EFFECT OF CHANGES IN CHLOROPHYLL CONCENTRATION ON PHOTOSYNTHETIC PROPERTIES

I. FLUORESCENCE AND ABSORPTION OF GREENING BEAN LEAVES

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(Received February 2nd, 1961)

SUMMARY

In order to obtain new information about the way of functioning of chlorophyll *in vivo* a study was made of optical properties and photosynthesis under condition of a low chlorophyll content in the leave. It was found that the fluorescence yield of greening bean leaves decreased from a value approximating the yield of chlorophyll in organic solution to that of chlorophyll measured in a green leave.

No transfer of absorbed light energy from carotenoids to chlorophyll *a* was found immediately after transformation of protochlorophyll. In the early stages of greening a relatively inefficient energy transfer developed.

The light intensity at which fluorescence induction phenomena appear was studied and found to decrease during greening.

Absorption measurements performed during greening showed the appearance of several shifts of absorption maxima thus confirming, at least partly, earlier measurements of SHIBATA. The effect of high light intensity and of freezing on the different chlorophyll forms was investigated.

INTRODUCTION

In the chloroplast the chlorophyll concentration is high. Also the chloroplast is not homogeneously pigmented, most probably the photosynthetic pigments are concentrated in a small fraction of its volume in rather thin layers[1]. Under the electron microscope layered systems can be seen. If we select one of these systems, the amount of chlorophyll in a chloroplast is in the right order of magnitude to cover the total integrated surface[2,3]. The pigment concentration in the photosynthetic apparatus proper thus may approach that concentration present in a crystal.

In chloroplasts the existence of crystalline properties has been shown by measurements of thermoluminescence[4], photoconductivity[5], paramagnetic electron spin resonance[6] and luminescence at liquid nitrogen temperatures[7]. As suggested by several authors, the existance of crystalline properties may be an essential prerequisite in photosynthesis[4,8,9].

The study of photosynthetic properties under condition of a much lower chlorophyll concentration in the chloroplast may provide evidence for, or against this hypothesis.

If all protochlorophyll present in an etiolated leave is transformed by light the chlorophyll concentration is approx. 1 % or less than the concentration in a fully green leave. As number and volume of chloroplasts are assumed to change relatively little in the period needed for greening, such a low concentration could be visualized schematically as follows:

1. The individual pigment molecules are situated about 10 times further apart than in the green state. With the formation of new molecules the distances between the pigment molecules decrease gradually during greening.

2. The individual pigment molecules are locally as close together as in the green state and, consequently, are grouped in "units". While greening, the number of these "units" increases but the "local" concentration of chlorophyll remains constant.

Of course, also a combination of 1 and 2 may exist.

If 2 holds it seems likely that a high "local" pigment density is essential in photosynthesis. If only the number of "units" increases we will expect that photosynthesis is a linear function of the chlorophyll content of the whole chloroplast, both in the light limited state (at low light intensities) and in the light saturated state (at high light intensities). If 1 holds, the "local" pigment density after illumination of an etiolated leave and before new protochlorophyll has formed (further called "post-etiolated state") is far removed from that in a crystalline structure. Thus, if such a structure were essential, photosynthesis could only occur after a considerable degree of greening.

Measurement of photosynthetic light curves during greening may enable to decide whether the presence of a very high pigment density is essential in the primary steps in photosynthesis. Also, if 1 holds, the gradually decreasing distance between pigment molecules will result in an increase in their mutual interaction. This may show up in a change in chlorophyll fluorescence yield, fluorescence polarisation, photostability, energy transfer from auxiliary pigments to chlorophyll *a* and location of absorption maxima. In this paper we will present some results of measurements on different aspects of fluorescence and absorption in greening leaves.

METHODS

Absorption spectra were determined in a Beckmann DK2 recording spectrophotometer. Spectra of intact leaves were obtained by the use of the opal glass method of SHIBATA[12]. The fluorescence yield of greening leaves was determined by comparing the fluorescence of the leave with that of a standard. For this standard a solution of chlorophyll *a* in methanol was taken. As allomerisation takes place in methanol resulting in a lower fluorescence yield, fresh chlorophyll solutions were prepared for each set of experiments.

Leaves and chlorophyll *a* solutions were illuminated successively with "monochromatic" light. A wavelength of 615 mμ was selected by a combination of a G.A.B. interference filter ($\lambda_{1/2}$ = 12 mμ), a 4 % copper sulfate filter (2 cm) and 2 Schott IR filters. This wavelength was chosen to avoid complications due to light absorbed by carotenoids. Also at this wavelength geometrical effects, such as flattening[10], increase of optical path due to scattering[11] and inhomogeneity of the leave influence the absorp-

tion measurements much less than at the wavelength of maximum chlorophyll absorption in the red, while absorption of residual protochlorophyll not transformed by light[12] is small. In order to minimize differences due to different reabsorption of fluorescent light, the absorption of the standard solution of chlorophyll *a* in methanol (cuvette, 1 mm optical path) was approximately equalized to that of the leave.

Fluorescence was detected by an infrared sensitive RCA 7102 multiplier in combination with a Schott 2 mm RG5 red filter. With chloroplast suspensions the absorption in the chlorophyll maximum was equalized to 60 % for all suspensions by dilution. Immediately after each fluorescence determination the absorption spectrum was recorded.

Chlorophyll concentration in the leave was determined by extraction with methanol (5 ml) after the leave surface had been measured. With leaves in the "post-etiolated" state a marked chlorophyll destruction occurred during extraction. In this case the absorption was taken from direct measurement with leaves. Measurements of leave absorption and absorption of extracted chlorophyll agreed fairly well in the half-green state at 615 mμ.

A correction was applied for the different amount of fluorescence absorbed by the Rg 5 filter, resulting from a slightly different location of absorption and fluorescence bands *in vivo* and *in vitro*.

Chloroplast suspensions were prepared by grinding the leaves in a 0.02 *M* phosphate buffer (pH 6.8) in the cold and in the dark. Chloroplasts and leave fragments thereoff were isolated by differential centrifugation.

RESULTS

Fluorescence

The fluorescence yield of chlorophyll in bean leaves in the "post-etiolated" state was found to be of the same order of magnitude as that of chlorophyll *a* dissolved in methanol. For ten different experiments the value of fluorescence yield relative to the yield of chlorophyll *a* ranged from 0.6 to 1.2. During greening the yield decreases to

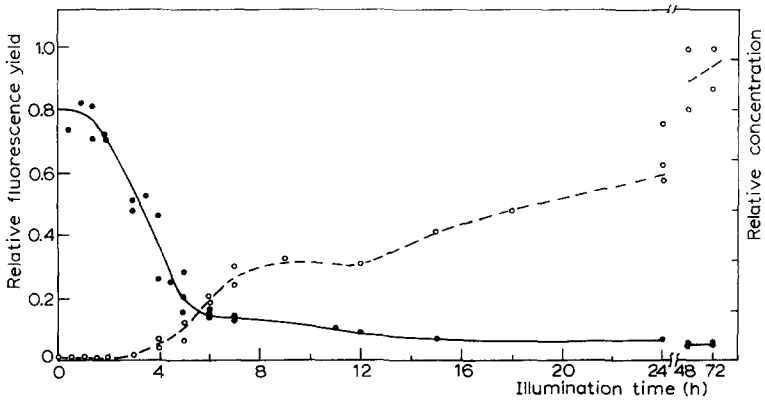

Fig. 1. Fluorescence yield and chlorophyll concentration in young greening bean leaves. Chlorophyll concentration is presented as a multiple of the concentration in the "post-etiolated" state. Chlorophyll fluorescence yield is given relative to the fluorescence yield of chlorophyll *a* in methanol (10^{-6} *M*). Wavelength of incident light is 615 mμ.

about 0.1 of the value in methanol. In Fig. 1 relative fluorescence yield and chlorophyll concentration are given as a function of time of greening in an experiment with "young" bean leaves (7 days after planting the soaked seed and keeping it at a temperature of 22°). This figure shows that chlorophyll concentration remained approximately constant during 2.5 h after first illumination. Then rapid chlorophyll formation begins, concurrently with a steep decline of the fluorescence yield. Illumination of the upper side of the leave yielded a fluorescence of about 20 % higher than that measured at irradiation of the lower side. This phenomenon can be explained by differences in reflection and scattering for both sides.

A decline of fluorescence yield during greening could also be observed with chloroplast preparations. However, only with chloroplasts which were at least light green reliable measurements could be obtained. Suspensions prepared from leaves in the "post-etiolated" state or shortly thereafter were unstable (chlorophyll bleached) at room temperatures and even in the dark. Their stability increases during greening. With extracts of green leaves no measurable change in chlorophyll absorption occurred. The stability of preparations from "post-etiolated" leaves was markedly increased if these leaves had been stored 24 h in the dark.

With greening corn leaves also a relative fluorescence yield of about unity was found for leaves in the "post-etiolated" state, the yield decreasing with increasing chlorophyll concentration.

The change of fluorescence intensity with time after etiolated leaves are brought into light was measured with both monochromatic and white incandescent light. In Fig. 2a the fluorescence time course is shown upon irradiation with light of 615 mμ (intensity 10^3 ergs/cm²sec). In Fig. 2b the results obtained with white light of two different intensities are given. Of a second leave of the same bean plant the absorption spectrum was measured after 5, 10 and 15 min illumination. These absorption measurements showed, that a shift of the absorption maximum from 681 to 670 mμ occurred within this time, while the fluorescence intensity did not change

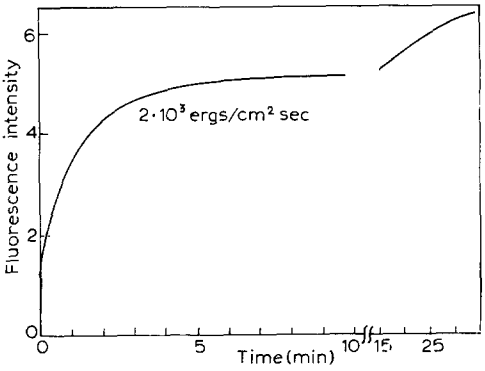

Fig. 2a.

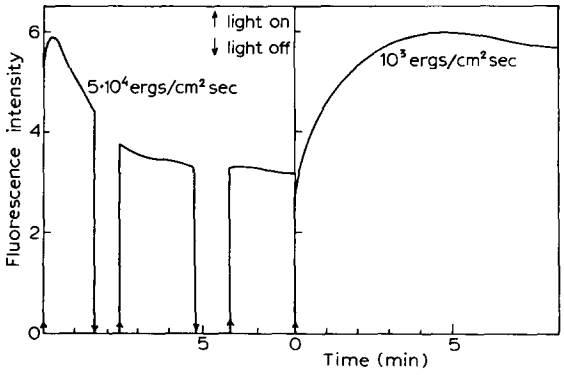

Fig. 2b.

Fig. 2. Fluorescence intensity time curve after illumination of etiolated bean leaves: a, with monochromatic incident light (615 mμ); b, with yellow incident light (570–690 mμ) of two different intensities. Interruption of illumination does not affect decrease in fluorescence.

markedly. The measurements also indicated that the strong decrease in fluorescence shown in Fig. 2b, which decrease continues in the dark when illumination is interrupted, is, at least partly, due to photobleaching.

Energy transfer from carotenoids to chlorophyll was investigated by measuring the fluorescence yield at several wavelengths at various stages of greening. All values are taken relative to that at 615 mμ. At this wavelength absorption is mainly due to chlorophyll in both the green and the "post-etiolated" state. Fig. 3 shows that the absorption in the carotenoid region does not increase markedly during the first stages of greening while the chlorophyll absorption in the red increases considerably. In the "post-etiolated" state the ratio of absorption at 485 mμ (mainly due to carotenoids) to that at 670 mμ (due to chlorophyll *a*) is about 10, while in the green plant this ratio is about 1. Moreover in the green plant the absorption at 485 mμ is partly due to chlorophyll *b*, which pigment is absent in the "post-etiolated" state. This implies that the difference in ratio is even greater. The fluorescence data of Table II indicate that there is no measurable energy transfer between carotenoids and chlorophyll in the post-etiolated state. If the chlorophyll content increases, a transfer from pigments other than chlorophyll *a* is developed.

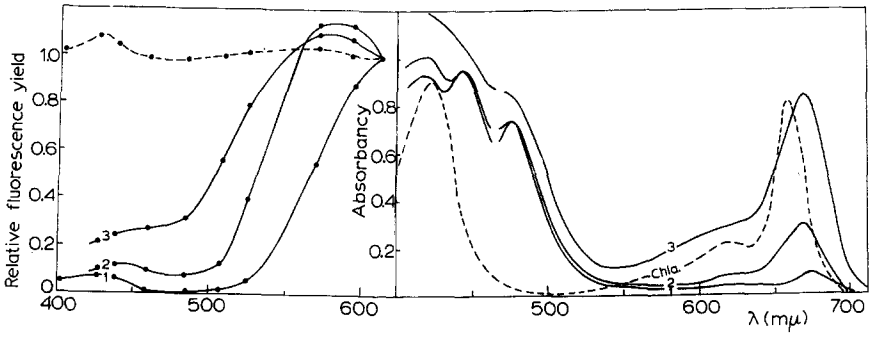

Fig. 3. Energy transfer to long wavelength chlorophyll *a* band. Fluorescence yield spectra are presented for bean leaves at various stages of greening and for chlorophyll *a* in methanol. The spectrum for chlorophyll *a* shows that the yield is unity over the whole spectral region considered, indicating that the quantum yield of fluorescence is independent of wavelength. The spectra for bean leaves show, that in the "post-etiolated" state light absorbed by carotenoids (overlapping the blue chlorophyll spectrum) is not used for chlorophyll fluorescence. While greening the effectiveness of blue light increases. The value at 615 mμ is taken as unity. Points are measured with interference filters ($\lambda_{1/2}$ about 10 mμ). The absorption spectra of the leaves indicate, that in 7-h greening the absorption at 670 mμ increases 900%, while the absorption at 480 mμ increases only 15%.

The minimum intensity at which fluorescence induction phenomena appear also was studied as a function of chlorophyll content in bean leaves. In these experiments the leaves were placed in a cuvette which could be flushed with air enriched by 5% CO_2. The induction phenomena are rather complex and, especially with respect to the first induction spike, not always reproducible. Slight induction phenomena appear sometimes 5 min after protochlorophyll transformation, but they occur only if very high light intensities are used ($3 \cdot 10^5$ ergs/cm^{2}sec). More often no trace of induction is visible. While greening continues, the complete fluorescence induction system appears at gradually lower light intensities. In Fig. 4 a characteristic set of induction curves is given obtained after various periods of greening.

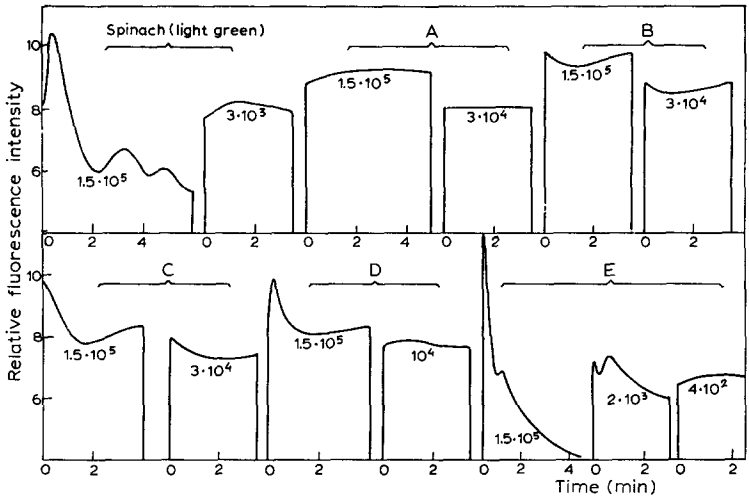

Fig. 4. Fluorescence induction curves of bean leaves at different stages of greening and of spinach leaves. The curves show that the lowest light intensity at which induction phenomena appear decreases markedly during greening. Leave A received 30 min light, no induction is visible at an incident light intensity of $3 \cdot 10^4$ ergs/cm^2 sec, while at $1.5 \cdot 10^5$ ergs/cm^2 sec (highest intensity used in this experiment) a slight induction appears. Leaves B, C, D, and E were used after an illumination of 3.5, 4.5, 6 and 40 h. Induction phenomena are visible at intensities exceeding $3 \cdot 10^4$, $3 \cdot 10^4$, $7 \cdot 10^3$ and $4 \cdot 10^2$ ergs/cm^2 sec respectively. Leaves were flushed by air enriched with 5% CO_2.

Absorption

According to SHIBATA[12] illumination of an etiolated bean leave results in the appearance of a chlorophyll *a* form with a maximum at 684 mμ. After some time in the dark, a shift to 673 mμ occurs. In general we could confirm this behaviour, not only with bean leaves, but also with corn, oat and crocus leaves. The mentioned shift depended strongly on age and condition of the leave. In bean leaves used 7 days after planting the soaked seeds (in vermiculite, temperature 22°) the shift—from 682 to 670 mμ as measured by us—took place at 20° in about 10 min. In old leaves, used 15 to 20 days after planting, the shift either took 1 h to complete or did not take place at all. In very young leaves, used as soon as sufficient protochlorophyll was present to allow measurements, the location of the peaks was at 670 mμ either nearly instantaneously or within 5 min.

Besides an influence of age also an influence of temperature was noticeable. At 4° the time needed to complete the shift was at least 4 times that at 20°. After storage at —6°—the leaves were frozen at this temperature—the maximum is located at 668 mμ at once and irrespective of the age of the leave. In such frozen and thawed leaves the protochlorophyll band is narrowed, while the maximum is shifted from 645 to 630 mμ. Confirming SMITH AND BENITEZ[13] practically no transformation to chlorophyll *a* was found to occur in those leaves after thawing.

In several cases also an effect of wavelength of light used for protochlorophyll transformation could be observed. Blue light (437 or 485 mμ) appreciably accelerated the 682–670 mμ shift, whereas red light (645 mμ) had the same effect as white incandescent light. The effect, however, was not always reproducible.

Formation of new protochlorophyll seemed to be somehow related with the shift

from 682 to 670 mμ. Such a production was detected spectroscopically by a decrease in protochlorophyll absorption and an increase in chlorophyll absorption after a second illumination. As long as the chlorophyll maximum was situated at 682 mμ—as it occurred for a prolonged time in old leaves and at low temperatures—no formation of protochlorophyll was observed. If the mentioned shift occurred in a very short time, protochlorophyll formation sometimes proceeded immediately and greening was a linear function of time from the point of first illumination. Usually, a time lag occurred between the first illumination and appearance of new protochlorophyll. With young leaves, this time lag was approx. 2 h (*cf.* Fig. 1), with old leaves 10–24 h.

TABLE I

CORRELATION OF FLUORESCENCE AND ABSORPTION IN GREENING BEAN LEAVES

Chlorophyll concentrations are given as a multiple of the concentration in the "post-etiolated" state. Chloroplast preparations are diluted to give an equal absorption in the chlorophyll maximum. Fluorescence yield values are calculated by making use of a value of 0.30 for the fluorescence yield of chlorophyll *a* in methanol.

Relative chlorophyll concentration	*Fluorescence yield Leaves*	*Fluorescence yield Chloroplasts*	*Location of red absorption maximum*	*Minimum light intensity (ergs/cm²sec) for obtaining fluorescence induction*
1	0.28		670	$1.5 \cdot 10^5$
3.5	0.09	0.08	671	$1.5 \cdot 10^4$
10	0.04	0.05	673	$2 \cdot 10^3$
20	0.02			
50	0.02	0.03	677	$4 \cdot 10^2$

SHIBATA[12] mentions the occurrence of a second absorption shift from 673 to 677 mμ after prolonged storage in the dark (106 min). Usually, however, such a shift could not be observed in our experiments. After several hours storage in the dark the chlorophyll maximum still was at 670 mμ. If the leaves were illuminated after protochlorophyll had been formed, a shift occurred due to the formation of the 681 mμ form, but after 10 min in the dark the maximum again was at 670 mμ. Only after further accumulation of chlorophyll the maximum shifted gradually to 677 mμ (681 mμ in chloroplast suspensions (*cf.* Table I)). In a few cases, however, the absorption band became asymmetric already at low chlorophyll concentrations.

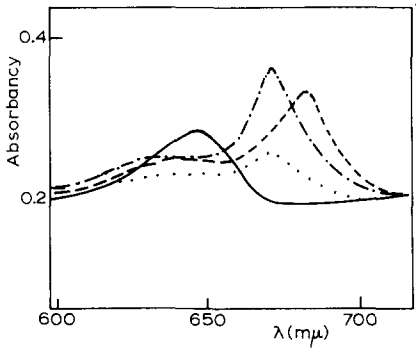

Fig. 5. Effect of high intensity illumination ($1.5 \cdot 10^5$ ergs/cm² sec, wavelength 570–690 mμ) on the red absorption band of chlorophyll in bean leaves at the "post-etiolated" state. ——, Protochlorophyll absorption; – – – –, spectrum after 20 sec illumination; · · · · ·, spectrum after 10 min illumination; · – · –, spectrum after 20 sec illumination followed by a 35-min dark period.

Photobleaching of chlorophyll in the leave was strong in the first minutes after its transformation from protochlorophyll. Both the 682- and the 670-mμ form were labile, but the 670-mμ form usually became photostable within 15 min. In Fig. 5 the effect of high light intensity on etiolated bean leaves is given. This picture shows that both the 682- and the 670-mμ form disappear after prolonged illumination. The change of the 682-mμ form might be due to its change into the 670-mμ form. In old leaves however, the disappearance of the 682-mμ form occurs without the appearance of a 670-mμ band. The quantum efficiency for irreversible bleaching was of the same order of magnitude as that for chlorophyll *a* in methanol. Chlorophyll absorption can be decreased 2.5 fold by photobleaching without the occurrence of a noticeable change in carotenoid absorption. Therefore, photobleaching of carotenoids and chlorophyll seems to be unrelated.

DISCUSSION

Fluorescence and absorption measurements show that, during greening, these phenomena are subject to rather complex changes. The observed decrease in fluorescence yield during greening can be brought about by different mechanisms, such as; (a) Change in the molecular structure of the chlorophyll molecule. (b) Change in the state of chlorophyll, *e.g.* due to a different protein binding, complex formation or change in chloroplast structure. (c) "Chemical" quenching of fluorescence, due to a flow of the major part of the excitation energy into the photosynthetic processes or to quenching photosynthetic products. (d) "Physical" quenching of fluorescence, *i.e.* concentration quenching.

No change in the absorption spectrum or fluorescence yield of the extracted pigment was found during greening. Hence, (a) is improbable. No change in fluorescence yield was found when chlorophyll *a* changed from its 682-mμ form to its 670-mμ form. A change in state of chlorophyll, resulting in this shift, thus is not responsible for the drop in fluorescence yield. As Fig. 1 shows, a slow decrease in fluorescence yield occurs in the period of stationary chlorophyll concentration for about 2 h after the first illumination. As soon as chlorophyll production as a consequence of initiated protochlorophyll formation sets on, this drop in yield is accelerated. This may suggest that as soon as new protochlorophyll is formed the state of the chlorophyll already present changes from a fluorescent to a non-fluorescent one. The decrease in yield then could be associated with a change in state of chlorophyll, due *e.g.* to some structural change.

In this respect the following remarks should be made. In several experiments the usual time lag for chlorophyll formation (*cf.* Fig. 1) did not appear. Here greening proceeded immediately after the leaves had been placed in light. With such leaves polarographic measurements showed that substantial oxygen production could be measured within 0.5 h after protochlorophyll transformation, provided very high intensities of white light (10^6 ergs/cm^{2}sec) are used, while usually no photosynthetic activity could be measured within the first few hours after illumination started. Most probably this behaviour divergent from the usual one should be sought in the pretreatment of the beans. The beans were soaked and sometimes germinated before planting. If roots of the beans had appeared with a length of about 1 cm and these roots had received some light, (not resulting in any chlorophyll appearance) new protochlorophyll was formed within 0.5 h after transformation of protochlorophyll present

in the dark grown leave. If the beans had been handled in complete darkness, no formation of new protochlorophyll could be observed after illumination for several hours. Both greening and appearance of photosynthetic activity thus seem to be influenced by light absorption during germination.

It may also be remarked that in some experiments a decrease in fluorescence occurs immediately after protochlorophyll conversion if high illumination intensities are used (Fig. 2b). This decrease continues in the dark if illumination is interrupted. Although, as mentioned before, part of this decrease will be due to photobleaching, the amount of chlorophyll lost is too small to account for the total loss of fluorescence, while it seems less likely that photobleaching proceeds in the dark. Part of the decrease in fluorescence thus may well be caused by a change in state of chlorophyll.

In organic solutions besides concentration quenching of fluorescence also concentration depolarisation occurs[1,15]. With chloroplast suspensions prepared from greening bean leaves a decrease in chlorophyll fluorescence polarisation from $p = 0.14$ to $p = 0.03$ was found[16]. Thus both a decrease in fluorescence yield and a decrease in polarisation with increasing chlorophyll concentration may be found in chloroplasts as well. Hence the decrease in fluorescence yield may also be due to mechanism (d). Of the 4 mentioned possibilities only mechanism (a) thus can be excluded. Possibly the other mechanisms occur simultaneously, but this cannot be decided as yet.

The quantum yield of fluorescence of chlorophyll *a* dissolved in methanol at low concentration is approximately 30% (see refs. 17, 18). This indicates that in the "post-etiolated" state an appreciable fraction of the absorbed light energy is emitted as fluorescence.

Concentration quenching and depolarisation are indicators of an energy transfer between neighbouring pigment molecules. The absence of any measurable energy transfer between carotenoids and chlorophyll in the "post-etiolated" state as demonstrated in Fig. 3 may indicate that most of the carotenoids in this state are not located closely to chlorophyll molecules. A definite, but low percentage energy transfer from pigments other than chlorophyll *a* was measured about 1 h after illumination started when little or no additional chlorophyll *a* had been formed and fluorescence still was high (Table II, *cf.* also BUTLER[21]). As the chloroplast structure is known to change from a granular type to a lamellar type during greening[19] an increase in pigment interaction could also be established by a spatial reorganisation of the available pigment.

TABLE II

RATIO OF CHLOROPHYLL FLUORESCENCE EXCITED BY BLUE LIGHT (CAROTENOID AND BLUE CHLOROPHYLL ABSORPTION) TO FLUORESCENCE EXCITED BY LIGHT ABSORBED IN THE RED CHLOROPHYLL ABSORPTION BAND (614 mμ)

The incident intensities are reduced to an equal number of light quanta. Values are presented for leaves after various periods of greening and for chlorophyll *a*.

	10 min	*1 h*	*4 h*	*8 h*	*Chlorophyll a (in methanol)*
424 mμ/614 mμ	1.15	1.40	1.33	1.37	3.5
437 mμ/614 mμ	1.12	1.12	1.25	1.35	2.95
458 mμ/614 mμ	0.21	0.32	0.70	0.89	0.51
483 mμ/614 mμ	0.09	0.15	0.54	0.78	0.19
511 mμ/614 mμ	0.13	0.18	0.51	0.57	0.22

A third possibility is that energy transfer from carotenoids to chlorophyll *a* proceeds in these bean leaves only via chlorophyll *b* (*cf.* DUYSENS[20]). This pigment being absent in the "post-etiolated" state[22], the absence of energy transfer could be ascribed to the lack of chlorophyll *b* immediately after protochlorophyll transformation.

The age and temperature dependent shift of the chlorophyll absorption maximum from 682 to 670 mμ most likely is due to a change in some pigment–protein linkage. This is indicated by the observation that denaturation of the protein by freezing and subsequent thawing results in an immediate shift to 668 mμ in all cases. Also the position of the red absorption maximum of the extracted pigment in methanol is identical, irrespective of the position of this band *in vivo*. The change from a photolabile to a photostabile state of the 670-mμ band, without the appearance of a measurable absorption change, indicates that other dark reactions are required before light energy is used effectively in photosynthesis. Measurements with an oxygen polarograph showed that substantial oxygen production occurred with the chlorophyll maximum in the 670-mμ position. The second measured absorption shift from 670 to 678 mμ occurring when the chlorophyll concentration increases appreciably, might be ascribed either to another change in pigment-protein linkage or to an increase in mutual interaction of the pigment molecules (in chlorophyllide crystals the absorption maximum is located at 735 mμ (see ref. 23)). With leaves of green plants in general the red chlorophyll maximum is situated around 678 mμ *in vivo*, but a complex structure of this red chlorophyll band has been demonstrated[24,25] indicating the presence of several forms of chlorophyll *a*. No shift of the red absorption maximum upon freezing and subsequent thawing in green bean leaves could be demonstrated. This indicates that the 678-mμ absorption maximum is not due to the 682-mμ chlorophyll *a* form in the "post-etiolated" state.

Fluorescence and absorption changes considered are in agreement with conception (1) as stated in INTRODUCTION. With such a mechanism the various changes can be explained by assuming the presence of a constant number of protochlorophyll-generating centres. The 682–670-mμ absorption shift then might represent the first step to enable a centre to resynthesize protochlorophyll. Evidence for such a mechanism might also be seen in the observation that no more protochlorophyll is formed in any dark period than the amount of protochlorophyll initially present in the dark (although the rate of formation may vary appreciably). The onset of fluorescence induction phenomena—which in green plants usually is seen when light saturation of photosynthesis is approached—at gradual lower light intensities during greening also fits into this picture.

Measurement of photosynthetic light curves during the process of greening may indicate whether or not effective photosynthesis is coupled with a high pigment density.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the animated discussions during his sojourn at the Carnegy Institution of Washington Department of Plant Biology Stanford with Dr. J. H. C. SMITH, which initiated these experiments with greening leaves.

He also wishes to thank W. H. VAN DEN DOOL for his cooperation in several experiments.

REFERENCES

1 J. C. GOEDHEER, *Thesis*, Utrecht, 1957.
2 J. B. THOMAS, K. MINNAERT AND P. F. ELBERS, *Acta Botan. Neerl.*, 5 (1956) 314.
3 J. J. WOLKEN AND F. A. SCHWERZ, *J. Gen. Physiol.*, 37 (1953) 111.
4 W. ARNOLD AND H. SHERWOOD, *J. Phys. Chem.*, 63 (1959) 1314.
5 W. ARNOLD AND H. K. MACLAY, *Brookhaven Symposium: the Photochemical Apparatus*, 1958.
6 B. COMMONER, J. J. HEISE AND J. TOWNSEND, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 710.
7 G. TOLLIN AND M. CALVIN, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 895.
8 E. KATZ, *Photosynthesis in Plants*, Iowa State College Press, 1949.
9 M. CALVIN, *Rev. Modern Phys.*, 31 (1959) 147.
10 L. M. N. DUYSENS, *Biochim. Biophys. Acta*, 19 (1955) 1.
11 W. L. BUTLER AND K. H. NORRIS, *Arch. Biochem. Biophys.*, 87 (1960) 31.
12 K. SHIBATA, *J. Biochem. (Tokyo)*, 44 (1957) 147.
13 J. H. C. SMITH AND A. BENITEZ, *Plant Physiol.*, 29 (1954) 135.
14 H. I. VIRGIN, *Physiol. Plantarum*, 11 (1958) 347.
15 T. FÖRSTER, *Fluoreszenz Organischer Verbindungen*, Vandenhoeck Ruprecht, Göttingen, 1951.
16 J. C. GOEDHEER AND J. H. C. SMITH, *Carnegie Inst. Wash.*, Yearbook, 58.
17 L. S. FORSTER AND R. LIVINGSTON, *J. Chem. Phys.*, 20 (1952) 1315.
18 P. LATIMER, T. T. BANNISTER AND E. RABINOWITCH, *Science*, 124 (1958) 585.
19 D. VON WETTSTEIN, *Brookhaven Symposium: the Photochemical Apparatus*, 1958.
20 L. M. N. DUYSENS, *Thesis*, Utrecht, 1952.
21 W. BUTLER, *Biochem. Biophys. Research Communs.*, 2 (1960) 419.
22 G. BLAAUW-JANSEN, J. G. KOMEN AND J. B. THOMAS, *Biochim. Biophys. Acta*, 5 (1950) 179.
23 E. E. JACOBS, A. E. VATTER AND A. S. HOLT, *Arch. Biochem. Biophys.*, 53 (1954) 228.
24 C. S. FRENCH, J. S. BROWN, M. B. ALLEN AND R. F. ELLIOT, *Carnegie Inst. Wash.*, Yearbook, 58.
25 R. GOVINDJEE, J. B. THOMAS AND E. RABINOWITCH, *Science*, 132 (1960) 421.

Biochim. Biophys. Acta, 51 (1961) 494–504

THE EFFECT OF MONURON ON OXYGEN LIBERATION IN PHOTOSYNTHESIS

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(Received February 9th, 1961)

SUMMARY

Evidence is presented that the herbicidal action of monuron, 3-(*p*-chlorophenyl)-1,1-dimethylurea, is due to the build-up of a phytotoxic substance on the oxygen-liberating pathway in photosynthesis. The rate of production of this substance in Chlorella can be varied by controlling the level of CO_2 in the culture.

INTRODUCTION

The herbicide monuron, 3-(*p*-chlorophenyl)-1,1-dimethylurea[1], has been shown to be a very potent inhibitor of the Hill reaction[2,3]. The site of monuron inhibition was

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** Contribution No. 627.