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LIGHT AND FUNDAMENTAL LIFE PROCESSES OF PLANTS

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BY

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Introduction

The phenomena of light are mainly studied by physicists, but the knowledge derived from their labours about the nature of light is of great importance to those who study the life processes of plants. If we go to the very root of things it becomes abundantly clear that there can be no life without light and the sensation that we know as light can only be experienced through the medium of life. Light is known to be the carrier and supplier of energy without which life cannot exist. It is therefore natural that the recent advances in the study of the physical nature of light have been of great interest to plant physiologists and are paving the way to the right understanding of the various processes taking place in plants under the influence of this all important phenomenon of Nature.

Plants by virtue of their unique activities entrap the energy that they receive from the sun's rays, work it up in a mysterious manner and produce the complex organic substances which not only maintain them in the state of living, but also maintain the multifarious forms of the animal life in the living state on the earth. The energy of the sun's rays that is harnessed and stored up in these products of plant manufacture is liberated again in an equally mysterious manner and becomes available for the important manifestations of the living organisms. Thus the living is made possible by the energy that is primarily derived from sunlight through the agency of plants whose strength, to use the quaint saying, was supposed to lie in standing still.

The sensation of light firstly makes us conscious of the entire Universe and all that it consists of. It has made possible the stock of human knowledge and experience that we now so proudly possess and that we so rapidly enlarge. Secondly the energy of light is the mainstay of living organisms. We are here more concerned with the second than with the first proposition. The first is the outcome of the second. It is therefore of paramount importance to direct our efforts to the study of these beneficent processes in plants that make the living possible. The knowledge of these processes will benefit mankind more than any other discovery in science and any small attempt to add a few facts to the existing stock of knowledge about these processes will be an attempt in the service of mankind. It would be therefore in my opinion a fitting occasion to speak on the present state of knowledge of the relations of light to these processes by which complex-organic food substances are manufactured, before this society which has, as its aim, the promotion of research in different fields of the plant sciences.

Light and Synthesis of Carbohydrates

The importance of light on synthesis of carbohydrates in leaves was first noticed by Ingen-Housz (1779). Senebier (1788) next observed the effect of light of different colours in the formation of starch in leaves. This work was extended by Gilby (1821), Daubeny (1836) and Dumas and Boussingault (1841). Draper (1844) next pointed out that the yellow region of the visible spectrum was the most effective but later Lommel (1871) showed that the photosynthetic activity was highest in the red yellow region. Timmiriazeff (1869-89) confirmed the findings of Lommel and he (1890) showed that the formation of starch increased from the red end of the spectrum towards the violet end. Englemann (1882-1884) on the other hand found by his bacteria method a secondary maximum in the rate of the process in the blue violet region. In all these experiments no attempt was made to keep the energy contents of the different rays of light the same. This defect was removed by Kniep and Minder (1909) in their experiments. These authors concluded that the rate of photosynthesis was the same in the red (620 $\mu\mu$ to infra red) and the blue (523-340 $\mu\mu$) regions and the process was at a standstill in the green region (524-512 $\mu\mu$). Though they made the intensities of the different rays equal in these experiments they used very low light intensity which acted as a limiting factor. They also employed an inaccurate method for the measurement of photosynthesis.

Ursprung (1912) further showed that the process of photosynthesis occurred only in the visible region of the spectrum of white light, the maximum being in the red region. The work of Ursprung (1917-18) fixed with certain degree of accuracy the exact spectral limits of photosynthesis, though they varied in the case of different plants. The photosythetic activity was also found by Lubimenko (1923) to be more intense in the red region (760-600 $\mu\mu$) than in the blue region (480-400 $\mu\mu$) of white light. The discovery of this fact has stimulated research on the effect of different wavelengths of light on photosynthesis. The difficulties of experimentation on this problem are so great and in some ways so insurmountable that experimental work done by different workers on this aspect of the problem is defective for one reason or another. In order to obtain reliable experimental data it is necessary to make the total intensity of incident rays equal in all cases. Even when that is done, it is not certain if the different rays like the red and the blue are absorbed by a leaf to the same extent. If that is not the case the rates of photosynthesis in the two parts of the visible spectrum cannot be compared.

Light of Different Wavelengths and Photosynthesis

Attempt was made by Wurmser (1920-21) to determine the general relations between the quantity of energy absorbed from lights of different wavelengths and the rate of photosynthesis and came to the conclusion that green light was utilised in the photosynthetic work to about four times the extent of the red. To express it in physical terms the utilisation factor, *i.e.*, the ratio of the quantity of the light energy absorbed to the amount which is transformed into chemical energy, increases from red to green from 60 per cent. to 70 per cent. The main objection to the results of Wurmser (1920-21) is that the method of calculating the amount of absorbed energy has no direct physical basis. This difficulty is met by Warburg and Negelein (1923) by using a silvered vessel for the assimilating material so that the incident light energy is taken as the energy absorbed. They found that the efficiency of the photosynthetic system decreases with decreasing wavelength. Thus the utilisation factor shows decrease from 60 per cent. to 40 per cent. which is in accordance with Einsteins's Law of Photochemical Equivalence. In these experiments by Warburg and Negelein in the errors in determinations of the energy absorbed by the assimilating organism are not removed. It is necessary to determine the fraction of light energy that is absorbed by the green pigments alone. Some energy is also absorbed by the colourless components of the tissues. Briggs (1929) tries to avoid the sources of error in measuring the absorbed amount of energy by an indirect method from the volume of oxygen evolved in photosynthesis, the energy utilised in the process is calculated from the heat of combustion of glucose to produce one cc. of carbon dioxide which is taken as equal to oxygen evolved. His results also confirm the findings of Warburg and Negelein (1923). In his experiments the energy incident on the leaves is not the same in the three regions, yellow, red, green and blue. The rates of photosynthesis are afterwards calculated for the same incident energy, *i.e.,* per 500 calories per 100 sq. cms. of the leaf area per hour. This is objectionable in the sense that the rate of photosynthesis in the different rays of light may not increase in the same proportion by the increase in their intensities. The incident light intensity employed by him is also very low.

The experiments done on the rate of reproduction in algae by Klugh (1925) also show that the rate of reproduction, *i.e.,* indirectly the rate of photosynthesis is highest in the red rays. Similar conclusions have been reached by Moore, Whiteley and Webster (1923) on the photosynthetic activity of the sea-weeds.

The main conclusion that can be drawn from the work quoted above is that the efficiency of the photosynthetic mechanism decreases with the decreasing wavelengths of light. All these costly and elaborate experiments do little more than confirm the findings of Senebier in 1788 with his simple technique of double-walled bell-jars containing coloured solutions.

The above conclusion does not however find support in the conclusions reached by Popp (1926) on the effect of the omission of the blue-violet and the violet regions of light on the growth of plants. The omission of these rays results in the greatly decreased production of carbohydrates in leaves. The results suggest that the blue-violet rays are important in the process in some way or the other.

Sunlight, Electric Light and Photosynthesis

Some fresh light on the question of the effect of different rays of white light on the photosynthetic activity is thrown by the experiments done in my laboratory on formation of carbohydrates in leaves exposed to sunlight and light from an electric lamp (Dastur and Samant, 1933).

The results clearly show that the photosynthetic process in green leaves does not proceed with the same speed in artificial light like a gas-filled electric lamp as it proceeds in diffused sunlight of the same total intensity. The formation of carbohydrates takes place very slowly and in small quantities in leaves of plants exposed to artificial light as compared with the formation of carbohydrates in sunlight. The spectrum analysis of the lights from the two sources showed that, though the spectral composition was the same, the distribution of energy in the different parts of the visible spectra was not the same. The artificial light was more intense in the yellow-red region of the spectrum than the diffused sunlight; while the latter was more intense in the blue-violet region than the former. The distribution of energy is fairly uniform in the different parts of the visible spectrum of sunlight while it is not so in the visible spectrum of light from the electric lamp. These observations open up the question of the effect of different wave-lengths of light on photosynthesis. The artificial light is more intense in the yellow-red region of the visible spectrum (at a distance of 50 cms. at which the plants are exposed) than the diffused sunlight. If the efficiency of the photosynthetic system decreases with decreasing wavelengths of light, the results obtained by us do not support this conclusion, as the artificial light is richer in those rays which are supposed to be photosynthetically efficient. These results suggest that either the blue-violet region of the visible spectrum is equally or more efficient in the process than the yellow-red region or that the whole region of the visible spectrum is photosynthetically effective, and the lesser proportion of any one region results in a depressed rate of photosynthesis. The total energy supplied by the different radiations of the visible spectrum is not the determining factor in the process, but the different radiations as such or the frequency of radiations are important for the process. If it is merely a question of energy derived from light radiations, the process of photosynthesis should go on normally in artificial light supplying the same amount of energy in terms of ergs or calories, as supplied by the sunlight. These findings make us look at the problems of the energetics and mechanism of photosynthesis from a new angle and it was considered to be of interest to extend these observations.

Photosynthesis in Lights from Different Sources

In order to obtain further evidence to support the above conclusions it was undertaken to determine the rate of photosynthesis in leaves exposed to lights from different sources. Four different artificial sources, an electric lamp, daylight lamp, an incandescent oil lamp and a carbon arc lamp were used. The measurements of the distribution of energy in the visible spectrum of each light were made in three different ways namely, (1) by micro-thermopile, (2) by photographic plates and (3) by taking spectrum photographs by means of Adam Hilger's constant deviation spectrameter. According to the intensities of the blue-violet regions the sources of light are found to be in the following order:—(1) Sunlight,

(2) Carbon arc lamp, (3) Daylight lamp, (4) Electric lamp and (5) Incandescent oil lamp. The quantities of carbohydrates formed in leaves exposed to these five sources of illumination are also found to be in the same order. The quantities of carbohydrates formed in sunlight are higher than that formed in the carbon arc lamp. Similarly the carbohydrate contents of the leaves exposed to the arc lamp are higher than those of the leaves exposed to the daylight lamp. The carbohydrates formed in leaves exposed to the daylight lamp are significantly higher than the carbohydrates formed under the electric lamp and so on. As the total intensities are kept the same and as the main differences in the energy distribution in the different parts of the spectrum are mainly in the blue-violet region the only conclusion that can be drawn is that the blue-violet region must be playing an important part in the photosynthetic process and for normal photosynthetic activity both the red and blue-violet regions are equally important. If any one of the two regions is either absent or is of a very low intensity the normal photosynthetic activity does not proceed. (*Dastur and Mehta, in course of publication in the Annals of Botany.*)

Photosynthesis in the Red and Blue-Violet Lights

In order to put to test these conclusions, it was undertaken to measure the rate of photosynthesis in leaves exposed to monochromatic red and blue lights and to white light of equal intensities in the three cases. It was not found possible to use an artificial source of light for obtaining large beams of monochromatic red and blue-violet lights of sufficiently large intensity in order that light intensity may not act as a limiting factor. So the experiments had to be conducted in open sunlight. Fortunately this was possible during the dry months of the year. For obtaining monochromatic lights solution filters had to be used. After several failures a solution of carmine in lithium carbonate one centimeter thick was used for the red light and ammoniacal solution of copper sulphate 1 cm. thick was used for the blue-violet light. The range of transmission of the red filter was 7,000 to 6,200 A° and that of the violet filter was 4,720 to 4,000 A°. The spectrum photographs of the filters showed no transmission in any other part of the spectra. The percentage transmission of the different wavelengths in the transmitted red and blue-violet regions were determined. The maximum transmission in the red region was 46·77 per cent. at 6,800 A° and in the blue-violet filters was 23·9 per cent at 4,200 A°. By determining the total percentage transmission of the two filters it was possible to make the total intensities of the two rays equal. As the micro-thermopile is not equally sensitive to the red and blue rays, an indirect and complicated method was employed to make the intensities equal. The intensities of the red light and white sunlight were reduced and made equal to that of the blue-violet light by interposing glass plates.

The glass plates used do not interfere with the transmitted red region or the white light except lowering the total intensities. This is verified spectrometrically. The results obtained with the three lights show that the formation of carbohydrates is highest in leaves exposed to sunlight, medium in red light of equal intensity and least in blue-violet light of the same intensity. The differences in the quantities of total carbohydrates formed in the red, blue and white lights of equal intensities are statistically significant. (*Dastur and Mehta, in course of publication in the Annals of Botany.*)

If the results obtained with red and blue-violet lights are alone compared, they will appear in agreement with those obtained by previous workers. But if the results obtained with the white light are taken into consideration, they support the conclusion that the efficiency of the photosynthetic mechanism is highest in the full visible spectrum of light than in the monochromatic red and blue-violet lights. The importance of blue-violet region in the process is again proved by these experiments.

In view of the results obtained the question of the energetics of photosynthesis acquires fresh aspects. It appears the energy carried by the different radiations is not the only determining factor in the rate of the process. If that was the case there should not have been marked and significant differences in the carbohydrate contents of the leaves exposed to sunlight, arc lamp, daylight lamp, electric lamp and also the sunlight and the red and blue-violet lights, all of equal intensities. Therefore the differences found could only be attributed to the differences in the distribution of spectral energy. The blue-violet rays are as important in the process as the red rays and so the frequency of the different rays is another determining factor. It is probable that for the different stages in the process all rays of different wavelengths are used as it is likely that for the activation of the different reacting molecules the rays of different frequencies may be essential.

In the case of *Helianthus annuus,* L., calculations show that the volumes of carbon dioxide that must have been decomposed in the white, red and blue-violet lights are 89, 22 and 7 cc. respectively. In case of *Raphanus sativus,* L. the volume are 60, 32 and 9 cc. It is apparent that with the same amount of radiant energy supplied the efficiency of the photosynthetic mechanism in the red light is nearly three times that of the blue-violet light, while in the white light it is nearly four times its efficiency in the red light in *Helianthus annuus,* L. and is nearly double in *Raphanus sativus,* L. Thus the number of light quanta necessary for transforming a molecule of carbon dioxide increases in the white, red and blue lights as the utilisation factor decreases in the same order. Warburg and Negelein (1923) have estimated that the number of quanta absorbed per molecule of carbon dioxide reduced, should be four or five and they should remain constant at four for all wavelengths in the visible spectrum. The results here show that that is not the case unless if it be assumed that the energy absorbed by the leaves is highest in the white light, medium in the red light and the least in the blue lights. But this assumption is not borne out by the results obtained with the different sources of light enumerated above.

Protein Synthesis in Lights from Different Sources

An interesting point arises from the results of carbohydrates formed in electric light and in sunlight. It is very likely that the lesser amounts of carbohydrates formed in leaves exposed to the electric lamp may be due to their rapid utilisation in protein synthesis, while in leaves illuminated by sunlight the formation of proteins may be taking place slowly leading to the accumulation of carbohydrates. If this supposition is found to be true it would again raise fresh issues on the effects of lights on protein synthesis. So far light is known to play an indirect rôle in the synthesis of proteins in as much as it is instrumental in the synthesis of the carbohydrates which are needed for the construction of proteins. Zaleski (1897-1901), Zaleski and Tutorski (1912), Stoklasa (1916), Muencher (1923), Pearsall and Loose (1933) have shown that the synthesis of proteins occurs four times as rapidly in sunlight as it occurs in absence of light. If that is the case, the lights of different spectral intensities may also bring about the differences in the rates of the synthesis of proteins. It was therefore necessary to determine the protein content of leaves of the same species exposed to sunlight, arc light, daylight lamp and electric lamp as were done in the case of carbohydrates. If the rate of photosynthesis is really depressed as the source of light is progressively poorer in the blue-violet rays, results of the total protein content of the leaves should also decrease in the same order.

The technique of experimentation employed is the same as before. The methods of extraction and determinations of protein nitrogen in different forms are carefully worked out and used. The leaves and petioles of plants exposed to these different lights of equal intensities are analysed for water-soluble protein nitrogen, polypeptide nitrogen, diamino nitrogen, monoamino nitrogen, amido nitrogen, acetone-soluble protein nitrogen, alcohol-soluble protein nitrogen, insoluble residual nitrogen and total nitrogen. The plants used were the same as before, *viz., Ricinus communis,* L. *Helianthus annuus,* L. and *Abutilon asiaticum,* G. Don. Results obtained are particularly striking and interesting in many ways. In the leaves water-soluble protein and polypeptides and diamino acids are definitely produced during the exposure, while monoamino acids and amides do not show any increase after exposure. It may be that amides and monoamino acids are rapidly converted into soluble proteins and polypeptides just as hexoses are supposed to be rapidly converted into starch. The results of the total protein contents of the leaves are in the order daylight bulb, arc light, electric light and sunlight, while the carbohydrate contents of the exposed leaves stand in the order sunlight, arc light, daylight bulb and electric lamp. If the carbohydrates supplied the basic materials for the formations of the proteins, the leaves exposed to sunlight should contain the largest quantity of proteins than those exposed to any of the three sources of light. If the supply of carbohydrates is not acting as a limiting factor the protein contents of the leaves should be equal in all the four lights. Why should there be an increased production of protein in the daylight bulb and arc lamp as compared to sunlight when the formation of carbohydrate is most rapid in the latter? It is evident that very small part of carbohydrates formed in the leaves by photosynthesis is utilised for protein synthesis as the protein content of the leaves exposed to electric light is slightly more than that of the leaves in sunlight, though the carbohydrates formed in the former is less than one half the amount formed in the latter. (*Dastur and Kanitkar, in course of publication*).

Conclusions

It is difficult to explain the differences in the synthesis of proteins in leaves exposed to different lights and still much more difficult it is to understand the low protein nitrogen contents of the leaves in sunlight where the photosynthetic activity is the highest. It may be possible that the machinery of the cells is clogged up owing to the very rapid production and consequent accumulation of the products of photosynthesis and hence the rate of synthesis of protein is depressed. In the case of electric light the protein synthesis goes on normally but its rate is inhibited by the depressed rate of photosynthesis, while in the daylight bulb and under arc lamp there is an increased production of proteins due to the increased rates of photosynthesis which however are not rapid enough to clog up the active cells. It is, however, difficult to understand why the protein synthetic activity should be inhibited in sunlight and the photosynthetic activity should continue unabated even after the cells are full of starch. It is also premature to say that the synthesis of protein is influenced by the differences in the spectral intensities like the synthesis of carbohydrates shown above.

It thus appears that the rays of different wavelengths of the visible part of the spectrum of white light are as important for the two fundamental constructive processes in plants as their energy contents. These results have added to our difficulties in understanding the energetics of the mechanism by showing that the respective intensities of the different radiations of white light are as important as their total energy contents. If the above findings are true, the synthetic processes of the plants are greatly influenced by variations in light intensities that normally occur during the day and season of the year. The growth of plants is the net result of the constructive and destructive processes and if the constructive processes are so affected by the diurnal or seasonal changes in the quality and the quantity of light, it is no wonder that the reproductive activities of the crop plants show such wide variations.

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