of temperature to 22°·7. The activity continued to be further enhanced, attaining its maximum between noon and 1 P.M. under the combined action of greater intensity of light and higher temperature. There were now 18 spaces in the record, showing an activity over four times that at 7.30 A.M. A decline of activity occurred in the afternoon, as seen in the diminished number of spaces, 12, in the record at 4 P.M. Henceforth the fall was extremely rapid : at 4.30 P.M. the number of spacings was 7, about the same as at 8 A.M. Owing to the diminishing intensity of light, the decline in activity was still more pronounced at 5 P.M., the number of spacings being reduced to 2. By 5.15 P.M. there was a complete arrest of photosynthesis.

## Determination of the Absolute Rate of Photosynthesis at Different Hours

The absolute rate was obtained by applying the various corrections for the successive volumes of oxygen evolved during photosynthesis. The rate of activity (N.T.P.) at various periods is given in Table XXIII. (p. 162), together with the intensity of light and of temperature.

In fig. 45 are given the three corresponding curves, of which two represent the environmental conditions, and the third the photosynthetic activity : the dotted curve gives the variations of intensity of light ; the curve in thin line gives the variations of temperature ; the curve in thick line gives those of photosynthetic activity.

I may begin the consideration of the curves by drawing attention to the combined effects of light and temperature in the forenoon and in the early afternoon, which present very interesting features. I have previously explained that though the intensity of sunlight is at its maximum at noon, the light reflected from the northern sky remains practically constant for a considerable length of time. This is seen in

the curve of light between 10 A.M. and 1 P.M., during which time the light remained approximately constant at about 68. It should, however, be remembered that there was always a slight fluctuation brought about by changes in the direction of the wind, charged as it was with relatively

TABLE XXIII.—GIVING VARIATION OF LIGHT, TEMPERATURE, AND THE RESULTING PHOTOSYNTHETIC ACTIVITY, IN THE COURSE OF THE DAY

(On the day of the experiment, January 24, 1923, the sun rose at 6.46 A.M. and set at 5.38 P.M.)

| Time | Intensity of light given in divisions of photometric scale | Temperature centigrade | Absolute activity (N.T.P.) in c.mm. O per hour |
|---|---|---|---|
| 6.10 A.M. | 3 divisions | 16°·5 | nil |
| 6.30 ,, | 15 ,, | 17°·0 | ,, |
| 7 ,, | 30 ,, | 18°·0 | ,, |
| 7.30 ,, | 61 ,, | 19°·0 | 135·8 |
| 8 ,, | 65 ,, | 20°·7 | 208·0 |
| 8.30 ,, | 67 ,, | 22°·7 | 348·4 |
| 9 ,, | 70 ,, | 24°·0 | 402·9 |
| 9.30 ,, | 65 ,, | 25°·3 | 465·6 |
| 10 ,, | 68 ,, | 26°·4 | 463·2 |
| 11 ,, | 69 ,, | 28°·2 | 481·9 |
| Noon | 68 ,, | 29°·3 | 531·4 |
| 1 P.M. | 68 ,, | 30°·5 | 530·6 |
| 2 ,, | 71 ,, | 30°·9 | 454·0 |
| 3 ,, | 64 ,, | 30°·3 | 453·5 |
| 4 ,, | 59 ,, | 29°·5 | 310·8 |
| 4.30 ,, | 57 ,, | 29°·0 | 189·0 |
| 5 ,, | 56 ,, | 28°·6 | 71·5 |
| 5.15 ,, | 40 ,, | 28°·1 | arrest |

dry and moist air. The temperature rose to a maximum of 30°·9 at 2 P.M.; it had already attained 30°·5 at 1 P.M., this temperature being above the general optimum for the light from the sky.

Such were the simultaneous variations of the two factors, light and temperature, producing resultant variations in photosynthetic activity which at first sight would appear to be almost inexplicable. For, while the light was practically at its constant maximum between 10 A.M. and 1 P.M., photosynthetic activity was being enhanced because of the

continuous rise of temperature ; but this rise was suddenly interrupted, and even reversed after 1 P.M., though the temperature was still rising. The reason of this decline is to be found in the fact that after 1 P.M. the optimum temperature had been exceeded, with the result of a fall in

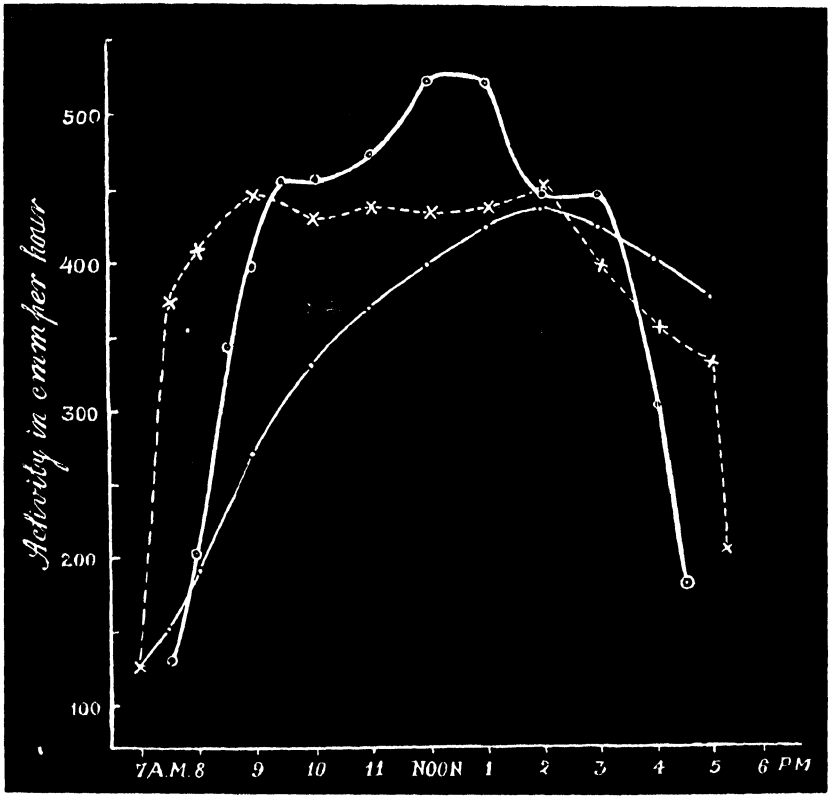



FIG. 45. The Curves for Light (dotted line), Temperature (thin line), and Resultant Photosynthetic Activity (thick line)

activity. This was temporarily arrested by the temperature falling to the optimum at 3 P.M. and by a transient increase of light which preceded that hour. But after 3 P.M. both light and temperature were falling, with the result of a very sharp decline in the rate of $CO_2$-assimilation. After 4 P.M. the fall in the intensity of light was rapid ; at 5 P.M. it had fallen to 56, in place of 68 at midday. The fall of

activity after 4 P.M. was therefore extremely rapid, and an arrest took place by 5.15 P.M., the sun setting at 5.38 P.M.

## SUMMARY

An account is given of an investigation for determining the changes of photosynthetic activity under the environmental variation of light and temperature at different hours of the day.

A description is given of the complete apparatus, consisting of (1) the Electric Photometer for determining the varying intensity of light, (2) the Thermograph for recording the variation of temperature, and (3) the Automatic Recorder for photosynthesis. The intensity of light reflected from the northern sky increased rapidly after sunrise at 6.46 A.M. (January 24, 1923); it remained approximately constant between 10 A.M. and 1 P.M.; the intensity fell in the afternoon, the decline being rapid between 4 and 5 P.M. There was practically no light after sunset at 5.38 P.M.

The temperature rose continuously from 17° C. at 7 A.M. to 30°·9 at 2 P.M., this being the thermal noon. It reached 30°·5 at 1 P.M., which was slightly above the optimum temperature for photosynthetic activity.

The resulting photosynthesis at various periods of the day can be readily explained as the expression of the combined effect of the factors of light and temperature. Under increasing intensity of light and temperature (up to an optimum), the activity was increased from 135·8 c.mm. at 7.30 A.M. to 531·4 c.mm. at noon, the increase being nearly four-fold.

After 1 P.M. the temperature rose slightly above the optimum; consequently there was produced a sharp fall of activity between 1 and 2 P.M. After this both light and temperature underwent a decline, with resulting rapid fall of activity.

Photosynthesis was arrested at 5.15 P.M., when the intensity of light had declined to 40 from 68 at noon. The sun set at 5.38 P.M.

# CHAPTER XX

## DETERMINATION OF THE PHOTOSYNTHETIC EFFICIENCY OF LIGHT OF DIFFERENT COLOURS

Contradictory views regarding the rays effective in photosynthesis—Investigation by the Method of Flotation and the Torsion Balance—New method of recording photosynthetic activity under light of different colours—Characteristic differences in response under different colours—Effect of intensity of radiation.

HAVING in a previous chapter determined the effect of increasing intensity of light on photosynthesis, there remains the question of the relative effectiveness of light of different colours. For the solution of this problem we require (1) monochromatic light of sufficient intensity to be physiologically effective; (2) a sensitive method of measuring the photosynthetic activity under a narrow beam of monochromatic light in a pure spectrum; and (3) the accurate measurement of the energy of the different rays. To obtain monochromatic light, liquid screens have been frequently employed, notably solutions of bichromate of potash for the yellow, and the ammoniated solution of copper sulphate for the blue rays. But the light transmitted through the bichromate solution is not pure red, but contains green also; similarly, copper sulphate solution allows the transmission not only of the blue but also of green rays. Additional screens may be used to cut off the impurities in the monochromatic light, but this procedure causes so great a diminution in the intensity of the light that it fails to produce any photosynthesis.

The spectrum of sunlight obtained by a prism has been employed for similar investigations, but its use is attended by various complications. The dispersion produced is

greater in the more refrangible portion of the spectrum, so that the intensity of light is relatively weakened in the blue-violet region. It is therefore necessary not merely to obtain a pure spectrum, but also to determine the energy of the different rays present in it. For the detection and qualitative determination of photosynthesis in the different regions of the spectrum, Engelmann devised the Bacterium-method, in which aerotropic swarmers are observed to congregate in larger numbers at the place of maximum evolution of oxygen during photosynthesis; this method, though extremely sensitive, is not suitable for quantitative measurements. Timiriazeff devised a highly ingenious method by which he was able to map out the photosynthetic action in different parts of the spectrum: he threw the solar spectrum on to a leaf, and after several hours developed it by first decolorising it with alcohol and subsequently treating it with iodine; the region of starch-formation under the effective rays then came out black. The most effective rays were found to lie between the Fraunhofer lines B and C. This method, again, is rather qualitative than quantitative.

Various attempts have been made to establish a relation between the wave-lengths of light and their photosynthetic efficiency. Timiriazeff (1877, 1885) and Reinke (1884) agree that the maximum $CO_2$-assimilation takes place in the red, though they differ in regard to the exact maximum point. Engelmann came to the conclusion that instead of a single point of maximum activity there are two maxima, one in the red and the other in the blue. Pfeffer, however, could not detect this second maximum, though he employed the same Bacterium-method. Timiriazeff and Richter (1902) attributed the smaller assimilation in the blue to the diminished intensity of radiation. Richter, indeed, was of opinion that it is not the quality but the energy of the light absorbed that determines effective assimilation. Jost thus summarises the general results of all the researches that have been made on the subject:

(1) 'Only light of wave-length between 770 $\mu\mu$ and 300 $\mu\mu$ conduces to assimilating activity in green plants; they are approximately the same rays that are visible to us.

(2) 'The assimilatory effect of different rays is unequal, but still not in such a way that some only are active whilst others lying beyond these are quite inactive. If we express the wave-length by abscissæ and the activity of assimilation by ordinates, we obtain a curve which does not in the least correspond with the curve expressing the energy of sunlight obtained by Langley.' [1]

The object of the following investigation is to obtain quantitative values of the relative effectiveness of the different rays of the spectrum. I employed for this purpose two independent methods, the Method of Flotation and the Method of the Bubbler. The principle of the latter has already been explained. I now describe in detail the Method of Flotation, which will be found to possess features which are novel.

A short length of *Hydrilla* plant floats in a beaker of water: on attaching a thin platinum wire to the young rosette of leaves at the upper end of the plant, the apex of the stem sinks down so that the cut end is uppermost. This end of the stem is then attached by means of a single waxed cocoon-thread to one arm of the Torsion Balance to be presently described. Adjustment is made (by adding a small weight to the pan P) such that the stem sinks about 1 cm. below the surface of the water in the beaker (fig. 46).

The balance consists of a light lever of aluminium, through which passes a thin torsion wire of phosphor-bronze used for galvanometer suspension. When a weight is placed on the right arm of the balance a torsion is produced, and the left arm is raised. The weight on the right arm is measured by the equivalent weight which has to be placed on the left arm in order to cause the balance to return to the zero position. This return to zero is more accurately determined by means of a beam of light reflected from a

[1] Jost, *Plant Physiology* (English edition), p. 127.

small piece of thin mirror M attached at the fulcrum, care being taken to place a small weight *w* below the point of suspension so as to make the system more stable by lowering the centre of gravity.

The cut specimen immersed in water undergoes a periodic variation of buoyancy under the photosynthetic action of light, in consequence of which the arm of the balance from

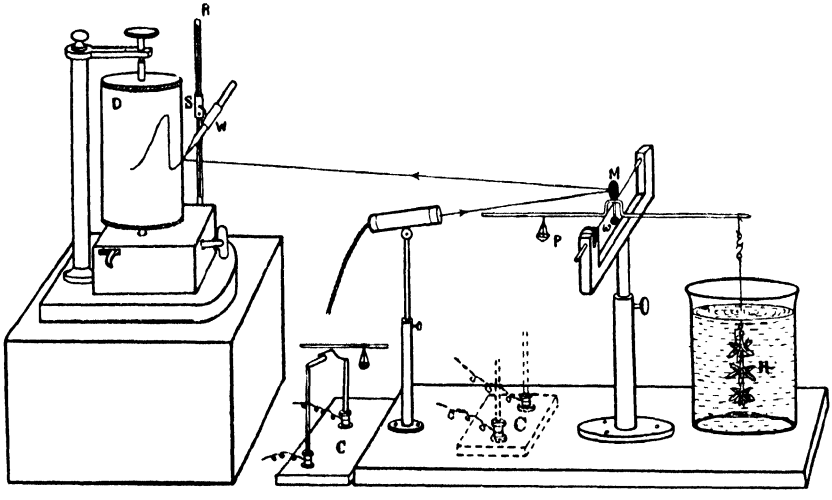



FIG. 46. Record of Photosynthetic Activity under Different Coloured Lights by the Method of Flotation

*Hydrilla* plant H suspended from one arm of balance; P, pan suspended from the second arm; the up and down movement of the *Hydrilla* plant under light completes an electric circuit by pressure of the left arm of the balance on two tinsels carried by rods on stand C; record is made on the revolving drum D by following the movement of the spot of light reflected from the mirror M with the writer W (see text).

which the plant is suspended moves up and down. This is due to the appearance of a bubble of oxygen at the cut end of the stem, which grows larger and larger, the rate of enlargement depending on the photosynthetic activity. Imagine a thin-walled balloon (with attached car) suddenly filled with gas: the balloon rises, and as the superincumbent pressure decreases the expanding gas bursts the envelope, the car being then precipitated down. The twig of the *Hydrilla* plant is the car, and the bubble of oxygen is the

balloon. The *Hydrilla*, suspended on one arm of the Torsion Balance, floats up till the cut end of the plant approaches the surface of the water, when the bubble bursts ; the twig now sinks, losing its adventitious buoyancy. The arm of the balance thus moves up and down, corresponding to the alternate flotation and sinking of the plant. Each periodic movement of the arm of the balance represents the photosynthetic production of a given quantity of oxygen. The next step is to obtain an automatic record of the evolution of the gas. This is secured by a device such that the periodic movement of the balance causes the completion of an electric circuit which includes the electro-magnetic writer previously described. Below the left arm of the Torsion Balance are adjusted two pieces of tinsel separated by a distance of 2 mm. The ascensional movement of the plant under the action of light causes up-movement of the right arm of the balance and down-movement of the left arm ; this latter presses one tinsel against the other, thus completing the electric circuit, with the result that a dot is made on the recording surface.

This method of recording photosynthetic activity may be rendered as sensitive as desired, first by increasing the sensitiveness of the Torsion Balance, and secondly by diminishing the depth of immersion of the cut end below the surface of the water. When this depth is reduced, the frequency of successive flotations becomes very rapid.

## Photosynthetic Activity under Different Colours

The frequency of oscillation of the floater thus enables us to measure the photosynthetic activity under different conditions. Experiments on this method were undertaken to determine the effect of different coloured lights, in which sunlight was reflected by a heliostat into the experimental room, different coloured lights being obtained by the interposition of suitable screens. Two coloured glasses, red and blue, were spectroscopically selected, each of which was

found to transmit light which was practically monochromatic. The red glass transmitted rays the wave-lengths of which extended from 760 to 605 λλ; the wave-lengths of light transmitted through the blue glass extended from 475 to 400 λλ, *i.e.* to about the G line. The method of experimental procedure was to determine the period of flotation under (1) direct sunlight, (2) under transmitted red light, and (3) under transmitted blue light.

The following is a summary of the results:

Period of successive flotations under sunlight, 50 seconds.
,, ,, ,, ,, red light, 175 ,,
,, ,, ,, ,, blue light, 400 ,,

The relative activities are inversely proportional to the respective periods of flotation. Hence—

$$S : R : B = \frac{1}{50} : \frac{1}{175} : \frac{1}{400}$$

In other words, taking the activity under blue light as 1, that under red light is 2·3; under direct sunlight it is 8.

$$B : R : S :: 1 : 2{\cdot}3 : 8 \quad . \quad . \quad . \quad (1)$$

## The Characteristic Differences in Response under Different Colours

The periods of flotation thus measure the relative activity under different lights; but they give us only the final results, not the entire history of the photosynthetic action. More complete and detailed information can be obtained by means of a continuous record of the rate of flotation. This is secured by an application of the optical method, already referred to. The spot of light reflected from the mirror attached at the fulcrum of the Torsion Balance moves downwards (represented up in the record) during the increasing buoyancy due to the formation of the bubble of oxygen. When this takes place rapidly under exposure to the more effective light, the rate of down-movement of

the reflected spot of light is correspondingly quickened. The less effective rays, on the other hand, cause a relatively slow movement. The records of the different rates of movement of the spot of light thus give a vivid picture of the relative effectiveness of the different rays. A continuous record of the response may be obtained photographically

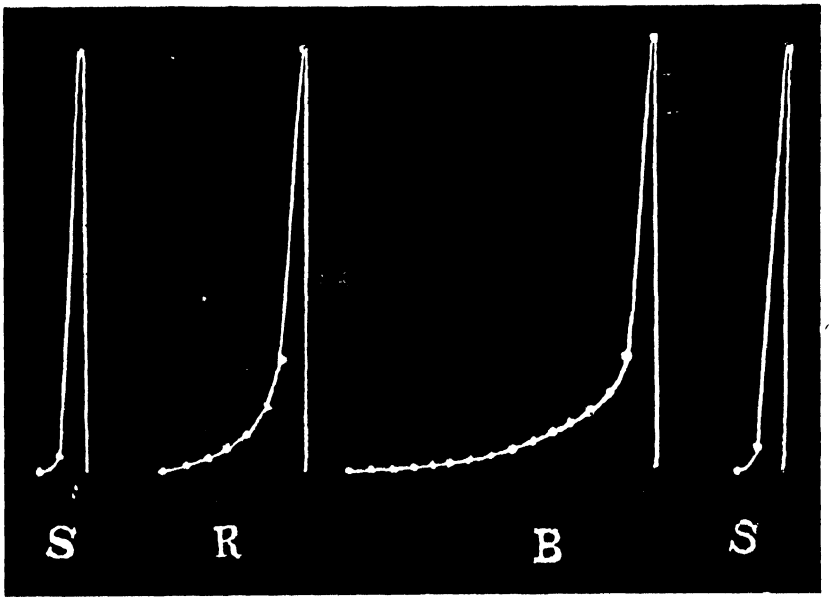



FIG. 47. Records of Response under Sunlight (S), Red Light (R), Blue Light (B), and Sunlight once more (S) (up-stroke represents flotation)

Successive dots at intervals of 30 seconds. Note the slow rate under blue light (B).

by means of sensitised paper on the recording drum; it can, however, be obtained more simply in the following manner. A split brass tube clasping a vertical rod R (fig. 46) acts as a spring and carries a writing-pencil W. The excursion of the spot of light up or down is followed by the hand, a thick dot being made at intervals of 30 seconds.

Fig. 47 gives records of responses under direct sunlight (S), under red light (R), and under blue light (B). A final record under sunlight was taken to make sure that the

sensitiveness of the specimen had not undergone any change during the whole period of the experiment. It will be noted that under sunlight the maximum flotation was attained in the course of 1 minute, the fall being practically instantaneous on the bursting of the bubble. Under red light the rate of ascent was, however, comparatively slow, and the ascensional movement was completed in the course of 3·5 minutes. A striking difference is observed in the curve under blue light. The bubbles were formed at a very slow rate, so that the slope of the response-curve is much flatter than that under red light ; the movement of flotation was completed in the course of 7·5 minutes. The last record under sunlight is practically the same as the first, proving that the sensibility had remained constant.

The relative ineffectiveness of blue light in comparison with red is thus demonstrated by two independent methods, which show different periods of flotation and the characteristic differences in the response-record, the response of the relatively ineffective blue light being characterised by a flatter curve.

The periods of flotation in two different specimens were as in the following table :

TABLE XXIV.—SHOWING PERIODS OF FLOTATION UNDER DIFFERENT LIGHTS

| | Specimen I. | Specimen II. |
|---|---|---|
| Period of flotation under blue light . | 455″ | 399″ |
| ,, ,, ,, red ,, . | 224″ | 186″ |
| ,, ,, ,, sunlight . | 55″ | 53″ |

The ratio of activities in the first case is :

$$B : R : S :: 1 : 2 : 8{\cdot}2 \quad . \quad . \quad . \quad . \quad . \quad (2)$$

In the second case it is :

$$B : R : S :: 1 : 2{\cdot}1 : 7{\cdot}6 \quad . \quad . \quad . \quad . \quad (3)$$

*Determination by the Method of Bubbling.*—The action of the same red and blue lights was compared by the Method of Bubbling. The sequence of experiment was: (*a*) Determination under direct sunlight; (*b*) under red light; (*c*) under blue light; (*d*) once more under sunlight. The following are the results:

| | | |
|---|---|---|
| (*a*) | Activity under sunlight . . . | 95 |
| (*b*) | ,, ,, red light . . . | 24 |
| (*c*) | ,, ,, blue light . . . | 11·6 |
| (*d*) | ,, ,, sunlight . . . | 94 |

B : R : S : : 11·6 : 24 : 95 : : 1 : 2 : 8·2 . . (4)

Thus with different specimens and by the employment of two different methods we obtain the following ratios:

| | | |
|---|---|---|
| (1) | B : R : S : : | 1 : 2·3 : 8 |
| (2) | ,, | 1 : 2·0 : 8·2 |
| (3) | ,, | 1 : 2·1 : 7·6 |
| (4) | ,, | 1 : 2·0 : 8·2 |

The relative values of photosynthesis under the different lights are thus seen to be practically the same in all the experiments.

Having determined the effects of the red and blue, I next attempted to find those of other lights; but the yellow, orange and green glasses did not give pure monochromatic light. It is possible to cut off the impure constituents in the light by the use of multiple screens, but the intensity of radiation then undergoes so great a diminution that the photosynthetic activity becomes diminished or even abolished. We have to remember that the colour or wavelength of light is but one of the two factors in its photosynthetic action, the other being its energy of radiation. This will be understood from the following experiment. The light transmitted through a single sheet of red glass was, as we have seen, more effective than that transmitted through a single piece of blue glass. If instead of a single

piece we use two such pieces of red glass superposed one over the other, we obtain the same red light, but there is a considerable diminution of intensity. The result is that the blue light under these circumstances is more effective than the red.

In order to avoid the indefiniteness in the result introduced by the use of coloured screens, we have to employ the solar spectrum itself, in which we obtain pure monochromatic light in the different regions, the wave-length of which is definitely known. A complication, however, arises on account of unequal dispersion ; it is therefore necessary to measure the energy of the different rays in the spectrum. Both important elements in photosynthesis, namely the wave-length and the energy of the particular ray, have therefore to be determined. An account of the successful solution of this problem will be given in the next two chapters.

## Summary

The results of previous researches on the photosynthetic activity of the different rays are not concordant. Engelmann regards the curve of photosynthesis as exhibiting two maxima, one in the red and the other in the blue ; this conclusion is controverted by others.

The important conditions in this research are : (1) a pure monochromatic light ; (2) a sufficiently sensitive device for the quantitative determination of photosynthesis ; and (3) the simultaneous measurement of the energy of the different rays.

Coloured screens do not, generally speaking, give pure monochromatic light.

A new method of measuring photosynthesis is described : that of recording the rate of the successive flotations of a piece of a water-plant suspended in $CO_2$-solution from one arm of a balance. The photosynthetic production of a bubble of oxygen causes flotation of the specimen, followed by sinking on the bursting of the bubble at the surface. The

resulting periodic movements of the arm of the balance are electrically recorded: the records exhibit characteristic differences in the efficiency of light of different wavelengths.

The relative photosynthetic efficiency of blue, red, and white light is found to be 1 : 2·1 : 8.

# CHAPTER XXI

## DETERMINATION OF THE ENERGY OF THE DIFFERENT RAYS OF THE SOLAR SPECTRUM

Securing a pure solar spectrum—Sliding of the spectrum on to the plant by a reflecting mirror—The Magnetic Radiometer—Distribution of energy in the spectrum.

THE investigation of the relative photosynthetic effectiveness of different wave-lengths of light involves the securing of a pure solar spectrum, and the determination of the energy of the different rays. I first describe the method of obtaining a pure spectrum.

### The Pure Solar Spectrum

For photosynthetic investigation the spectrum has to be fairly enlarged so that the plant may be exposed to rays which are approximately monochromatic. It is quite easy to do this by placing the plant at a considerable distance from the prism, but the enfeebled light is then ineffective for photosynthesis. A compromise has therefore to be made as regards the breadth of the spectrum, by the employment of a carbon disulphide prism. I secured a spectrum the visible size of which was 18 cm. from the extreme red at one end to the violet at the other ; it was also found to be continued into the invisible infra-red region for a distance of about 6 cm.

The plant-vessel with the attached Bubbler had to be as narrow as possible : a test-tube having a diameter of 1 cm. was used for the purpose.

The employment of the carbon disulphide prism accounts

for the presence of infra-red rays to the left of the visible spectrum. Though the dispersion is greater at the more refrangible end, this is not a serious drawback in the quantitative measurement by the method to be described for determining the distribution of energy in the different regions of the spectrum.

The following are the details of the method by which a pure spectrum was obtained. Sunlight is reflected by a self-

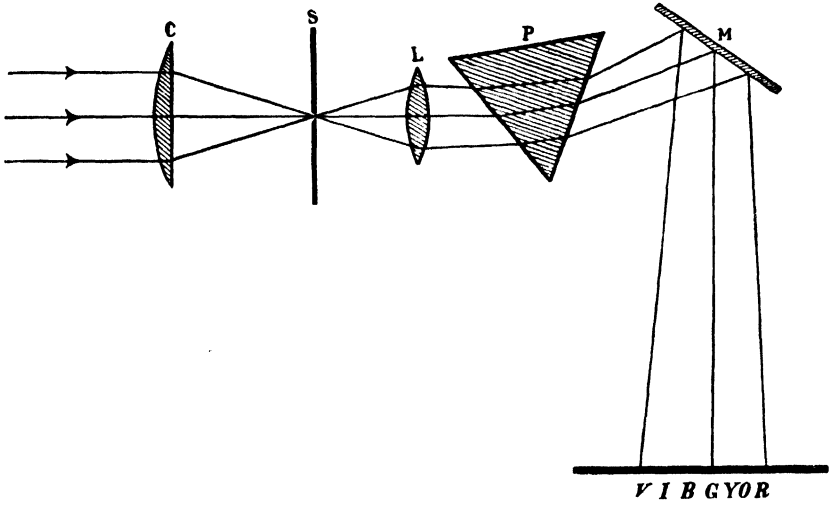



FIG. 48. Arrangement for obtaining a Pure Solar Spectrum

C, cylindrical lens focusses line of light on slit S; L, focussing lens; P, $CS_2$ prism; M, reflecting mirror on a stand with tangent screw (not shown), by which the different rays may be thrown in succession on the plant-vessel or on the sensitive strip of the Radiometer.

regulating heliostat on to a cylindrical lens C, which is fixed in the closed window of the dark experimental room. This gives a bright and intense line of light. A vertical and adjustable slit S, placed at the focus, cuts off all but the central rays. A photographic lens L forms a sharp image of the slit at a distance of 1 metre. The carbon disulphide prism is placed in the path of light and is adjusted at the angle of minimum deviation (fig. 48). The projected spectrum now shows the Fraunhofer's lines with great

distinctness. When the vertical slit is widened the spectrum is very bright, but no longer pure. The breadth of the slit is so adjusted that there is a maximum intensity of light without overlapping in the spectrum ; the Fraunhofer's lines become indistinct when there is any overlapping.

Instead of moving the plant-vessel from one region of the spectrum to the other, which might cause mechanical disturbance, it is preferable to slide the different spectral rays on to the plant-vessel kept undisturbed in its own place. The possibility of changing the position of the spectrum is absolutely necessary for experiments with the Radiometer for the determination of the energy of the different rays: for the highly sensitive Radiometer has to be kept fixed and protected from all disturbance.

The sliding of the spectrum is effected by means of a highly polished and perfectly plane mirror M, silvered on the front. The stand of the mirror has a fine tangent screw by which the different rays can be made to fall either on the plant-vessel or on the sensitive strip of the Radiometer placed near it.

For the measurement of the energy of radiation, some observers employ a linear thermopile, which is but moderately sensitive. Greater sensitiveness was obtained by Langley in his device of the Bolometer, where the change of resistance in a wire caused by rise of temperature, due to the incident radiation, is measured by a Wheatstone Bridge. The arrangement is somewhat complicated, and various precautions have to be taken to secure accurate results; but in Langley's hands it has done much in advancing astrophysical research. His values are, however, quite inapplicable at a different latitude and under different climatic conditions. The energy of the different rays is modified by the absorbing thickness of the strata of air through which sunlight is being transmitted ; changes in the intensity are thus introduced by meteorological conditions, the position of the sun in the heavens, the time of the day, and so on.

It was therefore necessary to devise a special Radiometer

for the determination of the different intensities of radiation at the time and place of observation almost simultaneously with that of photosynthesis. This is secured by throwing the particular rays first on to the plant-vessel and then on to the Radiometer. We thus secure the three elements necessary for quantitative research: (1) the wave-length of the rays, (2) their energy of radiation, and (3) the photosynthetic activity induced by each particular ray.

## The Magnetic Radiometer

I have succeeded in directly determining the energy of the different rays by measuring the elongation of a metallic wire coated with lamp-black. The particular spectral ray falling on the wire is absorbed, and thus raises the temperature proportionately to its energy of radiation. The rise of temperature is excessively feeble, being of the order of $\frac{1}{100,000}$° C.; the resulting increase of length is so minute as to be undetectable by any method of magnification which has been hitherto employed. I have, however, got over this difficulty by means of a magnetic device by which it is possible to obtain a magnification of about 50,000,000 times or even higher.

Fig. 49 is a diagrammatic representation of the instrument. W is the strip or wire which becomes lengthened by rise of temperature produced by the absorbed radiation. It is attached by a hook to the short arm of a long magnetic lever, the N end of which is lowered by any elongation of the sensitive wire. In front of the N end of the magnetic lever is suspended a small magnetic needle with an attached mirror. As the N pole of the magnetic lever is lowered it produces increasing deflection of the suspended needle, which is magnified by the spot of light reflected from the attached mirror. The sensitiveness of the apparatus is very greatly enhanced by the employment of a perfect system of astatic needles.

This method for measurement of radiation is extra-

ordinarily sensitive. For instance, there is an apparatus permanently adjusted in the Institute in which the sensitive element is a thin strip of ebonite which is enclosed in a tube with a narrow slit in front. When any person walks at some distance past the instrument, the indicating spot of

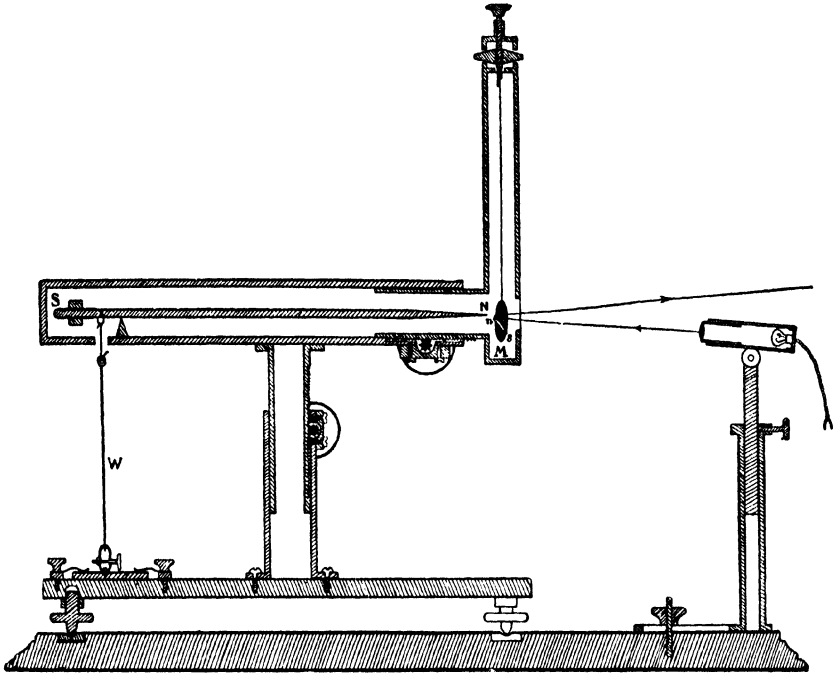



FIG. 49. The Magnetic Radiometer

SN, magnetic rod supported on fulcrum; short arm of magnet attached to sensitive strip of metal W. Elongation of strip lowers the N end, which causes increasing deflection of the suspended needle with attached mirror M. Deflection magnified by reflected spot of light.

light remains perfectly quiescent until the individual is in the line of sight of the sensitive strip; a sudden deflection is then produced by the radiation which is emitted from the human body through thick warm clothing and a heavy overcoat; when he walks past the line of sight the indicating spot of light returns to the original zero.

For our present purpose such a high sensitiveness is

not required. A thin strip of zinc 1 mm. in breadth and 10 cm. in length was therefore used as the sensitive element, zinc wire not being available. It was cut and filed sufficiently thin; the two ends of this strip were soldered to thicker pieces of brass in which large holes were bored, filled up with ivory. A small hole was bored in the ivory for the insertion of the upper hook which attached the strip to the magnetic lever; the second piece of brass soldered to the lower end was clamped between two jaws faced with non-conducting sheets of mica; a pin was passed through the narrow hole in the ivory which plugged the lower piece of brass to make the clamping more secure. The object of the thermal insulation of the sensitive strip is to prevent its receiving any heat by conduction from the rest of the apparatus.

The strip was surrounded by a wooden tube with a narrow slit for the passage of the spectral rays. The slit was open through a length of 10 cm., the brass piece to which the strip was soldered being protected from radiation. A very thin piece of mica covered the slit, to prevent air-currents getting access to the narrow chamber in which the sensitive strip was enclosed. This exceedingly thin piece of mica was found to have no effect in obstructing radiation. The whole apparatus was placed inside a larger wooden box covered with non-conducting felt. A round hole in front of the box (covered with a piece of thin glass) allowed the passage of the spot of light reflected from the mirror attached to the suspended magnetic needle. A second thin strip of mica closed the slit behind the box through which the radiation reached the sensitive strip.

It is necessary to give a detailed account of precautions to be observed, because the instrument is so sensitive that it detects the slightest difference in the temperature of different portions of the same mass of air. Gases are highly non-conducting, and their temperature hardly attains a perfect uniformity. In these circumstances it is best to take precautions against contact of the strip with the

outside air, which, again, should not be disturbed in any way. The observer never moves from his place in the dark room, which is closed on all sides.

A freshly mounted sensitive strip was found to be erratic in its responses, the zero-point being continuously displaced, as the result of the physical strain to which it had been subjected by previous filing and in mounting it in the apparatus. The instrument was therefore allowed to remain undisturbed in the experimental room for a fortnight, after which the responses became extraordinarily consistent; the zero-keeping quality also became very perfect. When the sensitive strip was exposed to a particular radiation, the maximum deflection was attained in the course of two seconds; stoppage of radiation was followed by an almost immediate return of the spot of light to the exact zero in a dead-beat manner. As regards the mica cover, a statement is often made that mica is opaque to certain radiations. This is true only when the thickness is sufficiently great; a thin strip put 'on' and 'off' the path of radiation did not produce any change in the deflection of the Radiometer.

The spectrum produced by the carbon disulphide prism was found, as already stated, to extend beyond the limit of the visible red, this extension into the infra-red region being almost 6 cm., or about one-third the breadth of the visible spectrum.

The extreme sensitiveness of the Radiometer was also exhibited by its discrimination of the radiation-components of the morning and the midday light. In the morning the intensity of the blue rays in the spectrum was found to be slightly less than at midday. This is due to the greater scattering of the short waves by the thicker stratum of the atmosphere through which the light has to pass in the morning.

The experiment on photosynthesis was carried out between 10 and 11 A.M. on March 10. The energy of radiation in different regions of the spectrum was measured on January 16 and 19, 1923, and on March 10 immediately

after the determination of the photosynthesis under the different rays. It should be remembered that the determination of the energy of the different rays was made upon the spectrum given by the carbon disulphide prism at a particular season of the year, from January to March. The following results for the 16th and 19th January are seen to be practically the same.

TABLE XXV.—SHOWING THE DISTRIBUTION OF ENERGY IN THE SPECTRUM PRODUCED BY THE $CS_2$ PRISM

| Wave-length | | Energy of radiation | | Mean |
|---|---|---|---|---|
| | | 16th January | 19th January | |
| Infra-red | 850 $\mu\mu$ (?) | 90 | 80 | 85 |
| | 790 ,, (?) | 148 | 146 | 147 |
| A | 760 ,, | 146 | 144 | 145 |
| *a* | 720 ,, | 135 | 129 | 132 |
| B | 680 ,, | 120 | 120 | 120 |
| C | 656 ,, | 96 | 98 | 97 |
| D | 590 ,, | 72 | 70 | 71 |
| E | 527 ,, | 43 | 41 | 42 |
| F | 486 ,, | 28 | 26 | 27 |
| G | 430 ,, | 12 | 8 | 10 |

Radiation was detected at some considerable distance beyond the extreme red. At about 850 $\mu\mu$ the mean deflection of the Radiometer was 85 divisions; at 790 $\mu\mu$ the energy was found to be 147, which was the maximum. At A (760 $\mu\mu$) the deflection declined to 145; at *a* (720 $\mu\mu$) it was reduced to 132; C (656 $\mu\mu$) gave a deflection of 97. At the sodium line D (590 $\mu\mu$) the reading was 71; the energy of the spectrum underwent a further and continuous decline: at E, F and G the deflections were 42, 27 and 10 respectively.

## SUMMARY

A pure spectrum of high dispersion was obtained by the employment of a $CS_2$ prism. Arrangements were made such

that the different rays could be thrown on to the stationary plant-vessel and on to the adjoining sensitive strip of the Radiometer quickly, one after the other. It was thus possible to make a simultaneous determination of the photosynthetic activity and of the energy of any particular ray of light.

The energy of radiation was determined from the elongation of a blackened strip of zinc, the expansion being magnified some 50,000,000 times by the Magnetic Radiometer. The Radiometer is capable of detecting a rise of temperature of $\frac{1}{100,000}$° C.

The maximum deflection of the Radiometer was attained within a few seconds, and the stoppage of radiation was followed by an exact return to zero in a dead-beat manner.

Radiation was detected at some considerable distance beyond the extreme red. The maximum energy was found to be in the infra-red at 790 $\mu\mu$, after which, towards the more refrangible blue and violet, the energy of the rays exhibited a continuous decline.

# CHAPTER XXII

## DETERMINATION OF THE PHOTOSYNTHETIC EFFICIENCY OF THE SPECTRAL RAYS

Difficulties in the quantitative determination of photosynthesis under enfeebled spectral rays—Results with exceptionally sensitive specimens —Enhancement of sensitiveness by rise of temperature—Vacuum-jacket for maintenance of constant temperature—Method of depletion of starch in enhancement of sensitiveness—The Heterostatic Method—The Auxiliary Stimulator—Additive effect of light—The Absorption-spectrum—Tropic movement under certain rays of the spectrum—Simultaneous determination of energy of rays and of the induced photosynthesis—Characteristic effects of different rays on photosynthesis —Complementary relation of A and D effects in photosynthesis and in phototropism.

HAVING fully explained in the last chapter the method of determining the energy of radiation, we may now proceed to obtain quantitative determinations of photosynthetic activity under different rays in a pure spectrum. This presents considerable difficulties, owing to the great enfeeblement of the light by the highly dispersing prism such that the intensity of radiation falls below the minimum intensity for photosynthesis. This may be obviated by increasing the intensity of light by widening the slit of the spectrum apparatus; but this advantage is discounted by the consequent overlapping of the different rays.

On several occasions, especially in spring, I obtained very sensitive specimens which reacted even under the spectral rays; but prolonged exposure to the feeble light induced such a depression that the plants soon became insensitive. The only possible way to secure a complete set of observations was to hurry through the determinations as quickly as possible. Out of thirty different attempts, I was fortunate in obtaining four complete determinations

of which a typical example is given below. The first column in Table XXVI. gives the colour of the light, the second the average bubbling-period, and the third the photosynthetic activity in c.mm. O per hour. In order that the different determinations given below may be easily compared with each other, the maximum activity which is manifested under red light is taken as 100, and the other activities proportionately.

TABLE XXVI.—SHOWING PHOTOSYNTHETIC ACTIVITY UNDER DIFFERENT SPECTRAL RAYS

| Light | Bubbling-period | Activity in c.mm. O per hour | Relative activities (Red = 100) |
|---|---|---|---|
| Red . . . | 18 seconds | 200·0 | 100·0 |
| Yellow . . | 42 ,, | 85·7 | 42·8 |
| Green . . . | 100 ,, | 36·0 | 18·0 |
| Blue . . . | 140 ,, | 25·1 | 12·6 |
| Violet . . . | 600 ,, | 6·0 | 3·0 |

The results obtained with four different specimens, and their mean, are summarised below.

TABLE XXVII.—GIVING PHOTOSYNTHETIC ACTIVITY OF FOUR DIFFERENT SPECIMENS

| Light | Relative activities | | | | |
|---|---|---|---|---|---|
| | I. | II. | III. | IV. | Mean |
| Red . . . | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 |
| Yellow . . | 41·5 | 40·5 | 46·1 | 48·0 | 44·0 |
| Green . . | 20·0 | 23·1 | 18·5 | 17·0 | 19·7 |
| Blue. . . | 12·0 | 13·5 | 12·0 | 10·0 | 11·9 |
| Violet . . | 4·0 | 2·3 | 3·0 | 1·5 | 2·7 |

The above results give a general idea of the relative efficiency of the different rays; there was not enough time to determine the exact wave-length of the red light which was most potent, for, as stated above, the determinations

had to be made quickly one after another to guard against the loss of sensibility in a protracted experiment.

## Methods for the Enhancement of Sensitiveness

In order to obviate the difficulties mentioned, various attempts were made to increase the sensitiveness of the specimen.

*Method of rise of temperature below optimum.*—One of the methods which appeared promising was to keep the specimen in warm water at a temperature slightly below the optimum. As this temperature of 28° C. was higher than that of the room, the temperature of the plant-vessel slowly fell, on account of loss of heat by convection and radiation. I attempted to remedy this by placing the vessel within a vacuum-jacket. The device was highly successful in maintaining the temperature constant; but the increase of photosynthetic activity obtained by this interesting method was not sufficiently great.

*Method of depletion of starch.*—From certain experimental results it had appeared that an excessive deposit of starch causes a partial inhibition of photosynthesis. It was therefore to be expected that depletion of starch would enhance the sensitiveness of the specimen. To test this I placed various specimens in complete and in semi-darkness, with the result that more or less of the starch disappeared. Partial depletion of starch was found to cause an enhancement of photosynthetic sensitiveness. A specimen kept in perfect darkness became insensitive and subsequently died. The maximum period in semi-darkness for optimum sensitiveness was found to depend on the physiological condition of the specimen. Though long investigation would undoubtedly enable us to determine the average duration of the requisite period of semi-darkness, the investigation is for the present postponed, as I have succeeded in discovering a more satisfactory method for rendering the plants efficient.

*The Heterostatic Method.*—The principle of this method of enhancing the sensitiveness of physical instruments is of much interest. A feeble electric charge cannot be easily detected; but by the previous charging of the indicating needle its deflection under a feeble electric charge in a neighbouring conductor becomes greatly increased. For physiological investigations we may secure greater sensitiveness by a somewhat analogous process, though the antecedent physical and physiological treatments are very different.

*The Threshold of Response.*—Let us consider the particular condition of the photosynthetic organ when it fails to respond to feeble incident radiation. The cause of this inefficiency is that a certain amount of energy must be absorbed before the plant-organ can manifest an external response by the evolution of oxygen. There is thus a preparatory limit, which I shall, for want of a better term, call the *Verge* or *Threshold of Response. The energy acting on the plant below this is outwardly inefficient, but it has brought the plant to the verge of response; the preliminary work has been accomplished, so that radiation, previously ineffective, will now precipitate response increasingly as it increases in intensity.*

*The Auxiliary Stimulator.*—The plant is brought to the verge of response by the employment of a 30-c.p. Pointolite for the auxiliary stimulation. The incandescent lamp is enclosed in a conical tube mounted on a suitable stand, so that by a slight inclination of the tube the rays may be thrown upon the plant-vessel (fig. 50); the lamp-tube is so fixed that it does not obstruct the spectral rays falling on the plant. The intensity of radiation is varied by moving the auxiliary light towards or away from the plant. It is so adjusted at first that bubbling is initiated. The source of light is now removed slightly away from the plant till bubbling just ceases. This is the adjustment for the *limiting verge.* There is no photosynthesis, but the plant is ready to exhibit it in response to additional radiation however

feeble it may be. The very great advantage of the auxiliary stimulator is that under its action the sensitiveness of the specimen remains constant for even very long periods. This will be seen from the account of the following experiment. The auxiliary stimulator was so adjusted that the specimen was on the verge of response; different rays of the spectrum were now thrown on the plant by means of the reflecting mirror M. Under red light (B, 680 λλ) the relative activity was 100; under blue light (486 λλ) the activity declined to 56: hence the blue rays are less effective than the

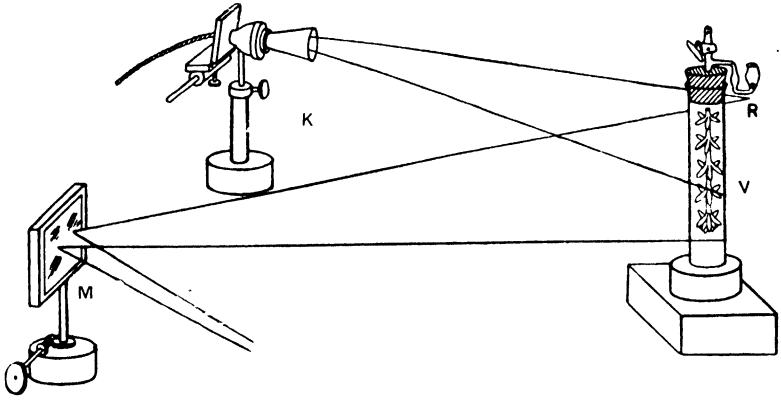



FIG. 50. Heterostatic Method for rendering ineffective Stimulus effective

Light from Pointolite K falls on the plant, and adjustment is made for inducing verge of stimulation. Rays from different regions of the spectrum are thrown on the plant by the reflecting mirror M, the stand being provided with a tangent screw.

red in the proportion of 56 : 100. The red was applied for a second time, and the activity was now found to be the same as before, namely 100. The activity under orange light (656 λλ) was 66; blue light was applied for a second time, and the activity was found to be the same as before, namely 56. Carrying the observations backwards and forwards gave similar results, showing the remarkable uniformity secured by the method of auxiliary stimulation.

*Additive effect of light.*—In order to prove that the effect of incident radiation is added to that of the auxiliary light I carried out the following experiments. The auxiliary

Pointolite was a 30-c.p. lamp mounted on the same base-board which carried the plant-vessel, so that the distance between the two was constant. This fixed distance was 33 cm., or 0·33 metre, and the intensity of light to which the plant was subjected was, therefore $\frac{30}{(\cdot 33)^2} = 275$ lux. This was slightly above the verge, and the evolution of oxygen by the plant was at a rate of 23·5 c.mm. per hour.

The plant-vessel and the auxiliary light were then placed on the experimental table, different points in which had been marked previously as 500, 750 and 1000 lux with a stronger Pointolite lamp of 100 c.p. After observation of the additive effect with 500 lux, the experiment was repeated with 750 and 1000 lux. The results given in the following table show that the combined effect of two distinct lights is additive.

TABLE XXVIII.—EFFECT OF INCIDENT LIGHT OF INCREASING INTENSITY SUPERPOSED ON AUXILIARY LIGHT

| | c.mm. O per hour |
|---|---|
| Effect of auxiliary light . . . . . | = 23·5 |
| Effect of 500 lux (without auxiliary light) . . | = 42·8 |
| Effect calculated with addition of auxiliary light: 42·8 + 23·5 . . . . . | = 66·3 |
| Observed effect . . . . . | = 66·7 |
| Effect of 750 lux . . . . . | = 64·3 |
| Effect calculated with auxiliary light: 64·3 + 23·5 | = 87·5 |
| Observed effect . . . . . | = 87·5 |
| Effect of 1000 lux . . . . . | = 92·3 |
| Effect calculated with auxiliary light: 92·3 + 23·5 | = 115·8 |
| Observed effect . . . . . | = 116·0 |

These results show (1) that, when a plant is exposed to light from two sources, the activity of photosynthesis is the sum of the effects, which are therefore additive; and (2) that an auxiliary light may be employed to bring the plant to the

verge of response so that it is possible to detect and measure the effect of incident radiation of even feeble intensity.

Before describing the effects of different rays of the spectrum on photosynthesis I must refer to two important factors which affect photosynthesis, namely, the absorption of particular rays by the chlorophyll and the utilisation of the energy of certain of these rays in phototropic stimulation.

## The Absorption-Spectrum of Chlorophyll

Spectroscopic observations were made to ascertain what absorption-bands are produced by the interposition of a single leaf of *Hydrilla* in the path of light. The spectrum showed (1) a feeble general absorption and numerous faint dark lines throughout; (2) an intense dark band between the red and the orange on both sides of B (680 $\mu\mu$), the darkest region being slightly to the left of B; and (3) a less dark absorption-band in the blue-violet region between F and G. This second band was inconspicuous when the spectrum was bright with strong light; but with feeble light it was more noticeable.

## Typical Experiments on the Photosynthetic Efficiency of the Different Spectral Rays

The following are the results of an experiment upon the relative effectiveness of light of different wave-lengths, made on the heterostatic method. For facility of comparison the maximum activity, occurring at B, is taken as 100. Photosynthesis was found to be initiated even in the invisible infra-red region: the activity was, however, feeble, being 7·6 at about 770 $\mu\mu$. At A (760 $\mu\mu$) the activity increased to 12; at *a* (720 $\mu\mu$) it rose to 35. The maximum activity of 100 was attained at B (680 $\mu\mu$). After this, photosynthetic action underwent a decline to 70 at C (656 $\mu\mu$), to 39·6 at D (590 $\mu\mu$), to 15 at E (527 $\mu\mu$), to 6·8 at F (486 $\mu\mu$), and to 2·0 at G (430 $\mu\mu$).

I give in the following table results obtained with three

different specimens including the above. On comparing them with those given in Table XXVII., with which they are in general agreement, the advantage of the heterostatic method is clear.

TABLE XXIX.—SHOWING PHOTOSYNTHETIC ACTIVITY EXHIBITED BY DIFFERENT SPECIMENS UNDER DIFFERENT RAYS OF THE SPECTRUM

(Activity at B taken = 100)

| Wave-length | Relative photosynthetic activity in c.mm. per hour | | | |
|---|---|---|---|---|
| | I. | II. | III. | Mean |
| Infra-red . . | 7·6 | 8·2 | 8·0 | 7·9 |
| A 760 μμ . . | 12·0 | 10·0 | 11·0 | 11·0 |
| *a* 720 ,, . . | 35·0 | 34·0 | 32·0 | 33·7 |
| B 680 ,, . . | 100·0 | 100·0 | 100·0 | 100·0 |
| C 656 ,, . . | 70·0 | 68·5 | 72·2 | 70·2 |
| D 590 ,, . . | 39·6 | 38·0 | 41·0 | 39·5 |
| E 527 ,, . . | 15·0 | 13·0 | 18·0 | 15·3 |
| F 486 ,, . . | 6·8 | 3·6 | 10·0 | 6·8 |
| G 430 ,, . . | 2·0 | 4·0 | 3·0 | 3·0 |

## Simultaneous Determination of the Energy of the Rays and of the Induced Photosynthesis

Determinations were made almost simultaneously by the Bubbler and the Radiometer; the former gave the photosynthetic activity and the latter the energy of the rays. The results obtained on March 10, 1923, are given in the following table.

TABLE XXX.—SHOWING THE DISTRIBUTION OF ENERGY IN THE SPECTRUM AND THE CORRESPONDING PHOTOSYNTHESIS

| Wave-length | Energy of radiation | Activity of photosynthesis |
|---|---|---|
| Infra-red 850 μμ (?) . | 120·0 | 0·0 |
| ,, 770 ,, (?) . | 144·0 | 6·0 |
| A 760 ,, . . | 143·0 | 11·0 |
| *a* 720 ,, . . | 136·8 | 41·0 |
| B 680 ,, . . | 120·0 | 100·0 |
| C 656 ,, . . | 97·0 | 75·0 |
| D 590 ,, . . | 64·0 | 43·0 |
| E 527 ,, . . | 38·0 | 20·0 |
| F 486 ,, . . | 24·0 | 10·0 |
| G 430 ,, . . | 10·0 | 2·0 |

The curves given (fig. 51) show the distribution of energy and of photosynthetic activity. The absorption-

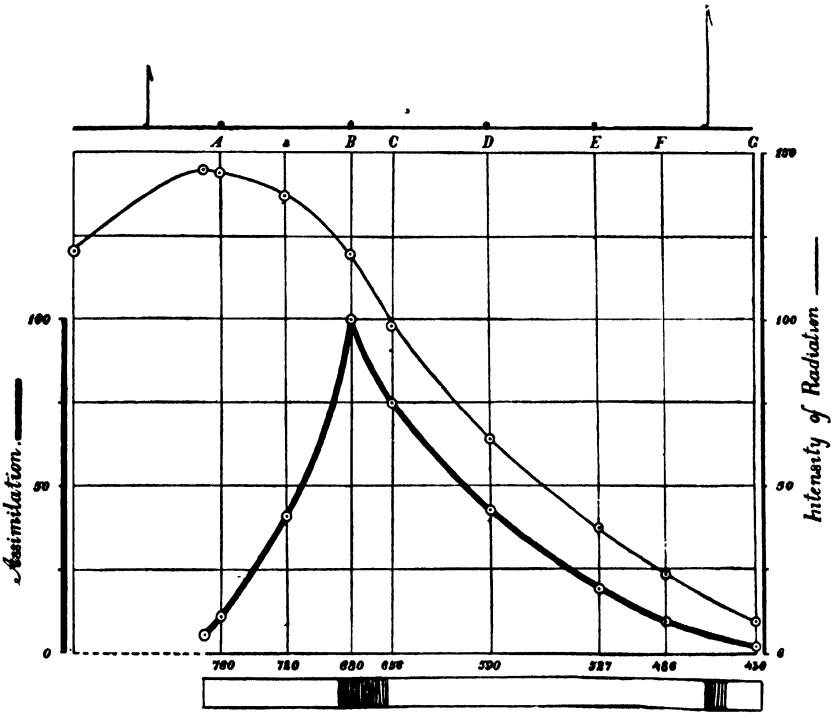



FIG. 51. Curves showing Distribution of Energy in the Spectrum (thin line) and corresponding Photosynthesis (thick line)

Absorption-bands of chlorophyll of the leaf are shown below: phototropic responses indicated above, effective rays being the infra-red to the left of A, and the rays between F and G.

bands of the visible spectrum are indicated below; the phototropic responses in the infra-red and the blue-violet are given as up-curves above.

## Characteristic Effect of Different Rays on Photosynthesis

There is a considerable difficulty in discovering the relation between either the intensity or the wave-length of radiation and the resulting photosynthesis. It has been thought

that photosynthesis depends only on the energy or intensity of radiation. But the curves of energy of radiation and of photosynthesis do not run a parallel course; for the intensity of radiation is at its maximum in the infra-red to the left of A, where photosynthesis is practically zero.

The less refrangible visible rays of greater wave-length have been held by others to be the most effective. But the least refrangible visible ray A induces hardly any photosynthetic action. Finally, it has been thought that it is the amount of energy absorbed, as indicated by the chlorophyll-spectrum, which is the chief factor in photosynthetic action. This view finds partial support in the occurrence of maximum activity at B, which is also the region of greatest absorption in the spectrum: but it fails to explain the considerable amount of photosynthetic activity in the orange and in the yellow, in which there is no great absorption. There is, again, very little activity corresponding to the absorption-band in the blue-violet.

These anomalies disappear if we take full account of all the factors and their combined effects.

1. Other things being the same, photosynthesis depends on the energy of radiation.

2. Photosynthetic activity is greatest in the region of maximum absorption in the spectrum.

3. When photosynthesis and absorption do not coincide, it may be inferred that the absorbed rays are utilised for some other work.

*Complementary A and D effects.*—The third factor in the list given above requires further amplification. It is obvious that the incident radiation can be utilised (1) for increasing the potential energy, or (2) dynamically in movement: the former takes place characteristically in photosynthesis; the latter in phototropic stimulation. The rays at the opposite ends of the spectrum—the infra-red and the highly refrangible blue-violet—being, as will be presently shown, the most effective in phototropism, are the least effective in photosynthesis.

Having before us all the operative factors, I now go on to explain the characteristic effects in the different regions of the spectrum. In the infra-red the energy is at its maximum, but there is no photosynthesis, the energy of the ray being utilised in phototropic stimulation. At B, where there is the maximum absorption, the photosynthetic activity is at its maximum. After this rise of activity there is a continuous fall, which is parallel to the decline in energy of the rays. The slight dark absorption-band in the blue-violet does not produce any increase of photosynthesis; how then is the absorbed energy utilised? In seeking for an answer to this question, I made the following experiments upon the phototropic activity of the different rays.

## Tropic Movement induced by Certain Rays of the Spectrum

I took a number of long-stalked leaves of *Tropaeolum*, which are very sensitive to the action of light. A row of these, mounted in small phials containing water, were exposed to the spectrum. In ordinary leaves the lamina is so large that it would intercept more than one region of the spectrum. This difficulty was overcome by growing the *Tropaeolum* plants in pots, and restricting the supply of food-material. The result of this was that the lamina was reduced to the size of about 1 cm. in diameter: so each leaf in the row could thus be acted on by monochromatic light in the different parts of the spectrum. Exposure of the leaves to the spectrum for about an hour brought out the differences in the phototropic action of the various rays. It was found that there was little or no curvature produced in the leaves placed in the red, orange, yellow or green light; there was, however, considerable phototropic bending of the leaf under the infra-red rays to the left of A; the phototropic response in the blue-violet between F and G was even greater. Records of responses in the infra-red

and blue-violet were taken for the equal exposures of seven minutes' duration; the amplitude of these responses is given at the upper part of fig. 51.

From these experiments it appears that the rays corresponding to the absorption-band between F and G of the chlorophyll-spectrum are chiefly active in phototropic stimulation. This naturally raises the general question as to the absorption of phototropically active rays by plants, more specially by parts of plants without chlorophyll, but it does not fall within the scope of the present work.

## Summary

Photosynthetic activity is reduced or abolished by prolonged exposure to the feeble radiation of the spectral rays.

In order to measure photosynthesis under the spectral rays, which are relatively feeble, it is necessary to enhance the sensitiveness of the specimen. This may be partially effected by raising the temperature short of the optimum, constancy of temperature being maintained by a vacuum jacket. The sensitiveness may also be increased by producing depletion of the deposit of starch by prolonged previous maintenance of the plant in partial darkness.

The best means for increasing the effective sensitiveness to radiation is the Heterostatic Method. By means of an Auxiliary Light-stimulator, the plant is brought to the verge of photosynthesis. The incident rays now induce $CO_2$-assimilation in proportion to their respective photosynthetic efficiency.

By the device of the Auxiliary Stimulator the sensitiveness of the plant can be maintained constant for a long time.

The relation between photosynthesis and the energy of the incident radiation was ascertained by the simultaneous determination of the photosynthetic activity by the Bubbler, and of the intensity of radiation by the Magnetic Radiometer. Curves were obtained showing the relation

between the energy of radiation in different parts of the spectrum and the corresponding activity of photosynthesis.

Photosynthesis is feebly initiated even under infra-red radiation. In the visible spectrum the activity at first increases gradually; at B the maximum of activity is reached, though the intensity of radiation is here lower; beyond B, the activity undergoes a decline parallel to the fall in the intensity of radiation of the successive rays.

The maximum of activity at B is due to absorption of these particular rays by the chlorophyll as shown by its spectrum. There is, however, no second maximum at the second absorption-band in the blue-violet between F and G.

Experiments are described which tend to prove that these blue-violet rays are concerned in inducing phototropic curvature. Hence the A and the D effects of light are complementary to each other. The rays which are most effective for photosynthesis are ineffective for tropic stimulation and *vice versa*.

The effects characteristic of the different regions of the spectrum are due (1) to the energy of the rays; (2) to the degree of their absorption; and (3) to the complementary A and D reactions in induction of photosynthesis and of phototropism.

# CHAPTER XXIII

## DETERMINATION OF THE INCREASE OF WEIGHT DUE TO PHOTOSYNTHESIS

Errors in determination of the photosynthetic increase of weight by the half-leaf method—Indirect determination from absorption of carbon dioxide—Indefinite value of the carbohydrate-factor—Necessary conditions for accurate results—Direct determination of the increase of weight of the living plant due to photosynthesis—Theory of the method—Advantages of the new method—Torsion Balance for determining increase of weight—Determination by Chemical Balance—Determination of photosynthesis under diffused light and under sunlight—Maximum rate of formation of carbohydrate per unit area.

UNDER the action of light, organic substances, mainly carbohydrates, are formed in the leaf as the photosynthetic product. No *direct* method of measuring the increase of weight under the action of light has hitherto been found possible. It has generally been determined from the intake of $CO_2$ by the assimilating leaf, the amount of carbohydrate being estimated by multiplying the quantity of absorbed $CO_2$ by the carbohydrate-factor. This factor, as will be presently seen, is not a constant, but varies in different plants. The $CO_2$-absorption is, moreover, not the most reliable method of estimating photosynthesis.

There is the other possibility of the indirect estimation of the carbohydrates from the evolution of oxygen: but, so far as I am aware, no attempt has been made in this direction, probably on account of the inaccuracies in the older bubbling-method. These have now been completely eliminated, and I will presently show how great is the reliability of the oxygen-method.

It is desirable at this stage to consider the advantages and the disadvantages of the different methods of which

detailed accounts are given below. It should be borne in mind that, under ordinary conditions, the dry substance formed is not merely carbohydrate but proteid also. But no allowance has hitherto been made for the possible formation of proteids, nor any attempt made to reduce it.

## Estimation of the Carbohydrate Product by the Half-leaf Method

Sachs, in his classical work on the subject (1884), attempted to measure the amount of the photosynthetic product by the relative increase of the dry weight of the leaf after exposure to light. He selected large leaves, *e.g.* of *Helianthus* growing in the open, and cut off one longitudinal half of the leaf early in the morning, the other half being allowed to remain attached to the plant and exposed to sunlight for a definite period. Equal areas of the exposed and unexposed halves of the leaves were dried, powdered and weighed ; the weight of the sun-exposed half was found to be the greater, and the difference of weight was taken as the amount of carbohydrate produced under the action of light for the given period. In *Helianthus* the rate of increase in weight was found to be 0·914 grm. per hour for a square metre of leaf-surface. This value can be taken only as an approximation, for there are various inevitable errors in the half-leaf method. These arise (1) from possible difference in the retention of water in the two halves of the leaf after drying ; (2) from the different thickness and lack of symmetry in the two halves ; and (3) from the shrinkage of area of the exposed leaf caused by insolation and by greater transpiration. Brown and Morris, from their experiments on the subject, came to the conclusion that the error introduced by the half-leaf method may even amount to 100 per cent.

There are other complications in the half-leaf method due to loss by respiration, and to the translocation of soluble carbohydrates from the leaf to the body of the plant. As

regards loss by respiration, Sachs made a rough allowance of 1 grm. per square metre during fifteen hours of active assimilation. The translocation of carbohydrates he estimated by finding the loss of weight in a half-leaf during ten hours of continuous darkness at night. For obvious reasons a different leaf had to be taken for this determination ; but the loss for one leaf cannot be applied to another, since the physiological characteristics of the two leaves cannot be the same. Again, translocation is dependent on temperature ; the calculated value of the loss at night must be different from the actual loss during daytime under light, the temperature by day being considerably higher than by night.

The absorption of nitrogenous and other substances from the soil would also produce an increase of weight not due to $CO_2$-assimilation.

## Indirect Estimation from the Absorption of Carbon Dioxide

Reference has already been made to the method employed by Kreussler for this purpose. He experimented with isolated twigs in an appropriate bell-jar through which was made to pass a definite volume of air containing a known percentage of carbon dioxide. ' He next determined how much of the carbon dioxide passed out of the apparatus, and at the same time worked out how much carbon dioxide was produced in the same period as a result of respiration. In this way the amount of carbon dioxide actually absorbed by the plant could be accurately determined. Since Kreussler worked with air containing a relatively high percentage of carbon dioxide, and since the material experimented with was illuminated by electric light, his results furnish us with no information with reference to the amount of carbon dioxide decomposed under *normal conditions of assimilation.* Brown (1899) employed the method in a modified form. He worked with ordinary

atmospheric air whose percentage of carbon dioxide was accurately determined, and, further, employed ordinary daylight. Brown also used in his bell-jars only single leaves, which, however, remained attached to the plant, so more easily guarding against withering than was possible in the severed branches.'[1] In their later researches Brown and Escombe (1905) estimated the formation of carbohydrate by multiplying the weight of the absorbed carbon dioxide by a 'carbohydrate-factor' of 0·640. The carbohydrate formed under light, per sq. m. per hour, for *Helianthus annuus* was thus estimated to be from 0·4 to 0·5 grm.

The carbohydrate-factor of 0·640 given above cannot be taken as a constant, for Krasheninikov (1901) found it to vary in different plants from 0·60 in the cherry-laurel to 0·67 in the sugar-cane. An explanation must, therefore, be sought for these differences, and further inquiry made as to whether the carbohydrate-factor of a given plant remains constant or undergoes variation under changed external conditions.

## Necessary Conditions for Accurate Results

The difficulties in the older methods of estimation of the carbohydrate formed in photosynthesis suggest that a perfect method should fulfil the following conditions:

1. The complete experiment should be carried out with one and the same specimen, since the physiological activities of two different specimens are not the same.

2. The increase of weight measured should be entirely due to $CO_2$-assimilation, and not to the production of other substances.

3. The experiment should, as far as possible, be carried out under the normal conditions to which the plant had been accustomed in nature.

4. There should be no shrinkage of the specimen under light in consequence of increased transpiration.

[1] Jost, *Plant Physiology*, translated by Gibson (1907), p. 116.

5. The loss of weight due to translocation of carbohydrate, as also the gain of weight due to inorganic substances derived from the soil, should be prevented.

6. The loss due to respiration should be determined or its effect eliminated.

7. The increase of weight should be ascertained directly in the living condition of the plant.

8. The fact that the increase of weight is due to photosynthesis should be demonstrated by proving that it is dependent on the intensity and duration of light.

9. The experimental investigation should be completed in as short a time as possible.

The ideal conditions detailed above are fulfilled in the following investigations carried out with the water-plant *Hydrilla*. They were undertaken on two independent methods—(1) the direct determination of the increase of weight on the living plant; and (2) the indirect determination of the carbohydrate from the amount of oxygen evolved during the same period. It is thus possible to test the reliability of each of the two methods by comparing the respective results.

## Determination of the Increase of Weight of the Living Plant due to Photosynthesis

Before describing the method for the determination of increase of weight, a few words may be said of the choice of experimental specimens. As the experiments are to be carried out with cut specimens, it is desirable to select very sturdy plants in which the activity is vigorous and persistent. Weak specimens are liable to exhibit increasing depression, culminating with the death of the plant. Middle-aged specimens prove to be more sturdy than very young ones. The physiologically vigorous specimens can be easily picked out after some experience: such cut specimens continue to exhibit vigorous photosynthesis even for days in succession.

*Theory of the method.*—Let us consider the case of *Hydrilla* growing in water containing carbon dioxide in solution. On exposure to light, carbohydrates are formed inside the cells, which therefore become *heavier* than before. The volume of water displaced by the immersed plant remains the same, and the increase of weight produced should, therefore, be capable of measurement by means of a delicate balance. Imagine a closed and weighted hollow bulb *sunk under water* and attached by a fine thread to one pan of a delicate balance with exact counter-weight in the other pan, the condition of equilibrium being assured from the position of the index at zero. Suppose in some way heavy particles are formed in the interior of the closed bulb ; as the volume of water displaced by the bulb remains constant, the displaced index can only be brought back to zero by placing an additional weight in the second pan of the balance exactly equal to the increased weight produced in the bulb. Now the cells of the plant correspond to the closed bulb ; the carbohydrates are the heavy substances formed in the interior under the action of light, the weight of which may thus be determined directly. There may be some misgiving that the volume of the plant might undergo a change by growth ; against this it is to be said (1) that the hypothetical growth of the plant for the short time of the experiment must be slight ; (2) that growth is lowered or even arrested under the action of strong light ; and (3) that the experiments to be described were carried out not with growing but with fully-grown specimens.

In this new method of investigation the experimental conditions fulfil all the requirements previously enumerated.

1. *Complete investigation with a single plant.*—The sprig of *Hydrilla* for the experiment is about 10 cm. in length ; a small piece of platinum wire is attached to the upper end and the twig suspended upside down from one arm of a sensitive balance. The plant is completely immersed in oxygenated distilled water, previously boiled, which has a definite content of about 7 mg. of $CO_2$ per 100 c.c. The

specimen is first weighed in the dark, and then at different intervals after exposure to light of moderate or strong intensity. The difference gives the increase of weight due to photosynthesis. The complete investigation is thus carried out on an identical plant with constant physiological characteristics.

2. *Increase of weight due solely to $CO_2$-assimilation.*—The sprig of *Hydrilla* is cut off from all connection with the soil from which it might otherwise derive nitrogenous or other substances. The increase of weight must, therefore, be entirely due to $CO_2$-assimilation.

3. *Natural conditions of the experiment.*—The experiment is carried out with the plant placed in a large vessel of water, so that it is not under restraint; the conditions of the experiment are thus as natural as it is possible to make them. The temperature of the plant is the same as that of the large mass of water in which it is immersed. There is thus no indefinite rise of temperature in the plant due to local heating under strong light, as is the case in the leaves of aerial plants placed within a bell-jar and subjected to strong light.

4. *Absence of transpiration and shrinkage.*—The plant being immersed under water, there is no transpiration with a resulting shrinkage of the area of the leaves.

5. *No diminution of weight by translocation of carbohydrates.*—In the half-leaf method, an amount of carbohydrate is lost by translocation from the leaves to the body of the plant. In the new method no isolated leaf is used, but the sprig as a whole bearing the leaves. The increase of the weight of the sprig gives the total quantity of carbohydrate formed, however it may be distributed.

6. *Allowance for, or elimination of, loss due to respiration.*—Two different methods of experiment have already been described on which allowance for loss by respiration can be made. The effect of respiration is, however, eliminated by the particular method of the experiment described in the next chapter.

7. *Direct determination of increase of weight.*—The in-

crease of weight of the living plant by the new method is directly measured, and not after drying and powdering it, a procedure which must introduce many errors.

8. *Periodic determination during insolation.*—The fact that the increase of weight is due to photosynthesis is proved by periodic measurement of the increase of weight; the increase is found to depend on the intensity and duration of light.

9. *Reduction of period of experiment.*—Finally the increase of weight can be demonstrated after an exposure of the plant to light for as short a period as one hour.

I will next describe in detail the method of weighing.

## Torsion Balance for Determination of the Increased Weight

A simple form of Torsion Balance, previously described, was found to be sufficiently sensitive for the determination of the increase of weight. The two arms of the balance are formed of light aluminium of thickness sufficient to ensure rigidity. A very fine wire of phosphor-bronze, used for galvanometer suspension, is passed through the centre of the arms of the balance and kept taut by an adjusting screw. The two arms are bent into L-shape to throw the centre of gravity of the system below the point of suspension. The right arm of the balance carries two rings at its end, the upper one of which carries a rider (not shown in the figure) of aluminium wire 3·5 mg. in weight. This is used, as will be presently explained, for the purpose of calibrating the value of the movement of the horizontal index of the balance. The sprig of *Hydrilla* is suspended in water by a hook from the lower of the two rings. The suspending thread is a single cocoon-fibre, which is waxed to prevent it being wetted. The thread is so fine that its movement up or down in water produces no change in the weight caused by slight difference in the length of immersion. The left arm of the balance is prolonged into an

index, which moves along a scale engraved on a mirror. The left arm of the balance also carries a small pan, to which fragments of aluminium are added till the pointer is adjusted at the zero of the scale. There is a screw-arrangement with two jaws by which the arm of the balance may be clamped (fig. 52). The successive readings of the index are taken as follows. The plant is subjected to a short preliminary exposure to light, after which light is cut off by two opaque

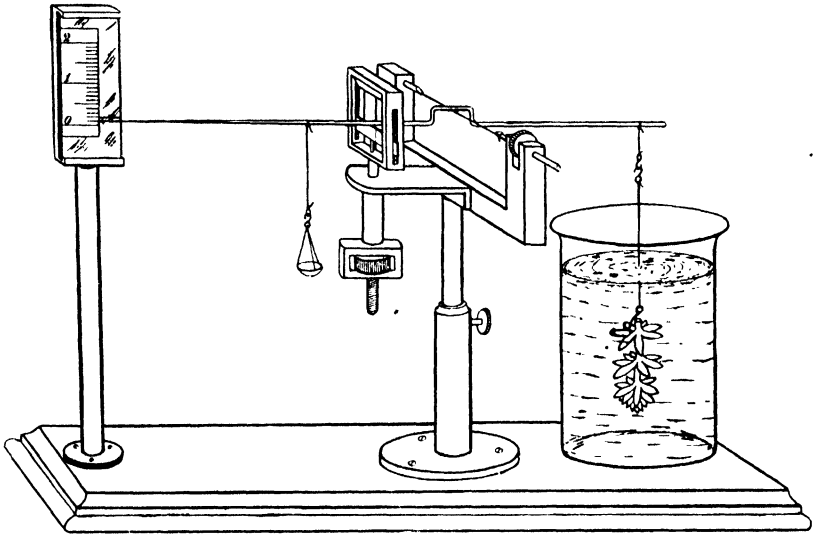


FIG. 52. The Torsion Balance for Measurement of Increase of Weight of the Living Plant during Photosynthesis
The rider and the two opaque semi-cylinders are not shown.

semi-cylinders. The index is now adjusted exactly at zero, and then the plant is exposed to light for about an hour. On account of the increase of weight due to photosynthesis the index moves upwards, and the exact amount of the increase is found from the previous determination of the value of each division of the scale. Certain precautions have, however, to be taken in obtaining the correct weight. When the plant is exposed to the light of the sky the rate of production of oxygen is relatively slow and the bubbles escape through the cut end; but under sunlight the rate

of evolution may become so greatly increased that the vent at the cut end of the stem is not large enough for the escape of all the oxygen. Small bubbles are thus forced out from the edges of the leaves and remain attached, thus producing a false buoyancy by which the weight of the sprig under water is reduced. The difficulty is, however, completely removed by slowly raising the plant above the water by means of the hook from which the plant is suspended, the balance being clamped in the meanwhile. The pressure of the water-column being removed, the small bubbles burst and escape through the film of water. The hook is replaced in its proper place and the balance is released.

We can ascertain the value of each division of the scale by removing the rider, weighing 3·5 mg., from the right arm of the balance. This was found to produce a downward movement of the index through 38 divisions of the scale. An up-movement of the index through one division of the scale thus indicates an increase of weight of $\frac{3\cdot5}{38}$ or 0·092 mg. With finer and longer torsional wire, it would not be difficult to construct a micro-balance by which a very small fraction of a milligram could be determined. The sensitiveness of the balance used is, however, quite sufficient for our present purpose.

I will give several results obtained with the Torsion Balance. The specimen was exposed to sunlight for two successive periods of one hour each. Exposure to sunlight from 3 to 4 P.M. gave rise to an up-movement of the index through 6 divisions of the scale. The increase of weight produced in one hour by photosynthesis is thus $6 \times 0\cdot092 = 0\cdot552$ mg.

The experiment was repeated for the next hour from 4 to 5 P.M. As the evening was approaching the intensity of sunlight was undergoing a rapid diminution. The movement of the index was now 4 divisions, and the increase of weight for the second hour is

$$4 \times 0\cdot092 = 0\cdot368 \text{ mg.}$$

It is thus seen that the production of carbohydrate under light can be directly measured by a sensitive balance, and that *the weight of the plant increases with the intensity and duration of the light to which the plant has been exposed.*

## Determination of Increase of Weight by a Chemical Balance

As the increase of weight under prolonged photosynthesis is sufficiently large, the increase of weight was next determined by a Chemical Balance with which it is not difficult to measure an increase of weight of a hundredth of a milligram, especially as the weight of the sprig is as small as 0·025 grm. or so. Details of the method of measurement will be fully given in the next chapter. We shall anticipate some of the results and obtain a general idea of the increase of weight produced during photosynthesis.

*Photosynthesis under diffuse light from the sky.*—The sprig of *Hydrilla* had 32 leaves ; the increase of weight after exposure to light from the sky for five hours from 10 A.M. to 3 P.M. was found to be 1·2 mg. (December 24, 1922).

Rate of increase of weight,
under sky light . . 0·24 mg. per hour . (1)

Another experiment was carried out on the same day, with this difference, that the plant was placed in sunlight. The area of the assimilating surface was in this case the same as before.

(*a*) For one and a-half hours between 9 and 10.30 A.M. the increase of weight observed was 1·3 mg.

Rate of increase of weight,
under sunlight (forenoon) . 0·86 mg. per hour . (2)

(*b*) The increase of weight was next determined for the next hour and a-half between 10.30 A.M. and 12 noon. The

sunlight was considerably stronger and the increase of weight was found to be 1·7 mg.

Rate of increase of weight,
under midday sun . 1·10 mg. per hour . (3)

It is thus seen that, while under light from the sky the rate of production of carbohydrate of a given specimen was 0·24 mg. per hour, the rate is increased to 1·1 mg., or nearly 5 times, under the light of the midday sun. The above results show how the formation of carbohydrate may be directly determined in the living plant by the method of weighing the submerged plant.

## Determination of the Rate of Production of Carbohydrate per Unit Area of Photosynthetic Surface

In the three examples given above, the total area of the leaf-surface in each case was 8 sq. cm. The following are, therefore, the rates of formation of carbohydrate per square metre of the surface per hour.

| | | | |
|---|---|---|---|
| Diffuse sky light—Rate per | sq. m. | per hour . | 0·30 grm. |
| Sunlight in forenoon | ,, | ,, . | 1·08 ,, |
| Midday sun | ,, | ,, . | 1·30 ,, |

In the last two cases sunlight struck the leaves in a slanting direction. It has been shown (p. 47) that the rate of photosynthesis is at its maximum at perpendicular incidence of light. This condition was secured by means of a reflecting mirror, and the determination of increase of weight was made by the more sensitive method of evolution of oxygen to be fully described in the next chapter. The maximum rate of formation of carbohydrate in the leaf of *Hydrilla* under sunlight at perpendicular incidence was thus found to be 2·8 grm. per hour per square metre of the leaf-surface.

## Summary

There are various sources of error in the determination of photosynthetic increase of weight by the half-leaf method, as also by the method of intake of carbon dioxide.

The new method measures the photosynthetic increase of weight in the living plant during exposure to light, either by means of a sensitive Torsion Balance or by a Chemical Balance.

The advantages of the new method, which is direct, are (1) the natural conditions of the experiment ; (2) the elimination of loss of weight due to transpiration, and to translocation of carbohydrates. The accession of mineral as well as nitrogenous substances from the soil is also avoided in experiments with cut specimens.

The weight is found to increase with the intensity of the light and the duration of exposure. The formation of carbohydrate under sunlight is about five times greater than that under diffuse light from the sky.

The maximum rate of formation of carbohydrate in *Hydrilla* under perpendicular incidence of sunlight is 2·8 grm. per hour per sq. metre.

# CHAPTER XXIV

## SIMULTANEOUS DETERMINATION OF CARBOHYDRATE-FORMATION BY TWO INDEPENDENT METHODS

Direct and indirect estimation of photosynthetic production of carbohydrate—Indirect methods based on absorption of carbon dioxide and on evolution of oxygen—Oxygen-carbohydrate factor—Indirect determination of increase of weight from volume of evolved oxygen—Direct determination by weighing the plant before and after exposure to light—Simultaneous determination of carbohydrate by direct and indirect methods—The Eudiometer and the Balance—Modification of the carbohydrate-factor by external conditions—Effect of strong light and of semi-darkness.

In the last chapter the method of direct estimation of the photosynthetic product by weighing was explained and shown to give consistent results. The experimental methods and conditions were shown to be such as to eliminate many possible sources of error. The question now arises whether it is at all possible to devise an independent method for determining the increase of weight. Success in this would enable us to compare the results obtained by the two methods, the mutual agreement of which would offer a conclusive test of the reliability of each. Such a method is to be found in the accurate determination of the volume of oxygen evolved during photosynthesis.

It will also be shown that, in normal specimens of *Hydrilla* growing in a tank exposed to northern light from the sky and not to direct sunlight, the ratio of the increase of weight under light to the weight of oxygen evolved during the same exposure is remarkably constant. The increase of weight in normal plants may, therefore, be estimated by multiplying the weight of oxygen by the constant oxygen-carbohydrate factor.

The method of determining the increase of weight

from the volume of oxygen evolved has the advantage of greater simplicity and accuracy over the method of intake of $CO_2$. By determining the relation between the gain in weight and the volume of oxygen evolved, the unknown factor of the loss of oxygen in respiration is eliminated: for the loss is the same on both sides of the equation.

## Simultaneous Determination of Carbohydrate by Direct and Indirect Methods

*Direct Method of weighing.*—The sensitiveness of the chemical balance used was such that it was easy to weigh accurately to 0·01 mg. The balance was placed in an underground vault free from vibration, in which the temperature remained practically constant, as shown by the records of the thermograph kept in the room. The maximum variation of less than 1° C. occurred at noon; the temperature was, however, the same in the forenoon and in the afternoon, these being the times at which determinations were made of the difference in weight of the plant before and after exposure to light. The balance in the dark room was lighted by an electric bulb placed outside, the light entering through a small window covered with blue glass, blue light being relatively ineffective in photosynthesis. The object of placing the source of light outside was to guard against variation of temperature from the heat given out by the incandescent lamp. The light was only put on for the short period required for weighing. The general mode of experimental procedure is as follows. The cut sprig of *Hydrilla*, immersed in water containing 7 mg. of $CO_2$ in 100 c.c. of water, is weighed before and after exposure to light, the evolved oxygen being collected in a Eudiometer. The increase of weight due to the production of carbohydrate is directly found by weighing.

*Indirect Method.*—The production of carbohydrate can be indirectly estimated from the weight of the oxygen collected

in the Eudiometer: this multiplied by the carbohydrate-factor gives the calculated value of the carbohydrate produced under light. The results obtained by these two entirely different methods can then be compared.

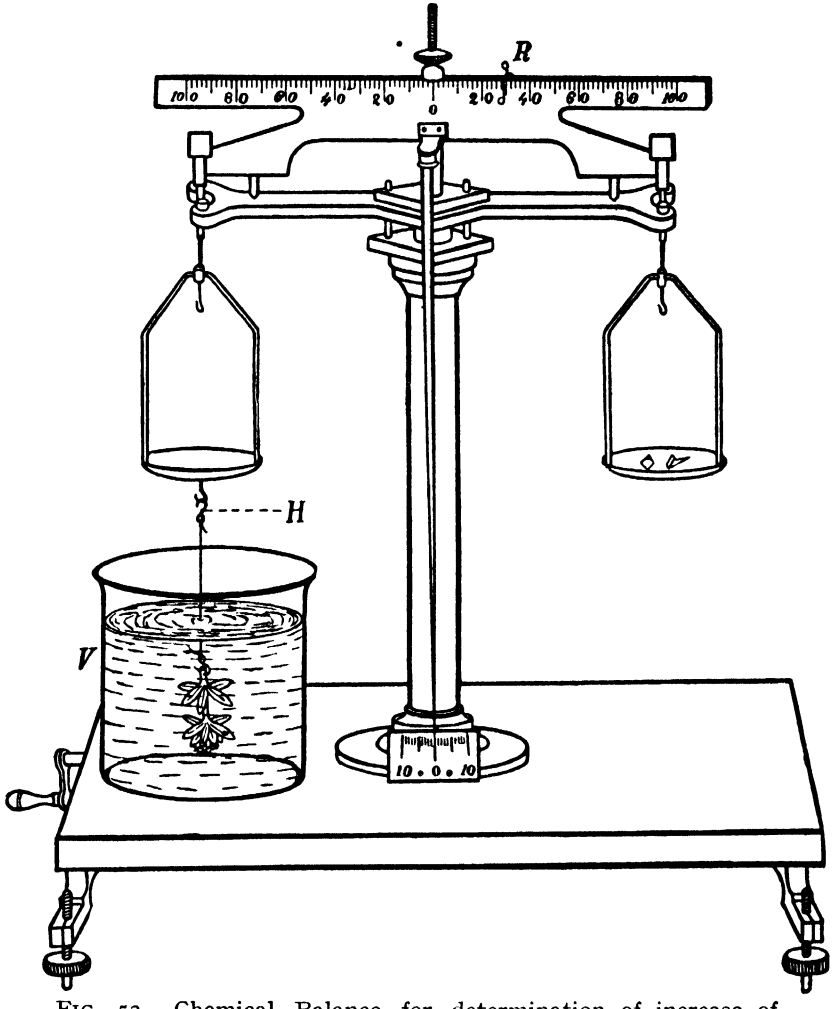



FIG. 53. Chemical Balance for determination of increase of weight of *Hydrilla* after exposure to Light
Vessel V always kept on the Balance.

*Experimental details.*—The following gives the experimental details of the procedure adopted. Two vessels filled with the same water containing the normal proportion of $CO_2$

were employed ; of this v is always kept inside the balance-room at the constant temperature. The second vessel $v_1$ is used for exposing the plant to light and for collecting the evolved oxygen. A specimen of the plant bearing about forty leaves is cut the previous night and placed (with the hook and the waxed cocoon thread) in the second vessel $v_1$. This specimen is exposed to light in the morning till bubbling takes place. The intercellular spaces are then filled with oxygen, the excess escaping at the cut end. The vessel $v_1$ is now taken into the balance-room, and the plant transferred to the vessel v and suspended from the balance by the hook. The plant immersed in water is exactly balanced by a counterpoise placed in the second pan, and by means of the rider the long index is adjusted to the zero of the scale (fig. 53). There may be a misgiving that the plant might get partially dried during transfer from one vessel to the other, thus resulting in a slight variation of weight. There is, however, no ground for this misgiving, as was proved by lifting the plant by the hook out of the vessel and reweighing it five times in succession. This did not produce the slightest variation in the weight, the index remaining exactly at zero. The plant, it must be remembered, is coated with a film of water which prevents contact with the air. The precaution of raising the plant and reweighing

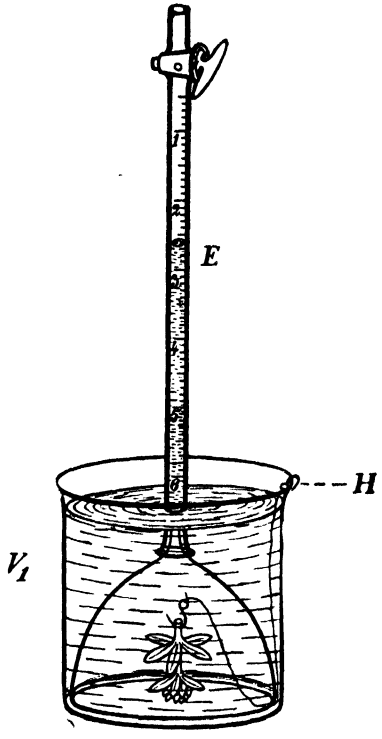



FIG. 54. Eudiometer E for collection of Oxygen evolved under Light
$v_1$, second vessel ; H, suspending hook.

was taken in order to guard against the possibility of false buoyancy produced by minute bubbles of gas that might have remained attached to the leaves.

After exact balance the counterpoise is left on the pan of the balance and the plant is transferred to the vessel $V_1$, which is taken outside and the plant exposed to light. The bubbles produced are collected in the Eudiometer E (fig. 54), a gentle tap detaching any bubble that may have remained attached to the cut end. The volume of the gas produced is noted, as well as the temperature and the barometric pressure. These data suffice for the *indirect* determination by calculation of the amount of carbohydrate produced during exposure to light. The amount of carbohydrate produced is then *directly* determined by weighing. The vessel containing the plant is taken into the balance-room, and the specimen transferred to the first vessel V and weighed. A short period is allowed for the plant to become adjusted to the constant temperature, and the additional weight now required to bring the index back to zero gives the quantity of carbohydrate produced.

*Determination of the oxygen-carbohydrate factor.*—The volume of oxygen collected during exposure to bright sky light from 10 A.M. to 3 P.M. was 1·10 c.c., at barometric pressure 762·5 mm., and temperature of 18° C. The tension of aqueous vapour was 15·5 mm. The N.T.P. volume

$$V_0 = \frac{1{\cdot}10 \times (762{\cdot}5 - 15{\cdot}5) \times 273}{760 \times (273 + 18)} = 1{\cdot}014 \text{ c.c.}$$

Since the weight of 1 c.c. of oxygen is 1·44 mg., the weight of evolved oxygen

$$W_0 = 1{\cdot}014 \times 1{\cdot}44 \text{ mg.} = 1{\cdot}460 \text{ mg.} \quad . \quad . \quad (1)$$

The actual increase of weight $W_1$ of the plant due to the production of carbohydrate was found to be

$$W_1 = 1{\cdot}30 \text{ mg.} \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

By dividing (2) by (1) we obtain

$$\text{the oxygen-carbohydrate factor} = \frac{1{\cdot}30}{1.460} = 0{\cdot}8906 \quad . \quad (3)$$

It will be noted that an evolution of 1·10 c.c. or 1100 c.mm. of oxygen accompanied an increase of weight of 1·30 mg. The evolution of 1 c.mm. of oxygen thus corresponded to the formation of $\frac{1 \cdot 30}{1000 \times 1100} = 1 \cdot 2 \times 10^{-6}$ grm. of carbohydrate. I have already explained how, by adjusting the capacity of the Bubbler, it is possible to record the evolution of even 0·2 c.mm. of oxygen. The Bubbling Apparatus can thus be made so sensitive as to record the photosynthetic production of carbohydrate smaller than a millionth of a gramme.

## Constant Value of Oxygen-Carbohydrate Factor in Normal Specimens

It has been shown that in normal specimens, grown in the pond exposed to the northern light of the sky, there is a definite relation between increase of weight of the plant and the weight of oxygen given out by it under the action of light, such that the increase of weight due to the production of carbohydrate may be found by multiplying the weight of oxygen produced by 0·8906. How extremely close is the actual increase of weight to the calculated value will be seen from the detailed results given of five other experiments carried out with specimens from the same pond, but on different days and under light of various intensity. In the following table, the first column gives the number of the specimen, the second column the duration of the experiment, the third column the volume of oxygen produced at temperature $t°$ C. and pressure $p$ in mm. of mercury. The fourth column gives the volume of oxygen at N.T.P.; the fifth the weight of the oxygen; the sixth the weight of carbohydrate obtained on multiplying the weight of oxygen by the oxygen-carbohydrate factor 0·8906. In the seventh column is given the actual increase of weight obtained by weighing.

TABLE XXXI.—DETERMINATION OF INCREASE OF WEIGHT DUE TO PHOTOSYNTHESIS BY INDIRECT AND DIRECT METHODS

| No. | Exposure. A.M.-P.M. | V. of O. at $t°$ and $p$ | | | $V_0$ of oxygen at N.T.P. in c.c. | Wt. $W_0$ of oxygen $V_0 \times 1{\cdot}44$ mg. | Calculated weight $W_0 \times 0{\cdot}8906$ | Observed increase of weight |
|---|---|---|---|---|---|---|---|---|
| | | V. in c.c. | $t°$ C. | $p$ mm. | | | | |
| 1 | 10–3 | 1·05 | 18° | 762·5 | 0·9680 | 1·394 | 1·2420 | 1·25 |
| 2 | 10–4 | 1·10 | 18° | 762·5 | 1·0140 | 1·460 | 1·3008 | 1·30 |
| 3 | 8–5 | 1·23 | 26° | 757·8 | 1·0830 | 1·560 | 1·3899 | 1·39 |
| 4 | 11–5 | 0·60 | 28° | 758·0 | 0·5227 | 0·7527 | 0·6707 | 0·67 |
| 5 | 9–3 | 0·70 | 25° | 764·0 | 0·6247 | 0·8996 | 0·8015 | 0·80 |

The agreement found between the calculated value and the actual increase of weight is so remarkable as to justify the conclusions (1) *that the oxygen-carbohydrate factor in normal Hydrilla plants under given conditions is definite; and* (2) *that the increase of weight can be accurately determined by multiplying the weight of oxygen evolved by the oxygen-carbohydrate factor.*

Though this factor is constant in normal specimens, I will presently describe experiments which show that it undergoes a definite variation under changed conditions in regard to the intensity of light to which the plant had been habitually exposed. It is a matter of interest and importance to ascertain the significance of the constant factor and its variation under definite external conditions.

It is commonly assumed that the first formed carbohydrate is a hexose glucose, and that its formation may be generally represented by an equation such as the following:

$$6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2$$

On this assumption the oxygen-carbohydrate factor will be

$$\frac{\text{glucose}}{\text{oxygen}} = \frac{C_6H_{12}O_6}{6O_2} = \frac{180}{192} = 0{\cdot}9375$$

But in no case was the value of the factor found to be so great as this: in normal specimens the observed value of the factor 0·8906 is 5 per cent. less than this calculated

value. The difference between the calculated and the observed value can only be accounted for by attributing it to the formation of some organic substance or substances other than glucose having a lower carbohydrate-factor; hence the variation in the value of the oxygen-carbohydrate factor indicates the varying proportions in which glucose and the other substance or substances are produced.

One of the carbohydrates produced in the assimilating leaf of *Hydrilla* after exposure to light is starch. The plant kept for about two days in the dark becomes, as tested by the iodine method, practically de-starched. After exposure to light, a profuse deposit of starch-grains is found in the leaves, and in very sensitive specimens after a period as short as ten minutes.

The observed increase of weight in the leaf is thus partly due to the production of starch of which the empirical formula is $C_6H_{10}O_5$. There is a general consensus of opinion that starch is formed as a secondary product from sugar.

Since the starch contributes its quota to the observed increase of weight found by direct weighing, we may try to obtain an approximate idea of the proportion of glucose and starch produced in normal specimens of *Hydrilla*. Let us imagine a particular case where the glucose and starch were formed in equal proportions. The ratio of the carbohydrate-factor in such a case to that where glucose alone was produced is

$$\frac{180 + 162}{2} : 180$$

$$171 : 180$$

that is to say, the carbohydrate-factor in the case of the mixed product would be 5 per cent. lower. Now this lower value of the carbohydrate-factor is what was actually found in the experimental determinations. This would seem to indicate that in normal specimens the glucose and starch were formed (the latter as a secondary product) in about equal proportions.

It is quite probable that, in the complex process of photosynthesis, carbohydrates are produced other than those just referred to. Accurate determination of variation of the carbohydrate-factor under change of external conditions will no doubt lead to a clearer insight into the chemical side of the problem. However, the following investigations show that the value of the factor is modified in a definite relation to the intensity of the light to which the plant had been previously exposed. I select the two extreme cases, those of excessively strong light and of semi-darkness. I will now describe the effect of these in modification of the carbohydrate-factor.

## Carbohydrate-Factor of Specimens grown in Sunlit Pond

All these specimens were found to be overloaded with starch. The following is a detailed account of a typical experiment continued from 8 A.M. to 4 P.M. under exposure to bright light from the sky. The volume of evolved oxygen measured at 21° C. and pressure of 766·6 mm. was found to be 1·00 c.c.; reduced to N.T.P. it was 0·914 c.c., the weight being 1·316 mg. The observed increase of weight of the leaf due to production of carbohydrate was 1·20 mg. The oxygen-carbohydrate factor was therefore $\frac{1\cdot20}{1\cdot316} = 0\cdot91$, which is 2 per cent. higher than the value (0·89) in normal specimens. Six others from the same pond gave likewise a higher value for the factor. This indicates that in plants kept in strong light the production of glucose is relatively the greater.

## Carbohydrate-Factor of Specimens Previously Subjected to Semi-Darkness

For the following investigation I took a specimen from the sunlit pond, and kept it in a semi-dark room for two days, during which the starch-content was reduced below

the normal. It was exposed to light of the sky from 9 A.M. to 3 P.M. The oxygen evolved was found to be 0·70 c.c. at 20° C. and pressure of 762 mm. The weight of the oxygen was 0·92 mg. and that of the carbohydrate produced was 0·80 mg., giving a carbohydrate-factor of 0·87. Determinations made with six other specimens gave very similar results. This would appear to indicate that, in specimens previously kept in feeble light, with a starch-content below the normal, the production of glucose is relatively the less.

The results obtained with plants grown in light of different degrees of intensity may be simply stated as follows. The constant value 0·8906 of the oxygen-carbohydrate factor in normal specimens undergoes a definite variation according to the previous conditions, plants from a sun-exposed pond exhibiting the higher value, those from semi-darkness the lower.

## SUMMARY

The method of determination of the amount of the photosynthetic product from the amount of oxygen evolved is free from many sources of error. It is simple and direct and requires no complicated chemical analysis.

The oxygen-carbohydrate factor for normal specimens of *Hydrilla* is 0·8906. The increase of weight in the plant may be calculated by multiplying the weight of oxygen evolved by the constant carbohydrate-factor.

The actual increase of weight due to photosynthetic production of carbohydrate is directly obtained by means of a sensitive balance. In simultaneous determination, the volume of oxygen evolved is collected in the Eudiometer, while the increase of weight is determined by the balance. The results obtained by the indirect and the direct methods are found to be in the closest agreement with each other.

The method of the investigation of photosynthesis has been rendered so sensitive that records may be obtained of

the evolution of oxygen corresponding to the production of an amount of carbohydrate as small as the millionth of a gramme.

The value 0·8906 of the oxygen-carbohydrate factor in normal specimens undergoes a definite variation according to the previous conditions, plants from a sun-exposed pond exhibiting a higher value, those from semi-darkness a lower.

# CHAPTER XXV

## EFFICIENCY OF THE PHOTOSYNTHETIC ORGAN IN STORAGE OF SOLAR ENERGY

Different determinations of the efficiency of the photosynthetic organ—Error arising from incorrect values of carbohydrate-factor and of heat of combustion of the photosynthetic product—Unsatisfactory determination of losses by reflection, by emission and by transpiration—Elimination of these losses in water-plants—Radiometric and calorimetric determinations of energy—Determination of the co-efficients of transmission and of absorption by the Radiometer and the Calorimeter—Simultaneous determination of energy absorbed and of energy stored—Typical examples—Comparatively high efficiency of the photosynthetic storage of energy.

THE economic life of the present age may be said to be dependent to a great extent on the utilisation of the solar energy that had been stored in past ages by vegetable life. What is the efficiency of the physiological mechanism by which this was accomplished? From Boussingault's result that a square metre of actively assimilating leaf-surface of *Nerium Oleander* forms 0·000538 grm. of starch in one second, Pfeffer (1871) attempted to form an approximate estimate of the efficiency of the plant as a transformer of energy. Assuming the heat of combustion of one gramme of starch to be 41,000 calories, he found 2·2 calories per square metre per second to be the amount of energy conserved by the leaf. Taking the energy of sunlight from Pouillet's results to be 333 heat-units per second per square metre, he estimated that the energy stored is about 0·6 per cent. of the incident radiation. It is obvious that the amount of solar energy received is not constant, but varies with the time of the day, the inclination of the leaves, the hygrometric state of the air, the altitude of the place, and so on. The result is therefore to be taken as only an approximation.

Detlefsen (1888) found that the leaf absorbed more energy when placed in an atmosphere containing carbon dioxide than in one without it. The intensity of radiation was measured by placing a thermopile behind the leaf. When there was no assimilation the temperature indicated by the thermopile was slightly higher; the results showed that the leaf did not utilise more than 0·3 to 1·1 per cent. of the incident radiation. The average efficiency may therefore be taken as about 0·7 per cent.

Puriewitsch (1914) measured the incident energy by a Bolometer, and the increase in the dry weight of the leaf after insolation by the half-leaf method. The leaf of *Acer platanoides* was exposed to sunlight for six hours; the increase of heat of combustion per sq. cm. after insolation was found to be 2·208 gm. cal. The total energy incident on the leaf per sq. cm. was 361·03 gm. cal. The efficiency in this case was found to be 0·6 per cent.

There are several sources of error in the above determinations. In prolonged experiments extending over many hours it is difficult to obtain an accurate estimate of the total radiation. The incident radiation is, moreover, subject to various losses, and finally the determination of the increase of dry weight by the half-leaf method cannot be regarded as highly accurate.

## Difficulties in Estimating the Photosynthetic Efficiency of Leaves of Terrestrial Plants

Brown and Escombe (1905) carried out the most elaborate and painstaking investigation on the subject, making allowances for the various losses of the incident radiation. Though every care was taken, the inherent difficulties of the problem stood in the way of obtaining accurate results. This will be understood from the following considerations.

*Measurement of the energy stored.*—Brown and Escombe assumed certain values of the carbohydrate-factor and of

the heat of combustion of the product which are not fully justified. They calculated the dry weight produced by multiplying the weight of carbon dioxide absorbed by the carbohydrate-factor 0·64. This factor is, however, not a definite constant, but varies from 0·60 to 0·74 in different plants. The carbohydrate-factor would depend on the proportions of different carbohydrates formed, which will affect the calculation, the heats of combustion of starch and glucose being 41,000 and 37,600 gm. cal. respectively. Brown and Escombe adopted the lower value in their calculation of the energy stored in photosynthesis.

Turning next to the exact determination of the energy absorbed for the internal work of assimilation, the difficulties encountered are even more serious. For a large proportion of the incident energy is lost in various ways ; (*a*) in transmission through and (*b*) reflection from the surface of the leaf ; (*c*) by re-radiation of heat from the leaf ; (*d*) by convection-currents in the surrounding air ; and finally (*e*) a portion of the energy absorbed is utilised for the maintenance of transpiration by the leaf. Of the total energy of the incident radiation only a small proportion is used in assimilation.

The various factors in the problem may be indicated as follows :

$I$ = The incident energy of light.
$E_A$ = Energy absorbed in photosynthesis.
$T$ = Radiation transmitted through the leaf.
$r$ = Radiation reflected from the surface of the leaf.
$e$ = Loss by emission, which includes loss by re-radiation and convection by air-currents.
$t_r$ = Loss of energy in transpiration.

$E_A$, or energy absorbed in photosynthesis, is then

$$= I - T - r - e - t_r.$$

Brown and Escombe measured the energy of the incident radiation, I, and that of the radiation transmitted, T, through

the leaf, by means of differential platinum thermometers, one of which was black and the other bright. The glass chamber did not at first contain the leaf when the full intensity of radiation I was measured; the plant was then placed in the chamber, and the intensity of transmitted radiation T observed.

The losses by reflection ($r$), by emission ($e$), and by transpiration ($tr$), are incapable of exact measurement, as will be seen from considerations to be presently given. The determination of the energy absorbed for photosynthesis is thus subject to considerable errors, since the absorbed energy has to be indirectly estimated by subtracting the various more or less indefinite losses from the energy of the incident radiation.

*Loss by reflection.*—Brown and Escombe regard the loss by reflection as negligible; but this assumption is not fully justified. The loss would have been negligible had the index of refraction of the substance of the leaf been the same as that of air; but this is far from being the case.

*Loss by emission.*—This depends on the rise of temperature produced in the leaf by the absorption of light. It is extremely difficult to measure the actual rise of temperature of the substance of the leaf, and the loss of energy by re-radiation and in convection-currents of air cannot be found with any degree of accuracy.

*Loss of energy in transpiration.*—From the heat for vaporisation of water the loss of energy could be estimated if we knew the exact quantity of water transpired by the leaf; this is not at all an easy matter, the difficulty being accentuated by the fact that transpiration does not remain constant, but undergoes change with the unavoidable variation of temperature and of light during the course of prolonged experimentation.

The following tabular statement gives the results obtained by Brown and Escombe in their experiments on *Polygonum Weyrichii.*

TABLE XXXII.—SHOWING ENERGY-RELATION IN PHOTOSYNTHESIS OF 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 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 | 57·07 | 58·67 | 35·32 | 6·01 |
| 4 | 1·32 | 35·64 | 36·96 | 35·28 | 27·76 |
| 5 | 0·49 | 52·72 | 53·21 | 35·30 | 11·49 |

The accuracy of an estimation of the efficiency of the photosynthetic organ depends on the reliability of the experimental determination of the energy absorbed in photosynthesis. This, it should be remembered, cannot be found directly, but only by subtracting from the amount of incident energy the various losses, none of which can be found with any degree of accuracy. These losses, moreover, are very considerable compared with the calculated value of the absorbed energy. Thus, taking the experiment number 4 in the above Table, we find that, while the proportion of energy used in photosynthesis is only 1·32, the various losses are proportionately very much higher, 35·64 for transpiration and 27·76 for re-radiation and convection. The inevitable errors in the determination of these losses must seriously affect the calculated value of the energy absorbed in photosynthesis, and must thereby introduce considerable error into the determination of the efficiency.

The estimates hitherto made by different observers agree generally in assigning a very low efficiency to the photosynthetic organ, of the order of 0·6 per cent. or so; a few, however, ascribed a much higher value to the efficiency. But no great reliance can be placed on these results, since the methods employed were subject to serious shortcomings. First, the period of experiment was necessarily prolonged, so that it was impossible to obtain any accurate measure

of the sum-total of the fluctuating energy of light. There were again the various losses of the incident energy which were more or less indeterminate. Finally, no accurate method was available for the determination of the energy stored in photosynthesis.

## Determination of the Photosynthetic Efficiency of *Hydrilla*

An ideal method for determining the efficiency should include (1) a brief period of experiment; (2) the practical elimination of the indeterminate losses of the incident radiation; and (3) the simultaneous determination of the energy absorbed and of the energy stored, the measurements being carried out with the highest degree of accuracy.

These objects have been attained by the method which I have been able to devise for determining the photosynthetic efficiency of *Hydrilla*. The period of a complete experiment has been reduced to a few minutes, so that the external conditions can be maintained absolutely constant. Very sensitive and accurate methods have been adopted for the determination of the energy absorbed and of the energy stored by the plant. The energy absorbed is determined by two independent methods rapidly following each other, and the agreement of the results gives assurance of their accuracy. Finally, the determinations of the absorption and of the storage of energy are made under an identical intensity of light.

*Elimination of loss by reflection, emission and transpiration.*—These losses are eliminated when the plant under experiment is immersed in water, as in my investigation with *Hydrilla verticillata*. The sap contained in the cells of *Hydrilla* has an index of refraction not very different from that of water in which the plant is immersed, hence the loss by reflection is practically negligible. The loss by emission depends on rise of temperature in the plant above its surroundings; but the plant and the water in which it is immersed

are at the same temperature, there being no variation of temperature in the plant-vessel caused by the passage of light which had its heat-rays previously absorbed by a thick stratum of water ; the experiment, moreover, lasts for three minutes only. These precautions are sufficient to maintain a constant temperature of the water in the vessel. Finally, the serious and unknown loss through transpiration is completely absent in aquatic plants immersed in water.

The efficiency of the photosynthetic organ is the ratio of the energy stored to the total energy absorbed. The energy stored can be accurately determined from the photosynthetic evolution of oxygen. The energy absorbed is found from the difference in intensity of the incident and of the transmitted light, as measured by the two independent methods of the Calorimeter and the Radiometer.

I will describe the experimentation for the determination of photosynthetic efficiency in the following order :

*Experimental methods and appliances.*

The Bubbler.
The Magnetic Radiometer.
The Calorimeter.
Measurement of energy by the Calorimeter.
Thermo-electric couple for measurement of temperature.
Elimination of loss of heat by radiation.

*Determination of coefficients of transmission and absorption.*

(1) By Calorimetric Method.
(2) By Radiometric Method.

*Determination of the stored energy.*
*Determination of the photosynthetic efficiency.*

## Experimental Methods and Appliances

A diagrammatic representation of the apparatus and method of experimentation is given in the illustration (fig. 55). A is the aperture $2 \times 1 \cdot 5$ cm. through which

sunlight reflected by a heliostat enters the experimental room after having passed through a thick stratum of water for absorption of the heat-rays. The experiment was carried out in the morning, when the intensity of light is about 0·4 S, *i.e.* less than half the intensity at midday. There is no advantage in the employment of a stronger light,

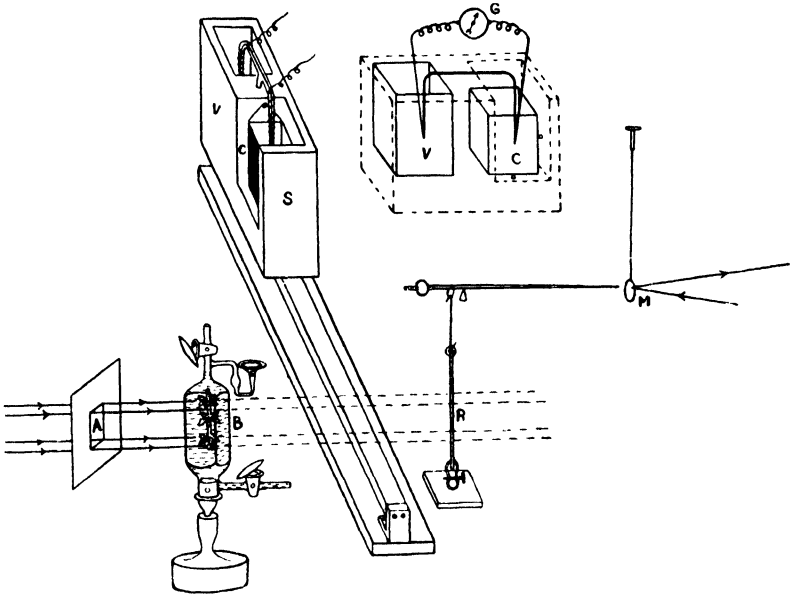



FIG. 55. Apparatus for Determination of Photosynthetic Efficiency
Sunlight enters by rectangular aperture A, and falls on plant-vessel B (only one shown in figure). Energy of radiation measured by the calorimeter C, or by the radiometer R. Thermo-electric couple shown in the upper right-hand figure (see text).

since there is little further increase in photosynthetic action above the intensity of 0·4 S.

*The Bubbler.*—Two Bubblers in every way similar (of which only one is shown in the figure) are successively interposed in the path of light. They are mounted side by side on a stand which slides on a rail. Both the Bubblers are filled with water, containing 8 mg. of $CO_2$ in 100 c.c., but one of them, B′, is blank, while the other, B, contains the plant. Interposition of the blank Bubbler B′ allows

unobstructed light to pass through, the full intensity, I, being measured by the Radiometer or the Calorimeter. When B is interposed, the plant absorbs a certain amount of light, and the transmitted radiation, T, is measured as in the last case. The radiation absorbed is found from the difference between I and T.

*Magnetic Radiometer.*—This has already been fully described; it is diagrammatically represented here as R, placed opposite to the Bubbler. The full radiation, I, produces a deflection D of the Radiometer, whereas the radiation, T, transmitted through the plant causes a reduced radiometric deflection *d*.

*The Calorimeter.*—An independent measurement is made by the Calorimeter C, mounted on a sliding stand S; the slide is pushed along the guide-rail, till the Calorimeter C intercepts the light passed through the two Bubblers, one after the other. The incident radiation I and the transmitted radiation T are measured by the rise of temperature of the water in the Calorimeter. The temperature is accurately measured by a thermo-electric couple in circuit with a sensitive Galvanometer, the deflections of which measure the incident radiation I and the transmitted radiation T.

*Measurement of energy by the Calorimeter.*—The size of the calorimetric vessel, made of thin sheet silver, is $1 \cdot 5 \times 2 \times 2$ cm. It is highly polished on three sides to diminish loss by radiation. The front surface is coated with lamp-black for the absorption of the incident light. The weight of the Calorimeter is 2·7 grms., the water-equivalent being $2 \cdot 7 \times 0 \cdot 06 = 0 \cdot 16$ grm. The quantity of water in the Calorimeter is always kept the same, namely 5 grms.

*Thermo-electric couple for measurement of temperature.*—The energy of the incident radiation and that of the transmitted radiation are directly measured from the number of heat-units produced in the Calorimeter. This is accurately determined from the rise of temperature of the contained water by means of a sensitive thermo-electric couple made of fine copper and nickel wires. One junction *t'* is placed in

the Calorimeter and the other $t$ in a larger vessel v filled with water. Under ordinary circumstances the temperatures at the two junctions are the same, and the deflection of the sensitive Galvanometer in circuit is zero. The distance of the scale from the Galvanometer is so adjusted that a difference of temperature of 1° C. in the two vessels produces a deflection of 50 mm. It is thus easy to measure a rise of temperature in the Calorimeter as small as 0·02° C.

*Elimination of loss of heat by radiation.*—In calorimetric measurements special precaution has to be taken in guarding against loss of heat by conduction and by radiation. Loss by conduction is avoided by suspending the calorimetric vessel by a silk thread in the recess of a sliding block of wood. The loss from radiation is eliminated as follows. The duration of the incident light is in all cases about three minutes only, and the resultant rise of temperature in the Calorimeter is $t°$. The water in the Calorimeter is, to begin with, at a temperature of $\frac{t°}{2}$ below that of the room. During the first half period of the experiment the Calorimeter is at a lower temperature than that of the room, and therefore absorbs heat from its surroundings; during the second half, when its temperature is rising above that of the room, it is losing heat. The gain and loss being equal there is no resultant loss. This will be clear from the following concrete example. The rise of temperature in the Calorimeter after three minutes' exposure to light is, generally speaking, 1° C., which corresponds to a deflection of 50 mm. of the Galvanometer. The temperature of the water at the beginning is adjusted half a degree below the temperature of the room, indicated by a deflection of − 25 mm. of the Galvanometer. The incident radiation is allowed to fall on the Calorimeter for three minutes with the resulting rise of temperature through 1° C., indicated by an increase of galvanometric deflection from − 25 to + 25 mm. The maximum difference of temperature between the water in the Calorimeter and the temperature of the room is only

± half a degree, the loss by radiation being thereby reduced to a minimum. This very small loss is, however, completely eliminated by the adoption of the particular method which has been described. A very high degree of accuracy is thus attained in the measurement of the energy of the incident radiation I and of the transmitted radiation T in terms of calories.

## Determination of the Coefficients of Transmission and of Absorption

The mass of water in the Calorimeter plus its water equivalent is, as already stated, equal to 5·16 grms. If G be the galvanometric deflection due to rise of temperature in the Calorimeter under full radiation, and $g$ that produced by transmitted radiation under similar conditions, then since 1 mm. deflection represents a rise of $\frac{1}{50}$° C., the units of heat H and $h$ produced in the two cases are :

$$H = 5{\cdot}16 \times \frac{G}{50}$$

$$h = 5{\cdot}16 \times \frac{g}{50}$$

The coefficient of transmission $= \frac{h}{H} = \frac{g}{G}$ . . . (1)

,, ,, ,, absorption $= 1 - \frac{h}{H} = 1 - \frac{g}{G}$ . (2)

The energy absorbed by the plant may thus be found from the calorimetric determination of H $- h$, in which every precaution had been taken in securing the highest accuracy, notably by the elimination of the loss by radiation from the Calorimeter. The accuracy of the calorimetric measurement may be tested by the independent and almost simultaneous determination of the coefficients of transmission and of absorption by the Radiometer.

When the Calorimeter is out of the way, the full radia-

tion, I, produces a deflection D of the Radiometer, the transmitted radiation, T, producing a smaller deflection *d*.

$$\text{Coefficient of transmission} = \frac{d}{D} \quad . \quad . \quad . \quad . \quad (3)$$

$$\text{,, absorption} = 1 - \frac{d}{D} \quad . \quad . \quad . \quad (4)$$

Comparison of the independent determinations of the coefficient of transmission $\frac{g}{G}$ and $\frac{d}{D}$ given in the equations (1) and (3) affords means of testing the reliability of either method. Experimental results will be presently given which show the great reliability of each of the two methods.

## Determination of Energy stored by the Plant

The exact value of this is obtained from the number of bubbles given out by the Bubbler (with its known constant) whilst the plant is exposed to light for three minutes, during which the energy absorbed by the plant is being measured by the Calorimeter. We calculate the weight of this volume of oxygen and multiply it by the carbohydrate-factor to obtain the quantity of carbohydrate that had been formed during exposure to light. The energy stored is converted into heat-units by multiplying the quantity of carbohydrate by its heat of combustion in calories per gramme.

In the determinations of the stored energy made by previous observers there has been much uncertainty in the values of the carbohydrate-factor and of the heat of combustion of the product. In the present determination there is, however, no such uncertainty. Specimens of plants were taken for experiment from the pond exposed to the light of the northern sky, which gave a very constant carbohydrate-factor, the value of which indicates (p. 218) that glucose and starch are formed in these specimens in about equal proportion. It would therefore be better to take for the heat

of combustion the mean value $3 \cdot 9 \times 10^3$ gm. cal., the heat of combustion for glucose being $3 \cdot 76 \times 10^3$ and for starch $4 \cdot 1 \times 10^3$.

Having explained the theory of the method, I proceed to give experimental details. A dozen experiments were carried out, the results of which were practically the same, with plants from the same pond.

## Determination of Photosynthetic Efficiency

The photosynthetic efficiency is the ratio of the energy stored $E_S$ to the energy $E_A$ absorbed by the plant.

$$\text{Efficiency} = \frac{E_S}{E_A}$$

The observations were taken in the following sequence:

1. The Calorimeter out of the way: the intensity of full radiation I passed through the blank Bubbler B′ was measured by the deflection D of the Radiometer.

2. The Calorimeter was interposed and the number of heat-units H, produced by full radiation acting for three minutes, determined by the rise of temperature G, as indicated by the deflection of the Galvanometer.

3. The Calorimeter was put out of the way, and the Bubbler B containing the plant interposed in the path of light. The intensity of transmitted radiation *d* was now measured by the Radiometer.

4. With the Bubbler B containing the plant interposed in the path of light, the transmitted radiation was made to act on the Calorimeter for three minutes, and the number of heat-units *h* determined by the rise of temperature *g* indicated by the Galvanometer.

*While the energy of radiation absorbed was being measured, a simultaneous determination was made of the energy stored in photosynthesis by counting the number of bubbles of oxygen given out by the Bubbler* B *during the three minutes' exposure of the plant to incident radiation.*

The carbohydrate-factor for oxygen enables us to calculate the amount of carbohydrate formed under the action of light. The energy stored is found from the number of calories corresponding to the heat of combustion of the product.

The coefficient of transmission is determined from the sequence of radiometric observations (1) and (3), and of the calorimetric determinations (2) and (4). The equality in the values of the coefficient obtained by the two independent methods serves as a test of their reliability.

*Typical example.*—The mass of water in the Calorimeter, plus the water equivalent, is equal to 5·16 grm.

*Determination of coefficient of transmission.*—Rise of temperature in the Calorimeter :

Deflection G under full radiation . . . 50 mm.
,, *g* ,, transmitted radiation . . 20 mm.
Intensity of full radiation D by Radiometer 166 divisions.
,, transmitted radiation *d* ,, 67 ,,

Coefficient of transmission by Calorimeter $\frac{20}{50} = 0 \cdot 40$

,, ,, ,, Radiometer $\frac{67}{166} = 0 \cdot 40$

Coefficient of absorption $= 1 - 0 \cdot 4 = 0 \cdot 6$

The coefficients of transmission and absorption determined by the two methods are thus identical.

*Energy absorbed in photosynthesis.*—This is ascertainable from the calorimetric determinations given in (2) and (3).

Energy absorbed in photosynthesis $= H - h$.

The rise of temperature under full radiation for three minutes caused 50 mm. deflection of the Galvanometer, while the transmitted radiation caused a deflection of 20 mm. (50 mm. = 1° C.).

Energy absorbed $H - h$ in 3 minutes $= 5 \cdot 16 \times \frac{50 - 20}{50}$

$= 3 \cdot 096$ cal.

or 1·032 cal. per minute.

*Energy stored in carbohydrate products.*

Constant of the Bubbler = 0·0014 c c
Temperature during the experiment = 31° C.
Tension of aqueous vapour = 33·5 mm.
Barometric pressure = 752·3 mm.

The volume of oxygen evolved under radiation per minute = 0·014 c.c.
Weight of 1 c.c. of oxygen N.T.P. = 0·00144 grm.
The carbohydrate-factor for oxygen = 0·8906
Weight of carbohydrate formed per minute

$$= \frac{0\cdot014 \times 273 \times (752\cdot3 - 33\cdot5) \times 0\cdot00144 \times 0\cdot8906}{(273+31) \times 760}$$

$= 0\cdot00001512$ grm.

The heat of combustion per gramme of carbohydrate $= 3\cdot9 \times 10^3$ cal.
Hence energy stored per minute $= 0\cdot0000152 \times 3\cdot9 \times 10^3$
$= 0\cdot0595$ cal.

$$\text{Efficiency} = \frac{\text{Energy stored}}{\text{Energy absorbed}} = \frac{0\cdot0595}{1\cdot032} = 5\cdot76 \text{ per cent.}$$

The results of three other experiments are given in the following table:

TABLE XXXIII.—PHOTOSYNTHETIC EFFICIENCY OF LEAVES OF *Hydrilla*

| Experimental Data | Experiment 2 | Experiment 3 | Experiment 4 |
|---|---|---|---|
| Coefficient of transmission by calorimeter | $\frac{9}{33} = 0\cdot27$ | $\frac{9}{24} = 0\cdot375$ | $\frac{13\cdot5}{36} = 0\cdot375$ |
| Coefficient of transmission by radiometer | $\frac{31}{110} = 0\cdot28$ | $\frac{30}{79} = 0\cdot379$ | $\frac{42}{106} = 0\cdot387$ |
| Rate of evolution of oxygen per minute | 0·012 c.c. | 0·008 c.c. | 0·013 c.c. |
| Energy absorbed in calories per minute | 0·825 cal. | 0·516 cal. | 0·774 cal. |
| Energy stored in calories per minute | 0·04976 cal. | 0·03421 cal. | 0·05737 cal. |
| Efficiency | $\frac{0\cdot04976}{0\cdot825} =$ 6 per cent. | $\frac{0\cdot03421}{0\cdot516} =$ 6·5 per cent. | $\frac{0\cdot05737}{0\cdot774} =$ 7·4 per cent. |

The efficiency is modified to some extent by the physiological condition of the plant. The different values for the efficiency did not, however, vary to any great extent, the lowest efficiency observed being 5·8 and the highest 7·4 per cent.

It is interesting to compare the efficiency of transformation in an ordinary steam-engine with that of the photosynthetic organ. In the former the potential energy of coal is transformed into the kinetic energy of motion; in the latter the kinetic energy of radiation is transformed into the potential energy of complex chemical compounds. The efficiency of the photosynthetic organ may be taken as about half that of an ordinary steam-engine.

## SUMMARY

Previous determinations of photosynthetic efficiency suffer from the disadvantage of the absence of adequate means for the exact measurement of the incident energy, of the various indeterminate losses to which that energy was subject, and of the energy stored by the plant.

In the experimental determination of the photosynthetic efficiency of the leaves of *Hydrilla* described in the present chapter, the losses were practically eliminated by the fact that the plant was immersed in water.

The energy absorbed by the leaves was very accurately determined by a sensitive Calorimeter, in which the rise of temperature was indicated by a thermo-electric couple. The loss of heat by radiation was eliminated.

The accuracy of the calorimetric determination was tested by the independent method of the Magnetic Radiometer. Similar values for coefficients of transmission and of absorption were obtained by the calorimetric and the radiometric methods.

The method is so sensitive that a simultaneous determination of the absorption and the storage of energy was obtained in a time as short as three minutes.

The storage of energy is calculated from the evolution of oxygen by the plant, the carbohydrate-factor of which had been very accurately determined.

The photosynthetic efficiency of the leaves of *Hydrilla* is fairly high, being as much as 7·4 per cent. in a vigorous plant.

# CHAPTER XXVI

## THE PHYSIOLOGICAL SCALE AND THE LAW OF PHOTOSYNTHESIS FOR THE DIFFERENT FACTORS

Different variable factors in photosynthesis—The characteristic curve—Scale of measurement—Thermometric and absolute zero—Physiological scale and the determination of the zero point—Determination of the coefficient and the law of variation of photosynthesis under variation of temperature—$CO_2$-coefficient and the photosynthetic law for variation of $CO_2$-concentration—Coefficient for light and the law of photosynthesis for varying intensity of light—Photosynthesis under variation of tonic condition.

PHOTOSYNTHETIC activity undergoes change under the variation not of one but of many factors. It is therefore quite impossible to obtain any consistent results unless we succeed in ascertaining the effect of each individual factor, as well as the combined effects of the different factors. Without this the results would be as anomalous as those obtained by an investigator in physics who, ignorant of the effect of pressure, attempted to determine the changing volume of a given quantity of gas under variation of temperature. It was the study of the separate effects of pressure and of temperature, and then of their combined effect, that led to the formulation of a law which enables us to calculate the volume of a gas under variations of temperature and pressure.

The principal factors which modify photosynthesis are (*a*) the intensity of light, (*b*) the $CO_2$-content of the medium, (*c*) the temperature, and (*d*) the tonic condition of the plant; the problem of the effect of simultaneous variation of different factors on photosynthesis therefore resolves itself into the determination of the quantitative relation between the variations of each of the above factors and

the resulting changes in photosynthesis; this would enable us to determine the physiological coefficient for the variation of any particular factor. Having thus determined the coefficients for temperature, for $CO_2$-content, and for light, we have next to find out how the result is modified by simultaneous change in two of these factors. The object of this line of inquiry is to determine whether the resultant effect induced by changes in two variables can in any way be deduced from the separate effects of each, or whether the combined effect is to be regarded as additive or as something quite different. And finally we have to face the still more difficult problems which arise when more than two different factors undergo independent variation.

The effects of permutation and combination of various factors must be very numerous; according to the older methods each experiment would take several months, so that it would be hardly possible to complete the necessary investigations in the course of a lifetime. The method which I have adopted offers so many advantages, both in regard to accuracy and quickness of operation, that the problems which confront us are not so formidable as they at first sight appear to be.

## The Characteristic Curve

We have first to ascertain the modifying effect of variation of a single factor. It may be said in general that when A and B are so related that any change in B produces a corresponding variation in A, then A is said to be a function of B. We may discover the relation between B and A by plotting a curve in which the ordinate represents the induced change, and the abscissa the variation that induces it. For a concrete example we may take the expansion-curve of the volume of 1 grm. of water from 4° C. to 20° C. (fig. 56). From the character of the curve we are able to establish the formula by which the volume at any temperature may be found,

$$V_t = V_0 (1 + at + \beta t^2 + \lambda t^3)$$

It may be noted here that a somewhat similar relation was found between the increased photosynthetic evolution of oxygen and the increasing intensity of light (p. 34).

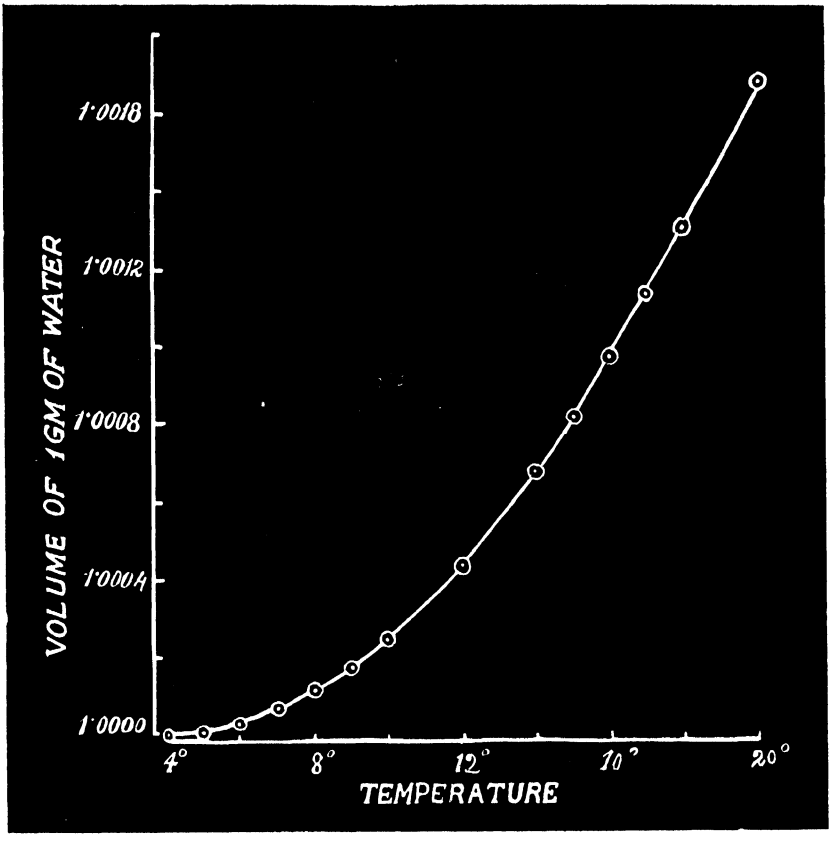



FIG. 56. Curve showing the Change of Volume of 1 Grm. of Water at different temperatures

The expansion-curve of water given in fig. 56 shows that it is practically straight in the middle range; limiting conditions are, however, imposed towards the extreme ends where the liquid becomes converted into a solid at the lower, and into steam at the upper, end.

Photosynthesis being a function of light, of $CO_2$-concentration, and of temperature, we study the effect of

variation of each factor at a time, as its intensity is gradually increased from a minimum to a maximum. We plot the results in the form of a curve, in which the ordinate represents the photosynthetic activity and the abscissa the intensity of the factor which is undergoing variation. The characteristics of the curve enable us to find the relation between the varying factor and the effect produced.

## Scale of Measurement

In all quantitative measurements it is necessary to adopt a suitable scale. For this we have to fix on two points of reference, a lower and an upper, the interval being divided into a convenient number of units. In the measurement of temperature, for example, the two definite points usually adopted are the temperatures at which water changes its condition to solid or to steam—the melting-point of ice or the boiling-point of water. According to the centigrade scale the interval is divided into 100 parts, the melting-point of ice being taken as zero. The fixing of the zero and of the higher point is, however, arbitrary. For there is no reason why a substance other than water should not have been chosen for the determination of the two points of reference; the melting-point of solid paraffin would have given a higher, and that of solid mercury a lower, zero-point. Fahrenheit attempted to discover the lowest temperature attainable in his time from a mixture of ice and salt. We have thus two temperature zeros, according to the centigrade or the Fahrenheit scale, the interval between the zero and the boiling-point being divided respectively into 100 and 212 parts. Again, the zero-point and the scale of measurement are by no means final; they are adopted as a matter of convenience to suit the particular investigation. The zero-temperature, according to Fahrenheit, was, as previously stated, determined from the lowest temperature that could be obtained in his time. Still lower temperatures were produced later in the liquefaction of gases; but the zero

was indefinite, for different gases are liquefied at different temperatures.

## The Absolute Zero

A certain characteristic property of permanent gases enables us, however, to obtain an absolute zero. The coefficient of expansion of gas is $\frac{1}{273}$; the curve representing the expansion of gas at different temperatures, when produced backwards, cuts the abscissa at the absolute zero ($-273°$ C.), where the volume of gas is imagined to have shrunk into nothing. The absolute zero is a hypothetical abstraction, for the volume of a gas can never be reduced to zero; the gas changes its condition and becomes liquefied before reaching $-273°$. The adoption of the absolute zero for the physical scale has, among others, the following advantage. The volume of a given quantity of gas is thus found (within limits) to be strictly proportional to the temperature as represented on the absolute scale. Thus,

$$\frac{V}{V'} = \frac{T}{T'}$$

where V and V′ are the volumes of the gas for the temperatures T and T′ represented on the absolute scale, pressure being supposed to be constant. The volume of a gas is thus a linear function of the absolute temperature.

*I will now show that, on ascertaining the proper physiological zero, photosynthetic activity is also found to be a linear function of temperature as represented on the physiological scale. We shall also find a similar relation when the varying factor is $CO_2$-content, or light, the physiological zero being appropriately determined for each of these factors.*

## Advantages of the Adoption of the Physiological Scale

I proceed to show that the adoption of the physiological scale not only enables us to formulate a general law of photosynthesis, but it affords us means of determining

the physiological constants with a high degree of accuracy. The photosynthetic curve in the median range is straight;

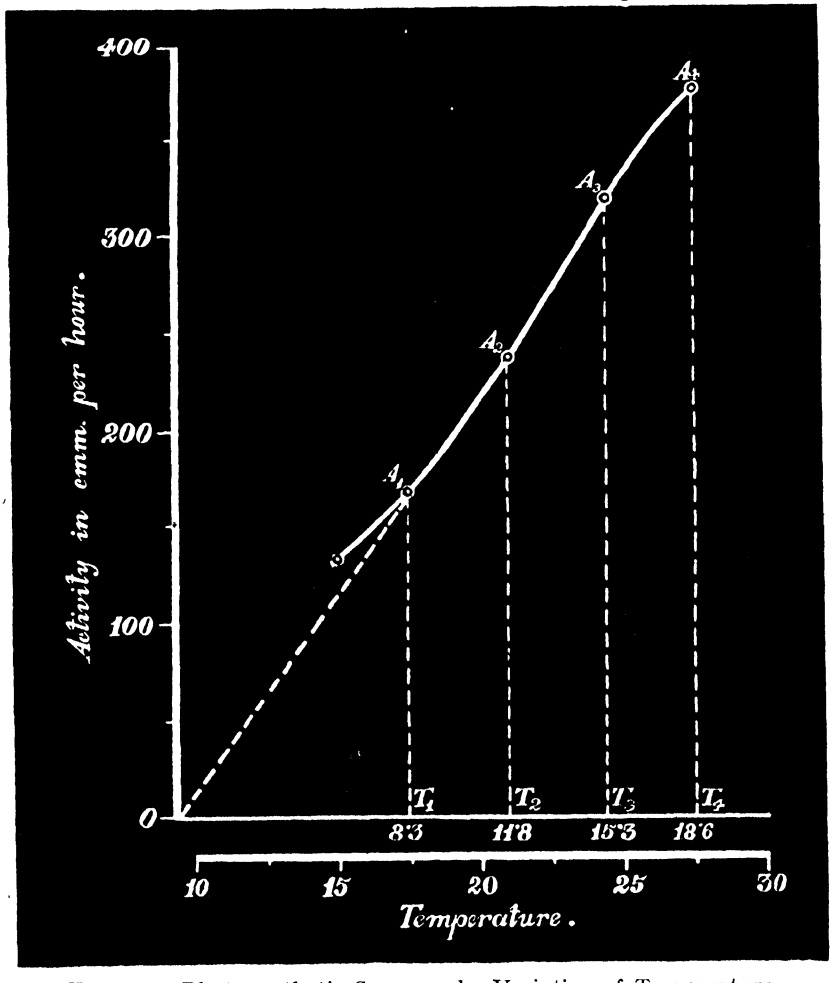



FIG. 57. Photosynthetic Curve under Variation of Temperature Curve produced backwards cuts abscissa at 9°·2, which is the physiological zero. The temperatures $T_1$, $T_2$, $T_3$, $T_4$ on the physiological scale are given in the upper and on the centigrade scale in the lower line.

when produced it cuts the abscissa at a point which is taken as the physiological zero. In the photosynthetic curve under variation of temperature given in fig. 57, the zero is at 9°·2. This physiological zero-temperature is not very

different from that at which photosynthesis becomes arrested. We obtain the absolute physiological temperature, indicated by the thick type **T**, by subtracting 9°·2 from the temperature on the centigrade scale. For example, when the physiological zero is at 9°·2, a temperature of 21° C., converted into the absolute physiological scale, becomes 21° − 9°·2 = 11°·8 = **T**. I will use the term *absolute determination* when measurements are made on the physiological scale.

In illustration of the advantages of the physiological scale, I give determinations of the coefficient for temperature in the following table, the data being those of the typical specimens I. and II., previously given in Table XIX.

TABLE XXXIV.—PHYSICAL AND PHYSIOLOGICAL SCALES AND THE DETERMINATION OF THE TEMPERATURE COEFFICIENT

| Specimen | Temperature in centigrade | Temperature **T** on physiological scale | Activity A in c.mm. O per hour | $K = \frac{A}{T}$ |
|---|---|---|---|---|
| I. | | | | |
| Physiological zero at 9°·2 | 17°·5 | 8·3 | 168·1 | 20·25 |
| | 21°·0 | 11·8 | 239·5 | 20·30 |
| Differential K = 20·3 | 24°·5 | 15·3 | 318·2 | 20·80 |
| | 27°·8 | 18·6 | 376·8 | 20·25 |
| II. | 17°·0 | 7·2 | 147·9 | 20·5 |
| | 20°·5 | 10·7 | 209·8 | 19·6 |
| Physiological zero at 9°·8 | 24°·2 | 14·4 | 288·4 | 20·0 |
| | 28°·0 | 18·2 | 360·1 | 19·8 |
| Differential K = 19·9 | 29°·8 | 201·0 | 400·4 | 20·0 |

*Absolute determination of the coefficient*

*Examples.*

Specimen I. Physiological zero . . 9°·2

(*a*) Temp. 17°·5 C.; **T** = 17·5 − 9·2 = 8·3: activity = 168·1
K = 20·25

(*b*) Temp. 24°·5 C.; **T** = 24·5 − 9·2 = 15·3: activity = 318·2
K = 20·8

Specimen II. Physiological zero . . 9°·8

(*a*) Temp. 17°·0 C.; **T**=17·0−9·8=7·2: activity=147·9
K=20·5

(*b*) Temp. 24°·2 C.; **T**=24·2−9·8=14·4: activity=288·4
K=20·0

In Table XXXIV. the first column gives the physiological zero, also the average of coefficient K obtained by the Differential Method. The second column gives the temperature in centigrade; the third column the temperature **T** on the physiological scale; the fourth column the activity A; the last column gives the coefficient K.

## Law of Photosynthesis for Variation of Temperature

It will be seen that the photosynthetic coefficient K, whether determined by the differential or by the direct method, is practically the same; while the former method gives only the average value, the latter gives it for every point in the median range. The coefficient for the median range is found to be a constant.

*The general law of photosynthesis under variation of temperature is—*

$$\frac{A}{T} \text{ is a constant.}$$

TABLE XXXV.—DETERMINATION OF COEFFICIENT FOR $Co_2$-CONCENTRATION FROM MEASUREMENTS ON THE PHYSIOLOGICAL SCALE

| Specimen | $CO_2$-concentration in mg. per 100 c.c. | **C** on physiological scale | Activity A in c.mm. O per hour | $K = \frac{A}{C}$ |
|---|---|---|---|---|
| I. | 3·0 | 1·80 | 70·96 | 39·4 |
| | 3·75 | 2·55 | 99·22 | 38·9 |
| Physiological zero at 1°·2 | 5·0 | 3·80 | 149·50 | 39·3 |
| | 6·0 | 4·80 | 194·75 | 40·5 |
| Differential K = 39·9 | 7·5 | 6·30 | 248·46 | 39·4 |
| II. | 2·7 | 1·50 | 60·50 | 40·3 |
| | 3·37 | 2·17 | 87·27 | 40·2 |
| Physiological zero at 1°·2 | 4·5 | 3·30 | 131·73 | 39·9 |
| Differential K = 39·6 | 6·55 | 5·35 | 215·88 | 40·3 |

## The Law of Photosynthesis under Variation of $CO_2$-Concentration

The photosynthetic curve for variation of $CO_2$-concentration of the typical specimen I. (p. 116), is given in fig. 58. The straight part of the curve cuts the abscissa at 1·2, which is

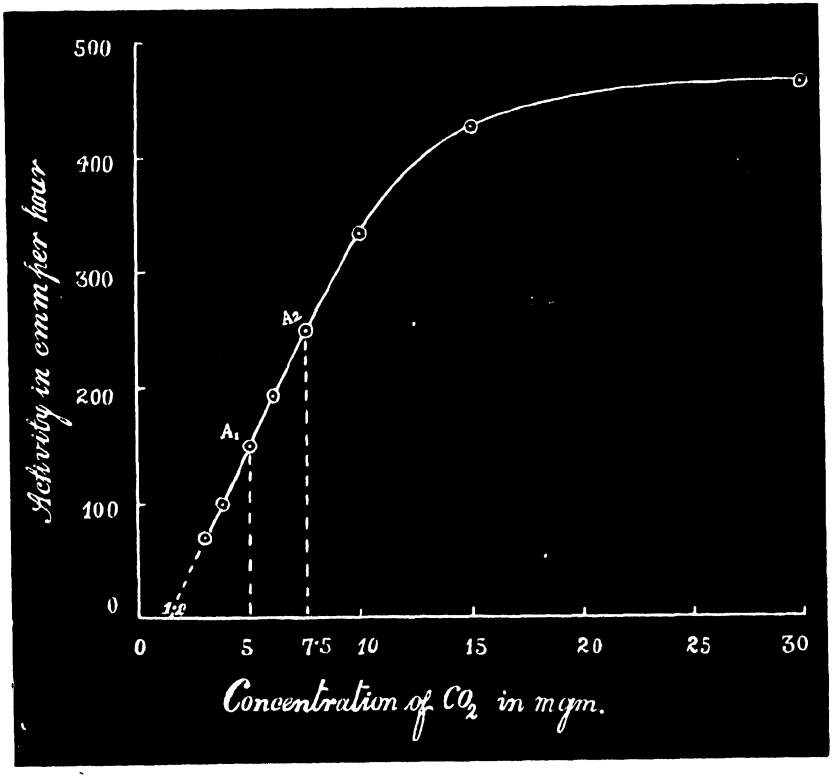



FIG. 58. Photosynthetic Curve under Variation of $CO_2$-Concentration
Physiological zero at 1·2 mg.

therefore the physiological zero for the specimen. The $CO_2$-concentration on the physiological scale will be represented by **C**. Table XXXV. (p. 246) gives the detailed results for the winter-specimens I. and II. previously referred to in Table XIVA.

The coefficients for different points in the median range

of the curve are found to be a constant and practically the same as those obtained by the differential method.

*The law of photosynthesis under variation of $CO_2$-concentration is that the ratio of activity to the concentration is a constant.*

This is symbolically expressed as—

$$\frac{A}{C} \text{ is a constant.}$$

## The Law of Photosynthesis under Variation of Intensity of Light

The curve given in fig. 59 relates to Specimen I. (p. 36),

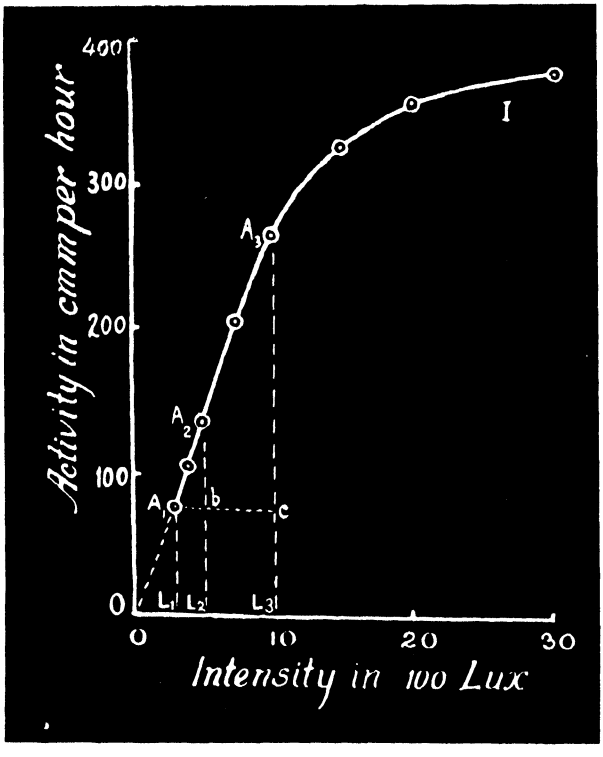



FIG. 59. Photosynthetic Curve under Variation of Intensity of Light

of which the physiological zero is at 0·13 (13 lux); this has to be subtracted from the intensity of light on the ordinary

scale to obtain the intensity of light L on the physiological scale.

The physiological zero does not differ greatly from the minimum intensity of light at which the evolution of gas becomes arrested. There is incipient photosynthesis at a minimum intensity of light though there is hardly any collection of gas in the Bubbler: a few bubblets are, however, formed at the cut end of the plant at as low an intensity as 50 lux. As it is here shown that photosynthetic activity is strictly proportional to the intensity of light measured on the physiological scale, and as the necessary correction is relatively small, the activity is approximately proportional to the intensity measured on the physical scale.

TABLE XXXVI.—DETERMINATION OF THE COEFFICIENT FOR VARIATION OF INTENSITY OF LIGHT

| Specimen | Intensity in 100 lux | L on physiological scale | Activity A in c.mm. O per hour | K $\frac{A}{L}$ |
|---|---|---|---|---|
| I. (Spring) Physiological zero at 0·13 Differential K = 26·9 | 3·00 | 2·87 | 77·1 | 26·9 |
| | 4·00 | 3·87 | 102·8 | 26·6 |
| | 5·00 | 4·87 | 131·4 | 27·0 |
| | 7·50 | 7·37 | 200·2 | 27·1 |
| | 10·00 | 9·37 | 265·6 | 26·9 |
| II. (Spring) Physiological zero at 0·48 Differential K = 24·8 | 2·00 | 1·52 | 37·4 | 24·6 |
| | 3·00 | 2·52 | 62·4 | 24·0 |
| | 5·00 | 4·52 | 102·2 | 24·7 |
| | 7·50 | 7·02 | 173·4 | 24·7 |
| | 10·00 | 9·52 | 234·8 | 24·6 |
| V. (Winter) Physiological zero at 2·0 Differential K = 13·2 | 5·00 | 3·00 | 41·00 | 13·6 |
| | 7·50 | 5·50 | 74·00 | 13·4 |
| | 10·00 | 8·00 | 109·00 | 13·6 |

The detailed results of measurement on the physiological scale are given in the table on p. 249, for the spring-specimens I. and II. and for the winter-specimen V. (*cf.* Tables V., VI., pp. 36, 37).

The coefficient determined on the physiological scale is thus found to be a constant for every point in the median range, its value being the same as that determined by the differential method.

*The law of photosynthesis under variation of light is that the ratio of activity to light is constant.*

$$\frac{A}{L} \text{ is a constant.}$$

## Photosynthesis under Variation of Tonic Condition

The experimental investigation of the effect of variation in tonic condition is rendered difficult, since the conditions for inducing a definite and quantitative variation in the tonic level are, generally speaking, beyond our control. A certain variation was produced by the addition of minute traces of certain chemical substances such as $HNO_3$, the enhancement of activity thus produced varying in different specimens from 50 to 200 per cent., the smaller increase taking place in cases where the plant was already very active (p. 68).

The change in the tonic condition due to the influence of season is, however, more definite than the foregoing. It is possible to obtain a measure of the tonicity from the coefficient for light; activity of an ascertained value of 13·2 in winter, increased to 24·9 in spring (p. 40). Hence the ratio of tonicity in spring $P_S$ to that in winter $P_W$ is :

$$\frac{P_S}{P_W} = \frac{24 \cdot 9}{13 \cdot 2} = \frac{1 \cdot 8}{1}$$

The tonicity for spring is thus 1·8 times that of winter.

The coefficient of activity for $CO_2$-concentration in spring-

specimens has been shown to be 71·1, while that for the winter-specimens was 39·9, the relative photosynthetic activities in spring and in winter being as 71·1 : 39·9 (p. 120). The ratio of tonicity in spring to that in winter has been shown to be as 1·8 : 1. The ratio of activity A to tonicity P is :

$$\frac{\text{Activity for } CO_2\text{-concentration } A_S \text{ for spring}}{\text{Tonicity for spring } P_S} = \frac{71\cdot1}{1\cdot8} = 39\cdot9$$

$$\frac{\text{Activity for } CO_2\text{-concentration } A_W \text{ for winter}}{\text{Tonicity for winter } P_W} = \frac{39\cdot9}{1} = 39\cdot9$$

*Hence the law of photosynthesis for variation of tonicity is that the ratio of activity to tonicity is a constant.*

$$\frac{A}{P} \text{ is a constant.}$$

## SUMMARY

On the physical scale for variation of temperature, the expansion-curve of permanent gases, when produced backwards, cuts the abscissa at the absolute zero. The volume of a given quantity of gas under constant pressure is found from the relation

$$\frac{V}{T} \text{ which is a constant.}$$

On the physiological scale, the straight part of the curve in the median range when produced cuts the abscissa at a point which is taken to be the physiological zero. A simple relation is then discovered between the variable factors as measured on the physiological scale and the resultant change in the photosynthetic activity.

It is shown for temperature, $CO_2$-concentration, light and tonicity, that the ratios to activity

$$\frac{A}{T}, \frac{A}{C}, \frac{A}{L}, \frac{A}{P} \text{ are constants.}$$

# CHAPTER XXVII

## PHOTOSYNTHESIS UNDER SIMULTANEOUS VARIATION OF DIFFERENT FACTORS

Problem of effect of simultaneous variation—Mutual interaction or independent reaction unaffected by other factors—Law of Product—Combined effects of increasing complexity—I. Two variable factors : $CO_2$-concentration and light—II. Variable factors : $CO_2$-concentration and temperature—III. Variable factors : light and $CO_2$-concentration ; light carried through half a cycle and $CO_2$-concentration in two stages—IV. Variable factors : light and temperature ; light of two intensities and temperature carried through a cycle—V. Three variable factors : light, $CO_2$-concentration and temperature—VI. Four variable factors : $CO_2$-concentration, light, temperature and tonic condition.

It has been shown that the effect of variation of the individual factors in photosynthesis may be expressed by a corresponding series of constants. We have now to consider the cases in which two or more of the factors vary, and to attempt to formulate a comprehensive law which will enable us to express the combined effects produced by simultaneous variation of the essential factors $CO_2$-concentration, intensity of light, temperature and tonic condition. Of these the first three are under our control, while the tonic condition cannot at present be modified in a quantitative manner. There is no doubt that even this last factor comes under the general law which it is our object to establish.

In the following discussion we represent the strong intensities by capitals (C, L, T), and the weak intensities by italic letters (*c*, *l*, *t*) : the values on the physiological scale are represented by thick capitals (**C**, **L**, **T**).

### Combined Effects of Different Factors

The main problem is to ascertain *whether the characteristic effect produced by variation of one factor is modified by the*

*interaction of another which is also varying independently, or whether each factor produces its own characteristic effect unaffected by the action of any other factor.*

An illustrative example borrowed from Physics will make the meaning clear. The volume of a given quantity of a gas is determined by the action of two different factors —temperature and pressure. Thus if T represents the absolute temperature, and if the pressure remains unchanged,

$$V \propto T \text{ or } \frac{V}{T} \text{ is a constant.}$$

When the temperature remains constant

$$V \propto \frac{1}{P} \text{ or } VP \text{ is a constant.}$$

If the pressure be doubled the volume will be halved. But this increased pressure will not in any way affect the ratio $\frac{V}{T}$ being a constant; that is to say each factor acts independently, so that we arrive at the general formula $\frac{VP}{T}$ which is constant under all variations of the constituent factors. It is to be understood that the above law holds good in regard to the median range of variation, *i.e.* as long as the physical condition of the gas does not undergo any transformation. The law is no longer applicable at the limit where the gas, under excessive pressure, becomes converted into a liquid. Both physical and physiological laws are understood to be applicable within certain normal limits.

I will now proceed to adduce experimental evidence proving that in photosynthesis *the characteristic effect of each factor is unaffected by that produced by others*; and further, that if the increase of photosynthetic activity

by change from $c$ to C be $x$ times
,, ,, $l$ to L be $y$ ,,
,, ,, $t$ to T be $z$ ,,

then the resulting variation in activity by simultaneous

change of all factors from *clt* to CLT will be $x \times y \times z$, the *product* of the partial effects induced by the individual factors. This, in contrast to arithmetical summation, will be designated as the *Law of Product* or *of Multiplication.*

In order to arrive at the above generalisation, it might be thought necessary to undertake a very extensive series of investigations on the effects of different combinations, not only of all the varying factors, but also of different intensities of the same factor. The number of such combinations would be so numerous that their investigation could hardly be completed within a lifetime. It is, however, possible to arrange a well-thought-out programme of about a dozen different experiments, the results of which will be crucial in the establishment of a photosynthetic law which will be of universal application. I give a list of these experiments under six heads, each including different combinations of increasing complexity.

i. Two variable factors: $CO_2$-concentration and light; the different combinations are *cl*, *c*L, C*l* and CL.

ii. Two variable factors: $CO_2$-concentration and temperature; the different combinations are *ct*, *c*T, C*t* and CT.

iii. Two variable factors: light and $CO_2$-concentration; light carried through half a cycle, $l_1$, $l_2$, $l_3$; $CO_2$-concentration in two stages, *c* and C.

iv. Two variable factors: light and temperature; light of two intensities and temperature carried through a cycle.

v. Three variable factors: light, $CO_2$-concentration and temperature.

vi. Four variable factors: $CO_2$-concentration, light, temperature and tonic condition.

## Method of Procedure

It is desirable that each set of experiments should be carried out with an *identical specimen*; for otherwise the observed variations might be ascribed to physiological differences of the specimens.

The employment of an identical specimen introduces, however, certain difficulties. For fatigue might be induced in the course of prolonged experiment, during which the different factors are being brought into various combination. This difficulty is obviated by bringing about the necessary changes in quick succession, celerity in manipulation being acquired from previous practice. The change in light may be produced quickly and with ease by moving the plant-vessel towards or away from the divergent beam from the Pointolite, the various intensities of which are marked on the experimental table. Greater dexterity is necessary in the adjustment of the temperature, for the change has to be brought about gradually to guard against thermal shock ; the definite temperature has, moreover, to be maintained perfectly constant during the period of the experiment. The necessary adjustment is secured, as previously explained, by the proper manipulation of the stop-cocks which regulate the flow of warm water through the water-jacket enclosing the plant-vessel. The introduction of water containing different proportions of $CO_2$ is accomplished, without causing any disturbance to the plant, by the method which has already been described.

The variation produced should, as far as possible, be forward and upward, in the direction of increasing intensity. For the plant responds more quickly to an upward change. Should it however be necessary to bring about a change in the opposite direction, it should be done slowly, so as to ensure physiological readjustment of the plant to the lower intensity.

The experiments are the following :

## I. Two Variable Factors: Light and $CO_2$-Concentration

Successive experiments and results are given in the subjoined account. The temperature was maintained constant at 22° C.

| | | | Activity |
|---|---|---|---|
| (*a*) | Light 500 lux, | $CO_2$-concentration 3·5 mg.; | 172·8 c.mm. O |
| (*b*) | ,, 1000 ,, | ,, 3·5 ,, ; | 333·0 ,, |
| (*c*) | ,, 500 ,, | ,, 7·0 ,, ; | 304·2 ,, |
| (*d*) | ,, 1000 ,, | ,, 7·0 ,, ; | 583·8 ,, |

From (*a*) and (*b*) we obtain the partial effect of light :

$$\frac{\text{Activity at 1000 lux}}{\text{Activity at 500 lux}} = \frac{333}{172\cdot8} = 1\cdot927 \quad . \quad . \quad . \quad (1)$$

From (*a*) and (*c*) we obtain the partial effect of $CO_2$-concentration :

$$\frac{\text{Activity for } CO_2\text{-concentration } 7.0}{\text{Activity for } CO_2\text{-concentration } 3\cdot5} = \frac{304\cdot2}{172\cdot8} = 1\cdot76 \quad (2)$$

The theoretical enhancement of activity by the combined effects of increase of light and $CO_2$-concentration (from 500 to 1000 lux and from 3·5 to 7 mg. $CO_2$-concentration) should, according to the Law of Product, be (1) × (2) times the original activity 172·8 c.mm.

The theoretical result is then

$$1\cdot927 \times 1\cdot76 \times 172\cdot8 = 585\cdot79 \text{ c.mm. O}$$

Observed result (*d*) = 583·80 ,,

The agreement between the theoretical and experimental results is very remarkable.

From the above results we find that

$$A_{LC} = A_{lc}\{f(L) \times f(C)\}$$

where $A_{LC}$ is the enhanced activity under the combined effects of stronger light and $CO_2$-concentration ; $A_{lc}$ is the original activity at lower intensity of light and $CO_2$-concentration ; $f$ (L) and $f$ (C) are the partial factors for light and $CO_2$-concentration.

*The resultant effect of the simultaneous variation of different factors is thus the* product *of their partial effects.*

Representing the partial activities as $l_a$, $c_a$ for the lower, and $L_a$, $C_a$ for the higher, values of the two factors, and the resultant activities as $A_{lc}$ and $A_{LC}$, then—

$$\frac{A_{lc}}{l_a \times c_a} = \frac{A_{LC}}{L_a \times C_a} = \text{constant.}$$

It has also been shown that $A \propto L$ and $A \propto C$.

Hence $\frac{A}{LC}$ is a constant.

## II. Two Variables : $CO_2$-Concentration and Temperature

The following gives the results of the experiments on the combined effects of $CO_2$-concentration and temperature, light being maintained constant at 1000 lux.

| | | | | Activity |
|---|---|---|---|---|
| (*a*) | $CO_2$-concentration 3·5 mg., | temp. 21°·2; | 133·7 | c.mm. O |
| (*b*) | ,, 7·0 ,, | ,, 21°·2; | 186·9 | ,, |
| (*c*) | ,, 3·5 ,, | ,, 26°·0; | 198·6 | ,, |
| (*d*) | ,, 7·0 ,, | ,, 26°·0; | 283·0 | ,, |

From (*a*) and (*b*) we obtain the partial effect of $CO_2$-concentration :

$$\frac{\text{Activity for } CO_2\text{-concentration } 7{\cdot}0}{\text{Activity for } CO_2\text{-concentration } 3{\cdot}5} = \frac{186{\cdot}9}{133{\cdot}7} = 1{\cdot}4 \quad . \quad (1)$$

From (*a*) and (*c*) the partial effect of temperature is obtained :

$$\frac{\text{Activity at } 26°}{\text{Activity at } 21°{\cdot}2} = \frac{198{\cdot}6}{133{\cdot}7} = 1{\cdot}49 \quad . \quad . \quad . \quad . \quad (2)$$

The theoretical result is the product of (1) × (2) × original activity :

$$1{\cdot}4 \times 1{\cdot}49 \times 133{\cdot}7 = 278{\cdot}9 \text{ c.mm.}$$

$$\text{The observed result } (d) = 283{\cdot}0 \text{ ,,}$$

It follows that $\frac{A}{CT}$ is a constant.

## III. Two Variables : Light and $CO_2$-Concentration ; Light carried through Half a Cycle

In these experiments the change in $CO_2$-concentration is from 3 mg. to 7 mg. ; light in each case was carried through half a cycle $l_1$, $l_2$, $l_3$.

(*a*) $l_1$ = 500 lux, $c$ = 3 mg. : activity = 108·0 c.mm. O
(*b*) $l_2$ = 750 ,, $c$ = 3 ,, : ,, = 164·4 ,,
(*c*) $l_3$ = 1000 ,, $c$ = 3 ,, : ,, = 219·0 ,,

From the above we find that

$$A_{500} : A_{750} : A_{1000} :: 108·0 : 164·4 : 219·0$$
$$:: 2·00 : 3·04 : 4·05$$

Thus the activities were very nearly proportional to the intensities of light.

The experiment was repeated with increased $CO_2$-concentration of 7 mg. per 100 c.c.

(*d*) $l_1$ = 500 lux, C = 7 mg. : activity = 217·4
(*e*) $l_2$ = 750 ,, C = 7 ,, : ,, = 327·4
(*f*) $l_3$ = 1000 ,, C = 7 ,, : ,, = 435·3

Altogether the above results form about eighteen combinations which supply evidence of the validity of the Law of Product. Of these we will consider in detail two representative examples :

*Example* 1.—From (*a*) and (*b*) we obtain the partial effect of light, $CO_2$-concentration being constant.

$$\frac{\text{Activity at 750 lux}}{\text{Activity at 500 lux}} = \frac{164·4}{108·0} = 1·52 \quad . \quad . \quad . \quad (1)$$

The results (*a*) and (*d*) give the partial effect of $CO_2$-concentration, light being constant.

$$\frac{\text{Activity for 7 mg. } CO_2\text{-concentration}}{\text{Activity for 3 mg. } CO_2\text{-concentration}} = \frac{217·4}{108·0} = 2·0 \quad (2)$$

Theoretical increase calculated from product of partial factors (1) and (2) :

1·52 × 2·0 × original activity 108·0 = 328·3 c.mm. O
Observed value (*e*) = 327·4 ,,

*Example* 2.—The partial effect of light is found from (*a*) and (*c*), $CO_2$-concentration being constant.

$$\frac{\text{Activity at 1000 lux}}{\text{Activity at 500 lux}} = \frac{219{\cdot}0}{108{\cdot}0} = 2{\cdot}02 \quad . \quad . \quad . \quad (3)$$

From (*c*) and (*f*) we obtain the partial effect of $CO_2$-concentration, light being constant.

$$\frac{\text{Activity for } CO_2\text{-concentration 7 mg.}}{\text{Activity for } CO_2\text{-concentration 3 mg.}} = \frac{435{\cdot}3}{219{\cdot}0} = 1{\cdot}99 \quad (4)$$

Activity for simultaneous change of light and $CO_2$-concentration is :

Calculated result, (3) × (4) × original activity, 108·0 c.mm. O
$2{\cdot}02 \times 1{\cdot}99 \times 108 = 434{\cdot}1$ c.mm.
Observed result (*f*) = 435·3 ,,

The Law of Product is fully verified by these facts, which prove once more that

$$\frac{A}{LC} \text{ is a constant.}$$

## IV. Two Variables : Temperature and Light ; Temperature carried through a Cycle

Though the experimental difficulties involved in producing more numerous and complex combinations are formidable, yet the results already obtained were so uniformly consistent that I ventured to subject the applicability of the Law of Product to a yet severer test. I employed the more intense light of the sun, which was varied in two stages, from 0·4 S to S. The temperature was carried through a cycle of ten stages, from 22° C. to 35° C., with each of these two intensities of light. I give first a table of detailed values of activity under each variation of temperature : (1) under 0·4 S, and (2) under S. The curves which show these relations are of extreme interest. The stronger intensity of light S is two and a half times greater than the lower intensity 0·4 S. The Law of Product is verified by the fact that the partial effect of the light-factor caused the same

increase of activity practically throughout the entire range of temperature-variation. The actual curve with light 0·4 S for all temperatures is indicated by the dotted line for comparison with that of S. (The curve should have been placed much lower down.) In order to show that the effect

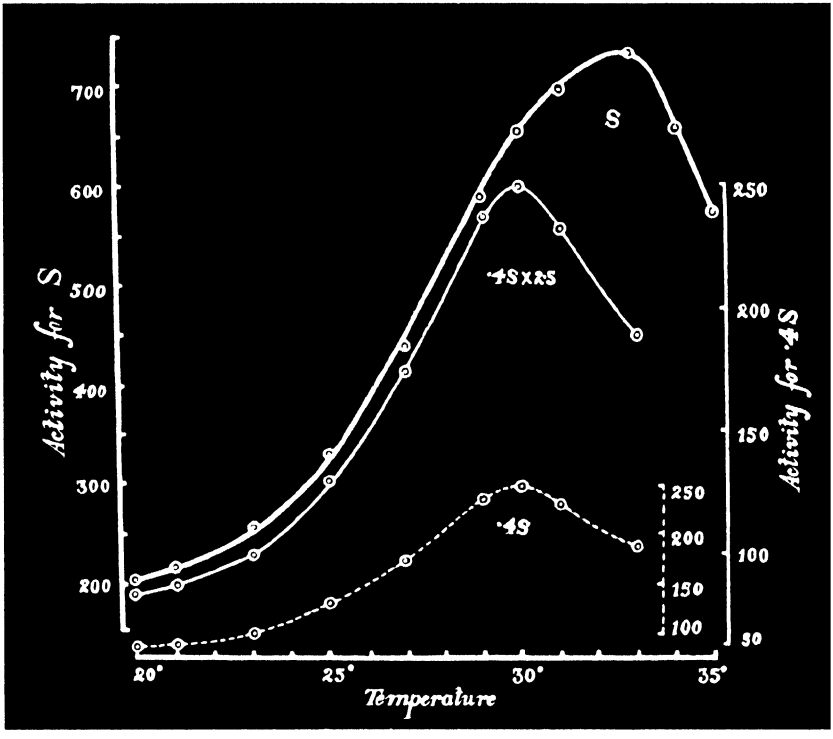



FIG. 60. Photosynthetic Curves under Variation of Temperature carried through a Cycle at Intensity of Light S (upper curve), and at Intensity 0·4 S (dotted curve). The middle curve is obtained by multiplying the activities under 0·4 S by 2·5, which is the ratio of the two intensities S and 0·4 S (see text).

of light S is 2·5 times that of 0·4 S, the activity for the lower intensity has been multiplied by 2·5, and the two curves for S and for 0·4 S × 2·5 are reproduced, the former in thick and the latter in thin outline (fig. 60). They had to be printed slightly apart from each other, otherwise one would have overlapped the other, which is a remarkable proof that the effect of change in the one factor, light, is unaffected by

change in the other factor, temperature, through a considerable range of variation.

TABLE XXXVII.—SHOWING COMBINED EFFECTS OF PARTIAL FACTORS TEMPERATURE AND LIGHT. TEMPERATURE CARRIED THROUGH A CYCLE, AND LIGHT IN TWO STAGES OF 0·4 S AND S

| Temperature in Centigrade | Activity under Light | | Ratio of Two Activities |
|---|---|---|---|
| | 0·4 S | S | |
| 20·0 | 86·0 | 202·0 | 2·35 |
| 21·0 | 88·5 | 215·0 | 2·4 |
| 23·0 | 100·5 | 253·0 | 2·4 |
| 25·0 | 131·7 | 328·0 | 2·5 |
| 27·0 | 175·3 | 436·0 | 2·5 |
| 29·0 | 236·5 | 585·0 | 2·5 |
| 30·0 | **248·4** | 650·0 | 2·6 |
| 31·0 | 232·0 | 694·5 | — |
| 33·0 | 189·0 | **732·0** | — |
| 34·0 | — | 653·0 | — |
| 35·0 | — | 575·5 | — |

The thick numbers represent the two optimum temperatures under 0·4 S and S respectively

*Translocation of the optimum temperature under increased intensity of light.*—Before discussing these results I would draw attention to the effect of intensity of light in modifying the optimum temperature already referred to on p. 139. We find in the table that for 0·4 S the optimum was at 30° C., the activity being 248·4. A rise of 1° C. caused a depression to 232·0. In contrast to this is the effect of the more intense light, under which the optimum is raised to 33° C., the activity being 732·0. Further rise of temperature of a single degree produced a fall to 653.

*Detailed consideration of the two characteristic curves.*—The two curves, for S and for 0·4 S (the latter multiplied 2·5 times), are seen to run parallel to each other. The noticeable divergence is after 30° C., the optimum for 0·4 S, after which the curve is reversed, whereas the curve for S continues to rise until the optimum is reached three degrees

higher. But through the long median range—that is, from 22° C. to 29° C.—the ratio between the two activities is exactly 2·5; it is slightly lower than this below 22° C., and higher after 29° C.

The fact that the ultimate value is the product of the partial effects is conclusively demonstrated by the various possible combinations, about a hundred in number, in the median range. The limitation of space compels me, however, to give only three typical examples.

*Example* 1.—Temperature-variation from 21° C. to 25° C.

*The factor of light.*—The activity under S is 2·5 times that under 0·4 S (through the range of 22° C. to 29° C.) . (1)

With constant light at intensity 0·4 S, the partial effect for temperature-variation is—

$$\frac{\text{Activity at } 25°}{\text{Activity at } 21°} = \frac{131·7}{88·5} = 1·49 \quad . \quad (2)$$

Hence the activity for S at temperature 25° C. is—

Calculated value, (1) × (2) × activity at 21°
= 2·5 × 1·49 × 88·5 = 329·7 c.mm.
Observed value = 328·0 ,,

*Example* 2.—Temperature-variation from 23° to 27°. Light changed from 0·4 S to S.

Partial factor for temperature-variation at 0·4 S is—

$$\frac{\text{Activity at } 27°}{\text{Activity at } 23°} = \frac{175·3}{100·5} = 1·74 \quad . \quad (3)$$

Hence activity for S at temperature 27° is—

Theoretically (1) × (3) × activity at 23° for 0·4 S
= 2·5 × 1·74 × 100·5 = 437·2 c.mm.
Observed value = 436·0 ,,

*Example* 3.—Temperature-variation from 21° to 29°. Light changed from 0·4 S to S.

Partial factor for temperature-variation at 0·4 S is—

$$\frac{\text{Activity at } 29°}{\text{Activity at } 21°} = \frac{236·5}{88·5} = 2·67 \quad . \quad (4)$$

Hence activity for S at temperature 29° C. is—

Theoretically (1) × (4) × activity at 21° for 0·4 S
= 2·5 × 2·67 × 88·5 = 590·7 c.mm. O
Observed value = 585·0 ,,

The agreement between the theoretical and the observed results is again most remarkable.

## V. Three Variables: $CO_2$-Concentration, Light, and Temperature

In this set of experiments all these three different factors underwent variation; the different combinations were:

*clt*; *Clt*; CL*t*.; CLT.

(*a*) $c = 3$; $l =$ 750 lux; $t = 21°$ activity = 50·4 c.mm. O
(*b*) $C = 7·5$; $l =$ 750 ,, $t = 21°$ ,, = 83·4 ,,
(*c*) $C = 7·5$; $L =$ 1500 ,, $t = 21°$ ,, = 163·8 ,,
(*d*) $C = 7·5$; $L =$ 1500 ,, $T = 26·6°$ ,, = 245·4 ,,

From (*a*) and (*b*) the partial factor for $CO_2$-concentration is:

$$\frac{\text{Activity at } CO_2\text{-concentration } 7·5}{\text{Activity at } CO_2\text{-concentration } 3·0} = \frac{83·4}{50·4} = 1·65 \quad . \quad (1)$$

From (*b*) and (*c*) the partial factor for light is—

$$\frac{\text{Activity at 1500 lux}}{\text{Activity at 750 lux}} = \frac{163·8}{83·4} = 1·96 \quad . \quad (2)$$

As regards the factor for temperature-variation, it has been previously shown that the physiological zero varies between 9°·2 and 9°·8. In the present case it is 9°·6.

$$\frac{A_T}{A_t} = \frac{T - 9·6}{t - 9·6}$$

Hence the partial factor for temperature variation from 21° to 26°·6—

$$\frac{26·6 - 9·6}{21·0 - 9·6} = 1·49 \quad . \quad . \quad (3)$$

From the above, the enhancement of activity from *c*, *l*, *t*, to C, L, T, is—

Theoretically, (1) × (2) × (3) × original activity
= 1·65 × 1·96 × 1·49 × 50·4 = 242·9 c.mm.
Observed value (*d*) = 245·4 „

Thus it is evident that when there are three variable factors the results conform to the Law of Product.

$$\frac{A}{LCT} \text{ is a constant.}$$

## VI. Four Variables: $CO_2$-Concentration, Light, Temperature, and Tonic Condition

Having determined the effect of simultaneous variation of $CO_2$-concentration, light and temperature, there remains only the factor of tonic condition. As previously explained it is not easy to produce a definite change of tonicity at will. The effect of season was, however, found to be fairly definite, the photosynthetic activity in spring being about 1·8 times that in winter (p. 40).

One of the means of raising the tonic level of the plant is by supplying it with infinitesimal quantities of certain chemical substances. It was found that an addition of 0·5 part of $HNO_3$ in a billion greatly increased the photosynthetic activity. In specimens already active the increase was about 50 per cent., while in less active specimens it was as high as 200 per cent.

In the experiments carried out to observe the effect of change in four variables, the sequence was as follows:—

(*a*) Activity for $CO_2$-concentration 3 mg., light 750 lux, temperature 20° C. without any trace of $HNO_3$ . . 110·5 c.mm. O

(*b*) Activity for $CO_2$-concentration 5 mg., light 750 lux, temperature 20° C. without trace of $HNO_3$ . . 183·0 c.mm. O

(*c*) Activity for $CO_2$-concentration 5 mg., light 1500 lux, temperature 24° C. with addition of 0·5 part of $HNO_3$ in a billion . . . . 748·2 c.mm. O

The enhancement of activity under these conditions may be calculated as follows :—

from (*a*) to (*b*)—

$$\frac{\text{Activity for } CO_2\text{-concentration } 5{\cdot}0}{\text{Activity for } CO_2\text{-concentration } 3{\cdot}0} = \frac{183}{110{\cdot}5} = 1{\cdot}65 \quad (1)$$

from (*b*) to (*c*)—

Increase of activity by doubling light from 750 to 1500 lux $= 2{\cdot}0$ (2)

Increase of activity due to variation of temperature from 20° to 24° C. (physiological zero at 9°·5 C.) found by the formula $\frac{24 - 9{\cdot}5}{20 - 9{\cdot}5}$ $= 1{\cdot}38$ (3)

As regards tonicity, since other factors had already enhanced the activity, the further increase is about 1·5 times $= 1{\cdot}5$ (4)

The resultant enhancement of activity is theoretically the product of (1) × (2) × (3) × (4) × original activity: hence the calculated result is

$$= 1{\cdot}65 \times 2 \times 1{\cdot}38 \times 1{\cdot}5 \times 110{\cdot}5 = 754{\cdot}8 \text{ c.mm.}$$

Observed result $= 748{\cdot}2$ ,,

It therefore follows that :

$$\frac{A}{CLTP} \text{ is a constant.}$$

## SUMMARY

It has been demonstrated that (1) when there is simultaneous variation of different factors in photosynthesis, each factor contributes its full independent effect, and (2) that the resultant effect of the simultaneous variation of factors is, not the *sum*, but the *product* of the effects of the individual factors.

This is the Law of Product, which is expressed by the general formula $\frac{A}{CLTP}$ which is a constant.

# CHAPTER XXVIII

## GENERAL REVIEW

THE living plant is in a state of unceasing activity, absorbing and storing energy supplied from without, setting free and dissipating it from within. The expenditure of energy may be manifested in movement, or it may not be externally perceptible, being employed in working the internal mechanism of the body—such, for instance, as the distribution of water, which, as I have elsewhere shown, involves a considerable expenditure of energy. The fundamental importance of photosynthesis is, that it is the process by which the plant absorbs the energy it requires, the radiant energy of sunlight, and stores it in the form of latent or potential energy in the organic products of the process. The energy so stored can readily be set free again and become kinetic, by the chemical decomposition of the organic substances, manifesting itself in heat, electric current or movement.

All these changes are effected by the living protoplasm and are the expression of its physico-chemical reactions. This is made clear by the observation that all the various manifestations of them that have been made accessible to investigation are affected in a similar manner by any given stimulus or change in internal or external conditions.

### The Physiological Factor in Photosynthesis

It is clear that the physiological factor is most important, inasmuch as any protoplasmic stimulation profoundly modifies the activity of this process. Thus a moderately

strong electric shock causes an arrest of photosynthesis, the period of arrest being prolonged with increased intensity of stimulus. This is analogous to the temporary abolition of the power of response in *Mimosa* after an electric shock, the irritability not being fully restored until after the completion of protoplasmic recovery.

The photosynthetic curve, showing the relation between the activity and the intensity of light which induces it, is similar to the phototropic curve.

Light induces both the anabolic A and the catabolic D reactions, the resultant being A—D. The $CO_2$-assimilation by the green leaf of *Hydrilla* is essentially an anabolic process, as exhibited by an electric response of galvanometric positivity (p. 78). In certain cases where the D reaction is predominant, the A is unmasked on the cessation of the action of light, when it is exhibited either as a positive after-effect, or an overshooting response in the positive direction.

*Effect of variation of temperature.*—The various activities of the plant are arrested at a definite minimum temperature characteristic of the species; the thermometric minimum is also modified to some extent by the conditions to which the plants had become habituated. In the tropical *Desmodium gyrans*, the pulsation of the leaflets is arrested at 17° C. in summer, and at 11° C. in winter. The minimum temperature for the arrest of photosynthesis in *Hydrilla* varies from 9° to 12° (p. 137). Growth and the ascent of sap are similarly arrested at a definite thermometric minimum.

The various activities of the plant are enhanced by rise of temperature, and reach a climax at an optimum temperature, which is nearly the same in a large number of tropical plants. The optimum temperature for photosynthesis under sunlight is 33°: the optimum temperature for growth and ascent of sap is also 33°. The excitability of *Mimosa* is at its highest at 34° C. The growth-curve under variation of temperature is practically a replica of the photosynthetic curve (p. 140).

*Effect of chemical agents.*—Excess of carbon dioxide arrests the pulsation of the *Desmodium* leaflet and the activity of growth; it also depresses the photosynthetic activity. Ether induces a depression of response in *Mimosa*, and diminution in the rate of photosynthesis. Removal of the anæsthetic is attended in both by restoration of normal activity.

Solution of copper sulphate depresses and abolishes all physiological activity: a solution of this substance, ten parts in a million, arrests photosynthesis. A solution of formaldehyde abolishes the pulsations of the *Desmodium* leaflet, the ascent of sap, and also the photosynthetic activity in *Hydrilla*. A minute dose of a poison often enhances various activities of the plant; photosynthetic activity is increased in a remarkable degree by traces of certain substances, ordinarily toxic in their action.

*Modification of tonic condition by age, season and unfavourable environment.*—In *Mimosa* moderately young leaves are found to be the most irritable; the old leaves are insensitive. Similar relation between age and activity is to be found in the pulsating leaflets of *Desmodium*, and in the photosynthetic activity of the leaves of *Hydrilla*.

The physiological vigour of the plant is greatly lowered after flowering, as seen in the depressed response of *Mimosa*, in the feeble pulsation of *Desmodium* leaflet, and in the very low rate of photosynthesis in *Hydrilla*.

The different activities of the plant are higher in spring than in winter. In *Hydrilla* the photosynthetic activity in spring is nearly double that in winter.

Under unfavourable external conditions, the tonic condition of the plant falls below par, with resulting depression of physiological response. In such sub-tonic specimens, the immediate effect of stimulus is to confer an enhanced activity to the organism, so that the stimulus which was formerly ineffective now becomes effective. Thus the activity of sub-tonic specimens of *Hydrilla* becomes enhanced on stimulation.

These remarkable similarities in the modification of different modes of response to definite external changes prove that the protoplasmic reaction of the protoplast is a most important factor in photosynthesis.

I will now give a short summary of the more important results obtained concerning photosynthesis.

## Determination of Photosynthetic Activity

The methods of investigation in photosynthesis with *Hydrilla* which I have been able to devise may be regarded as ideally simple, and as nearly as possible, perfect. The estimation of the activity of photosynthesis from the rate of evolution of oxygen is direct and requires no prolonged chemical analysis. Complications arising from the presence of stomata do not arise in *Hydrilla*. The oxygen method is, moreover, free from many sources of error unavoidable in the method based on the absorption of carbon dioxide.

The inaccuracies in the method of counting the bubbles from the cut end of the stem have been completely removed by the new method which I devised for determination of the frequency of successive bubbles representing *equal volumes* of pure oxygen given out by the special Bubbler. The Automatic Method of record by the Electro-magnetic Writer eliminates all personal error of observation. The entire investigation can be completed in the course of about forty minutes, during which the external conditions can be maintained absolutely constant. The temperature of the plant remains the same as that of the mass of water in which it is immersed. The difficulty in the maintenance of constant light has been completely removed by the employment of special devices.

As regards artificial source of light, Pointolite has been found most suitable, since it produces little heat, which is completely absorbed by an interposed stratum of water. The intensity of light can be varied from 100 to 4000 lux.

## Photosynthetic Induction

The total volume of oxygen given out in photosynthesis is proportional to the duration of the exposure to continuous light; but this quantitative relation does not hold good when the light is intermittent. The photosynthetic production of carbohydrates is brought about by a series of chemical dissociations and combinations. Preliminary to dissociation, a state of strain is induced in the molecule. When the light is temporarily discontinued at this stage, the induced state of molecular strain disappears; thus the preliminary work is undone. The undoing of the positive or uphill work brings about a prolongation of the *Induction-period* (p. 89).

*Effect of rapid intermission.*—When the intermission is slow, the effect on photosynthesis is as described above. But a very interesting phenomenon is discovered under rapid intermission. It is found that a rapidly intermittent light is photosynthetically more effective than continuous light. A probable explanation is that, since under continuous light the resultant effect is A − D, on the cessation of light A persists longer than D (p. 95). The results of a gradual variation from rapid to slow intermission are: (1) an effectiveness greater than under continuous light, (2) a decreasing effectiveness reaching a limit, and (3) a partial recovery (p. 96).

## Effect of Infinitesimal Traces of Chemical Substances on Photosynthesis

The photosynthetic activity of *Hydrilla* was found to be very greatly enhanced after a thunder-storm. The only plausible explanation for this was the production of oxides of nitrogen, by electric discharge during the storm, which were washed down by the rain into the pond and thus enhanced the activity of the plant. Investigation on the effect of

infinitesimal traces of $HNO_3$ showed that one part of this substance dissolved in two billion parts of water induced an increase in the rate of $CO_2$-assimilation of nearly 200 per cent. Minute traces of extract of thyroid gland, of iodine, and of formaldehyde, showed similar enhancement of photosynthetic activity.

The effect of a minute quantity of formaldehyde in enhancing activity is of special significance in regard to the possible formation of formaldehyde as the first product of photosynthesis. This substance is toxic only as a strong dose; before there could be any great accumulation of this substance in the cells it would have become polymerised into carbohydrate.

## The Photosynthetic Curve under Increasing Intensity of Light

Photosynthesis is feeble under low intensity of light; the activity then increases at a uniform rate, the curve being straight with a uniform slope. The turning-point occurs at or about 1200 lux, after which the curve tends to become horizontal. Under very strong intensity of light the curve exhibits a reversal, notably in summer.

In the median range of the curve, *i.e.* from about 200 to 1200 lux, the increase of photosynthetic activity is proportional to the intensity of light. The coefficient is found by dividing the increment activity by increment of light. In spring the average coefficient of *Hydrilla* for light is 25. In winter the coefficient is about half that in spring. The minimum intensity for photosynthesis in spring is about 100 lux, whereas in winter it is about 500 lux or more. The activity for intensity of light L is found from the following formula:

$$A_L = A_l + K\,(L - l)$$

The limiting maximum activity is found to be relative and not absolute. It increases with the intensity of light,

this particular increase being independent of the $CO_2$-concentration of the liquid in which the plant is immersed. The maximum under sunlight (0·4 S) was found to be nearly double that under 3000 lux (p. 43).

The *Hydrilla* plant may be used as a photometer, since its sensitiveness to light is very great. The physical and the physiological determinations of intensity of light agree with each other (p. 45). Photosynthesis increases with (1) the duration of exposure, and with (2) the intensity of light. It also increases with the sine of the directive angle. This may be summarised in the general statement that the amount of photosynthesis is proportional to the quantity of the incident light (p. 48).

## Relative Efficiency of the Different Rays

The relative effectiveness of light of different colours has been ascertained by the new method of Flotation (see p. 168), in which a piece of *Hydrilla* is suspended from the pan of a Torsion Balance.

The essential conditions for investigating the relative efficiency of light of different wave-lengths are (1) the securing of a pure spectrum, (2) a sensitive device for the quantitative determination of photosynthesis, and (3) the simultaneous measurement of the energy of the acting rays. Pure spectrum of high dispersion was obtained by a carbon disulphide prism. The different rays were successively thrown on the stationary plant-vessel and on the adjoining strip of the Magnetic Radiometer which detected a rise of about $\frac{1}{100000}$° C., so that the determination of the photosynthetic activity and that of the energy of the rays were practically simultaneous.

The special difficulty arising from the enfeebled intensity of radiation in a highly dispersed spectrum was overcome by the Heterostatic Method. By means of an Auxiliary Photic-stimulator, the plant is brought to the verge of photosynthesis. Exposure to the feeble rays of the spectrum

now induces photosynthesis in proportion to their respective effectiveness (p. 190).

The relation between photosynthesis and the energy of the incident radiation is ascertained by the simultaneous determination of the activity by the Bubbler and of the intensity of radiation by the Radiometer. Photosynthesis is found to be feebly initiated even under infra-red radiation. In the visible spectrum the activity at first rises gradually; at B (680 $\mu\mu$) the maximum is abruptly attained, though the intensity of radiation is here lower than at A. The abrupt rise of activity at B is due to absorption of these particular rays by the chlorophyll. Beyond B the activity undergoes a decline parallel to the decline in the intensity of radiation of the different rays. Though there is an absorption-band in the blue-violet there was no second maximum in this region: this is because the energy absorbed is utilised in other physiological work. At the opposite ends of the spectrum the thermal and the blue-violet rays are effective in inducing tropic reaction; they are, therefore, relatively ineffective in photosynthetic action. The characteristic effects in different regions of the spectrum are due (1) to the energy of the rays, (2) to their relative absorption, and (3) to the complementary A and D reactions in the induction of photosynthesis and of phototropism.

## The Photosynthetic Curve under Increasing $CO_2$-Concentration

The curve is straight up to the turning-point at $CO_2$-concentration of about 8 mg. per 100 c.c.; after this the curve tends to become horizontal, but gradually and never abruptly. Under very strong concentration of $CO_2$ the curve exhibits a reversal which is due to the toxic effect of the carbon dioxide. In winter the average coefficient for $CO_2$ is about 40; in spring the value of the coefficient is nearly double that in winter (p. 120).

## Photosynthesis in Absence of $CO_2$

In summer the temperature at thermal noon is 43° C. or even higher, which is in sharp contrast with the average temperature of 23° C. in spring. The minimum $CO_2$-concentration for initiation of photosynthesis in spring is about 1 mg. per 100 c.c.; but in the summer the photosynthetic evolution of oxygen was found to occur in the total absence of $CO_2$, as when the plant was immersed in distilled water. High temperature in summer suggests more active catabolism, greater oxidation and production of organic acids. As a matter of fact it was found that the juice of plants which was neutral in winter and spring was strongly acid in summer, the acids present being malic and oxalic, the latter in very small quantities.

On supplying the acid *Hydrilla* with strengths of malic acid solution increasing from 4 to 16 parts in 10,000 parts of water, the photosynthetic curve was found to be in every way similar to that under increasing strengths of $CO_2$-solution. In the photosynthesis of plants in an acid condition the organic acids serve as substitutes for $CO_2$ (p. 129).

The results obtained with acid *Hydrilla* plants offer a satisfactory explanation of the variations in the assimilatory and respiratory quotients found in different plants. In succulent plants the access of both oxygen and carbon dioxide from the atmosphere is restricted; organic acids are produced in these plants, which, as in the acid *Hydrilla*, render them less dependent on the supply of carbon dioxide.

In normal cases the volume of $CO_2$ absorbed is about equal to the volume of oxygen evolved: the assimilatory quotient $\frac{O_2}{CO_2} = 1$. But in acid plants the denominator $CO_2$ is less than the normal, and the value of the quotient is greater than 1. The respiratory quotient $\frac{CO_2}{O_2}$ is in normal

cases = 1; but in acid plants it is less than 1, and in extreme cases it may be zero.

The fact that photosynthesis may occur in the absence of $CO_2$ renders the method of estimating photosynthesis by the absorption of carbon dioxide less reliable than the method by the evolution of oxygen.

The catabolic activity is far more pronounced in summer, which may account for the acid condition of the *Hydrilla* in that season. The oxidation produces organic acids, and finally carbon dioxide under complete physiological combustion. When the leaves containing organic acids are acted on by daylight, the building up process begins, the acid taking the place of carbon dioxide. The result is more or less the same, whether the plant is provided with an external supply of carbon dioxide or whether it assimilates the organic acids produced within itself.

## Photosynthetic Curve under Variation of Temperature

An essential condition in the accurate determination of photosynthesis under thermal variation is the adjustment of temperature without any sudden change which causes a physiological shock. This was secured by a device for the gradual rise of temperature; arrangements were also made for the maintenance of constant temperature during the period of the experiment.

The increase of photosynthetic activity in *Hydrilla* is uniform in the median range between 17° and 28° C.; beyond the optimum there is an abrupt decline. A remarkable resemblance is observed between the curves of photosynthesis and of growth, under variation of temperature (p. 140).

A correction has to be made in respect of the respiratory loss in order to arrive at the absolute rate of photosynthetic evolution of oxygen. The loss due to respiration has been determined by two independent methods, which

give concordant results. The loss at 22° C. is about 4 per cent.

The temperature-coefficient is determined on the Differential Method by the formula

$$K = \frac{A_T - A_t}{T - t}$$

The value of the coefficient is about 20. The photosynthetic activity of *Hydrilla* becomes doubled under a rise of temperature of 7° C.

The activity at a higher temperature is found by the formula

$$A_T = A_t + K(T - t)$$

## The Tonic Factor in Photosynthesis

The condition of a plant can be gauged by the Tonometer; the ratio of response to stimulus measures the physiological tone of the plant. In photosynthesis the ratio, K, is found by dividing the increment of activity by the increment of the factor which induces it.

The tonic condition of a plant is modified by seasonal variation, by traces of chemical substances, by the starch- and chlorophyll-content of the cell, and by the action of stimulus.

Seasonal variation exerts a considerable influence on the tonic condition; the photosynthetic coefficient in spring is nearly double that in winter.

Infinitesimal traces of certain chemical substances induce a heightening of the tonic level as seen in the great enhancement in the rate of photosynthesis (p. 71).

The photosynthetic activity is greater in a young leaf than in a very young or old leaf. The chlorophyll-content is at its maximum in a young leaf.

In a sub-tonic specimen, stimulus stirs up the relatively inert tissue to activity. The tonicity is raised and the response becomes enhanced. In a vigorous specimen,

the precisely opposite effect is produced by strong stimulation.

In photosynthesis the after-effect of stimulus in modifying the tonic condition is found by the Method of the Cyclic Curve, the plant being subjected to light increasing to a maximum and then diminishing to a minimum. Three types of physiological *hysteresis* are observed under definite conditions; first, the *negative* hysteresis, where physiological activity becomes depressed under the previous and long-continued stimulation, the return-curve being to the right; second, the *positive* hysteresis, where the feeble activity of a sub-tonic specimen becomes enhanced in consequence of stimulation, the return-curve being to the left; and third, the *zero* hysteresis, where the forward- and return-curves coincide, the activity being unchanged (p. 154).

## Daily Variation in Photosynthesis

The *Hydrilla* plant growing in the open is subjected to two changing environmental factors—temperature and light. Special apparatus was devised to obtain simultaneous determinations of temperature by the Thermograph, of light by the Electric Photometer, and of photosynthesis by the Photosynthetic Recorder.

In a typical experiment at the end of January, photosynthesis was found to be initiated at 7.30 A.M., increasing to a maximum at noon. There was a fall after 1 P.M.; the decline of activity was rapid from 3 P.M. to 5 P.M., there being a complete arrest at 5.15 P.M.

The resulting photosynthesis at various periods of the day can be explained as the expression of combined effects of the factors of light and temperature. The activity is continuously increased with increasing intensity of light; it is also increased under rising temperature up to the optimum; the fall of activity at about 1 P.M. is explained by the rise of temperature above the optimum. The curves given (fig. 45) show the variations of light and temperature, and of the resulting change of photosynthetic activity.

## Determination of the Carbohydrate Product under Light

There are inevitable sources of error in the determination of the increase of weight by the half-leaf method. These arise from the difference in the retention of water in the two halves of the leaves after drying, from the different thickness and lack of symmetry of the two halves, from the shrinkage of area of the exposed half to light, and also from the translocation of soluble carbohydrate from the leaf. Nor can the indirect estimation of carbohydrate from the absorption of carbon dioxide by the leaf be regarded as highly accurate. The value of the carbohydrate-factor assumed is untrustworthy, since it is not constant, but varies in different plants, and even in the same plant under different conditions.

These errors are obviated in experiments with cut specimens of *Hydrilla*. There is no loss of carbohydrate due to translocation, nor is there any increase of weight through accession of nitrogenous or other substances from the soil.

Simultaneous determination was made by two independent methods. The first is the *direct* determination of the increase of weight of the living plant by a sensitive Torsion Balance, or by a Chemical Balance, for an exposure to light of a definite duration; the second, *indirect*, is the measurement by a Eudiometer of the volume of the oxygen evolved during the same period. The results obtained by the direct and indirect methods are in the closest agreement with each other (p. 217).

The rate of production of carbohydrate under sunlight per square metre of photosynthetic surface of the leaf of *Hydrilla* was found to be 2·8 grms. per hour.

The methods of determination of the rate of photosynthesis have been rendered so sensitive that records may be obtained from which it is possible to estimate the

formation of quantities of carbohydrate as small as the millionth of a gramme.

## Efficiency of Photosynthetic Storage of Energy

The estimates hitherto obtained indicate generally a low efficiency; but the experimental methods employed have been defective from absence of means for the exact measurement of the incident energy, of the indeterminate losses of this energy, and of the energy stored in photosynthesis.

I have been successful in obviating these difficulties by the new methods devised. The incident and the absorbed energy were determined by the Calorimetric Method, the rise of temperature being measured with great accuracy by a thermo-electric couple; the loss of heat by radiation was completely eliminated. The accuracy of the calorimetric determination was tested independently by the Magnetic Radiometer. The coefficient of transmission and that of absorption were both found to be practically the same by the two methods. The methods employed are extremely sensitive, the determination being completed in the course of a few minutes.

The energy stored was simultaneously found from the volume of oxygen given out by the plant, the carbohydrate factor of which had been very carefully determined. The photosynthetic efficiency of the leaves of *Hydrilla* is fairly high, being about 7·4 per cent.

## The Physiological Scale and the Law of Photosynthesis under Variation of Different Factors

The expansion-curve of a permanent gas when produced backwards cuts the abscissa at $-273°$, which is taken as the absolute zero of temperature. When the temperature T is measured on the absolute scale, the volume of a given quantity of gas is found from the relation

$$\frac{V}{T} \text{ is a constant.}$$

The volume is thus a linear function of the absolute temperature.

It may be said in general that when A and B are so related that any change in B produces a corresponding variation in A, then A is a function of B. The relation between B and A is discovered by plotting a curve in which the ordinate represents the induced change and the abscissa the variation that induces it. Photosynthesis is a function of light, of $CO_2$-concentration, of temperature and of the tonic condition of the plant. The relation between photosynthesis and each of these factors is found from the characteristics of the different photosynthetic curves.

It is shown that more satisfactory results can be obtained by basing the measurement upon a physiological rather than upon a physical scale. The physiological zero is found by producing backwards the straight median portion of the photosynthetic curve until it intersects the abscissa.

It is also shown that when measurements are taken on the physiological scale the photosynthetic activity is found to be a linear function of each of the different factors.

It has been explained how the average coefficient is obtained by the Differential Method, dividing the increment of activity by the increment of the particular factor. In the absolute determination we obtain the coefficient for every point in the median range by dividing the activity by the value of the factor on the physiological scale.

The general law of photosynthesis for variation of the individual factors of temperature, of $CO_2$-concentration, of light and of tonic condition is expressed by

$$\frac{A}{T} \text{ is a constant}; \quad \frac{A}{C} \text{ is a constant}; \quad \frac{A}{L} \text{ is a constant};$$

$$\frac{A}{P} \text{ is a constant.}$$

The change in photosynthetic activity under the variation of one factor is found from the laws of photosynthesis

established by the experimental results described in Chapter XXVI. In nature, however, all the different factors—$CO_2$-concentration, intensity of light, temperature and the tonic condition of the plant—are changing simultaneously, each in different ways. The possible different combinations of them are too numerous for experimental investigation of all of them. The results obtained in the cases selected prove that the characteristic effect of each factor is unaffected by the effect produced by others: that is to say, if the increase of photosynthetic activity due to three of the principal factors—

by change from $c$ to C be $x$ times
,, ,, $l$ to L be $y$ ,,
,, ,, $t$ to T be $z$ ,,

then the resulting variation in activity by simultaneous change of all the factors from *clt* to CLT will be $x \times y \times z$, or the *product* of the partial effects induced by the individual factors. This, in contrast with arithmetical summation, is designated as the *Law of Product* or *of Multiplication*. This law of combined effects of different factors in photosynthesis is expressed by the formula

$$\frac{A}{CLTP} \text{ is a constant.}$$

The investigations described in the present work establish the underlying unity of physiological mechanism in the plant. The diverse phenomena of mechanical and electrical response, of growth and of ascent of sap, and of photosynthesis, are but characteristically parallel effects of fundamental protoplasmic reactions associated with storage or expenditure of energy. Work is done *on* the plant by energy received, and work is done *by* the plant in the maintenance of its various life-activities. The resultant effect A — D is the difference between the uphill and downhill work. The greater insight into the physiological mechanism obtained from this wider outlook has led to the discovery of

new classes of phenomena, instances of which are found in the prolongation of the Induction-period under increasing duration of darkness, and of photosynthetic efficiency higher than normal under rapidly intermittent light. The nature of the change of activity under variation of one factor at a time has been ascertained from the characteristics of the photosynthetic curve. The adoption of the absolute scale in physiological measurements has led to the establishment of a simple law of photosynthesis. In nature the photosynthetic organ is acted on not by one but by many changing factors, the resultant effects of the simultaneous variation of which are innumerable. It is the high complexity introduced by the many possible combinations of the constituent factors which renders the analysis of life-processes so extremely difficult. An analogous case in physics will give some idea of the difficulties involved. Before the discovery of the law of dilatation of gases the changing volume of a gas under variation of pressure and temperature must have caused great perplexity. It was the establishment of the law that $\frac{PV}{T}$ is a constant that led to very important advances in physics; in chemistry not even an elementary advance would have been possible without the application of this law.

The complicating factors in photosynthesis are far more numerous; it is hoped that the introduction of measurement on the physiological scale and the establishment of the Law of Product will lead to as great an advance in Plant Physiology as the introduction of absolute measurement has accomplished in Physical Science.

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