EARLY STAGES IN THE DEVELOPMENT OF PLASTID FINE STRUCTURE IN RED AND FAR-RED LIGHT

SHIMON KLEIN, Ph.D., G. BRYAN, and LAWRENCE BOGORAD, Ph.D.

From the Department of Botany, The University of Chicago, Chicago, and the Argonne National Laboratory, Argonne, Illinois

ABSTRACT

Developmental changes in fine structure were studied in plastids of etiolated bean leaves during the time required for the protochlorophyllide-chlorophyllide transformation and the following lag phase prior to chlorophyll accumulation. In agreement with some other workers, two distinct stages of change in the fine structure of proplastids were found to occur upon illumination during this period. The first involves a dissociation of the previously fused units in the prolamellar bodies of the proplastids and occurs simultaneously with the protochlorophyllide-chlorophyllide conversion in light of 655 mμ, but not of 682, 700, or 730 mμ. The effect of the red light could not be reversed by a simultaneously supplied stronger far-red irradiation. The energy requirements for these structural changes parallel those for the pigment conversion. During the following step the vesicles which arose from the fused units of the prolamellar body were dispersed in rows through the stroma, and the prolamellar bodies themselves disappeared. For these changes to occur, higher light energies were required and the leaves had to be illuminated for longer periods. A red preillumination seemed to accelerate the development somewhat. The structural changes could be induced by light of 655 mμ, but also, to a lesser degree, of 730 mμ. No measurable additional chlorophyll accumulated during this period. Thus, the structural changes observed were independent of major changes in pigment content.

INTRODUCTION

A vesicular center, or prolamellar body, of frequently crystalline, lattice-like structure is characteristic of proplastids of dark-grown (etiolated) seedlings (7, 17, 23). These proplastids contain protochlorophyllide *a* and protochlorophyll *a*. Upon illumination the protochlorophyllide *a* present is converted to chlorophyll *a* while, during the same interval, the structure of the prolamellar body changes and its tubular elements give rise to vesicles. After some additional time in light, during a lag in chlorophyll accumulation, the vesicles disperse and line up in rows stretching across the plastid; finally, during a period of rapid chlorophyll synthesis, lamellae and stacks of lamellae (grana) appear (5, 22, 24).

According to von Wettstein and Kahn (24), Eriksson *et al.* (5), and Virgin *et al.* (22), all of the progressive changes in plastid fine structure occur only in light. Because the first visible structural change, *i.e.* the transformation of tubes to vesicles, occurs at about the same time, and in response to the same light quality as the conversion of protochlorophyllide to chlorophyllide, it was suggested that the two processes might be functionally re-

lated. However, Mego and Jagendorf (14) could not detect changes in plastid fine structure after illumination with more light energy than was required to convert all the protochlorophyllide present to chlorophyllide. The vesicles are reported to become dispersed through the plastid during a lag period in chlorophyll formation. The length of the lag phase in chlorophyll synthesis appears to be under the control of the red–far-red reversible phytochrome system (15, 21), but the relationship between the phytochrome system and changes in plastid fine structure has not yet been defined. In view of the discrepancies between the observations of von Wettstein's group (5, 22, 24) and those of Mego and Jagendorf (14), and the uncertainties about the relationship between the phytochrome system and developmental changes in plastid fine structure, we investigated the effect of monochromatic light of various wavelengths between 653 mμ and 730 mμ on plastid development, using the large biological spectrograph at the Argonne laboratories (16). In a number of experiments changes in plastid fine structure were investigated during the protochlorophyll-chlorophyll conversion, while in others structural changes were studied during the lag phase in chlorophyll accumulation.

MATERIAL AND METHODS

Red kidney bean seedlings (*Phaseolus vulgaris*) were grown for 10 to 14 days in the dark at 26°C. Two to 3 hours before the beginning of the experiments, the first pair of leaves was detached from the plants, placed between layers of wet filter paper, and transferred at 2 to 5°C. in darkness to the Argonne laboratories. All the handling of the leaves was done under a weak green safelight.

In most of the experiments, the *in vivo* absorption of the etiolated leaves between 550 to 750 mμ was measured in a Cary recording spectrophotometer. A piece of tissue was cut from each leaf and fixed in a 2 per cent $KMnO_4$ solution in the dark for ¾ to 1 hour; the remainder of each leaf was exposed to monochromatic light. Immediately after the irradiation, another small part of the leaf was fixed in the dark and the *in vivo* absorption of the unfixed parts of the leaves measured. The fixed segments were dehydrated in graded alcohols, embedded in Epon, and sectioned with a diamond knife on a Porter-Blum microtome. Occasionally sections were after-stained with uranyl acetate and investigated in either a RCA C3 or a Siemens electron microscope.

The biological spectrograph at Argonne National Laboratory (15), which has a dispersion of 1 A per millimeter was used as the source of monochromatic light (produced by a DC super-high-intensity carbon arc, Peerless Hy-condescent). The intensities were monitored on the basis of measurements taken with an Eppley spectral type thermopile, a Beckman DC breaker-amplifier (Model 14), and a recording potentiometer. The energies were reproducible within a range of 3 per cent. The amperage was adjusted to give a constant thermopile reading.

Blinders were placed at the harmonic filters to block out regions of the spectrum which were not studied.

In all cases mirrors were used to reflect the monochromatic light onto the horizontally places samples. Each leaf was exposed to a range of wavelengths not exceeding 10 A. The intensities at the focal curve were reduced by neutral density filters or increased with Fresnel-type condensers.

RESULTS

Structural Changes During the Period of Protochlorophyll-Chlorophyll Conversion

Leaves of etiolated, 10 to 14-day-old bean seedlings (var. Red Kidney) when grown under the described conditions contain proplastids[1] with a very regular, crystalline prolamellar body which, at this stage of development, is made up of interconnected tubules (12, 23). Also present are a small number of vesicles, either dispersed through the stroma or, more frequently, arranged in a few strands which are usually connected to the prolamellar body (Fig. 1). When starch grains are present they are usually in close proximity to one of the strands.

The detached etiolated leaves were exposed to monochromatic light for 10 to 30 seconds after their protochlorophyll content had been determined spectrophotometrically *in vivo*. After exposure to light, a part of each leaf was fixed immediately in the dark and the pigment content was again determined in the unfixed part of the leaves. Thus, both pigments and plastid structure were measured in the same leaves. The results are summarized in Table I, which gives the percentage of the initial protochlorophyll(ide) converted to chlorophyll and an estimate of the number of vesicular centers in the plastids, the appearance of which had changed during the treatment. The

[1] In this paper, proplastids are defined as plastids without a measurable amount of chlorophyll(ide). Chlorophyll(ide)-containing plastids shall be called chloroplasts, independently of their internal structure (4).

nature of the structural changes, whenever they occurred, was always similar and thus independent of light intensity or wavelength; the changes consisted of a disconnection and partial rearrangement of the previously interconnected tubules in the prolamellar body. Instead of having a regular crystalline appearance, the vesicular body consists now of an irregular mass of single, seemingly intertwisted, elongated vesicles or tubules (Fig. 2). It is essentially the phenomenon described by Kahn and von Wettstein (5, 24) as tube transformation. However, since in our etiolated leaves the units of the prolamellar body of the proplastids were uniformly connected, a transition from tubes to vesicles could not be ascertained. There is no doubt, however, that during short exposures to light the units become disconnected and consequently the prolamellar body loses its crystalline appearance.

The *frequency* of these changes depends upon both the wavelength and the energy supplied and seems to parallel closely the amount of protochlorophyllide converted to chlorophyll by the same treatment (Table I). At 655 mμ, structural changes in none or only a few proplastids in the leaf were observed upon illumination with light sufficient for the conversion of up to 25 per cent of the protochlorophyllide. With increasing light intensity more of the pigment is converted and an increasing number of plastids per leaf show the described structural changes. At this wavelength an energy level between 120 erg/mm²-280 erg/mm² is sufficient to affect the structure of practically all the proplastids and to transform all of the convertible protochlorophyll in the leaves. At 683 mμ, however, the same energy gave less than 25 per cent pigment conversion and had no effect on plastid structure.

In the near infra-red region, at 700 mμ and 730 mμ, the leaves were irradiated at considerably higher energy levels, but even when up to 2500 erg/mm² was given, no effect on either pigment or plastid structure could be observed and the prolamellar bodies remained unchanged. While irradiating the leaves with the 730 mμ beam, a small amount of visible stray light (amounting to ±1 per cent) was found to be present. However, this dose of visible light had no effect on either pigment or plastid structure during these short-term experiments.

In view of the widespread activity of the phytochrome system in morphogenetic phenomena (10),

TABLE I

Changes in Pigments and Plastid Fine Structure After Short Illumination

Per cent of protochlorophyllide converted to chlorophyllide and frequency of structural changes in the plastids after short illumination with red and far-red light of various intensities.

Wave length	Time	Total energy	Proto-chlorophyll	Structural conversion
mμ	*seconds*	*erg/mm²*	*per cent*	
655	25	25	0–25	−−−*
"	10	100	50–70	+−−
"	10	120	50–70	++−
"	10	281	100	+++
"	10	750	100	+++
683	3, 6	225	25	−−−
"	25	210	25	−−−
700	30	1590	0	−−−
730	30	1890	0	−−−
	45	2500	0	−−−
730	45	2500	100	+++
+655‡	15	+620		
"	40	2220	100	+−
" ‡	10	+281		
"	40	2220	100	+++
" ‡	10	+178		
"	40	2220	100	++−
" ‡	10	+178		

* −, no conversion; +, conversion.
‡ Applied simultaneously during the last 15 or 10 seconds of the far-red irradiation.

attempts were made to reverse the effect of the red irradiation with far-red light. In general, red light activates phytochrome by converting it to a far-red absorbing form. A simultaneous or subsequent illumination with far-red light changes the pigment back into the red absorbing form and renders it inactive. Because of the rapid response of the plastid structure to red light, the leaves were exposed simultaneously to red (655 mμ) and far-red (730 mμ) light. They were placed in the path of the 730 mμ beam for 40 to 45 seconds and light of 655 mμ was projected onto the leaves by help of mirrors during the last 10 seconds. (For technical reasons the red light could not be given during the earlier part of the illumination.) The energy of the 730 mμ radiation was kept constant at 2220 erg/mm² in most of the experiments, while the intensity of the 655 mμ light was changed in the various experiments from 120 to 620 erg/mm²,

thus varying the energy ratio of the simultaneously supplied red and far-red light between 1:1 and 1:5. Except for one experiment in which the results were questionable, no indication of an inhibition of the red light effect by far-red was found. In regard to plastid fine structure and, as expected, pigment conversion, the leaves behaved in the presence of the far-red as if they were exposed to red light alone.

Structural Changes During the Lag Phase in Chlorophyll Accumulation

Having confirmed the observation of von Wettstein and coworkers on plastid fine structure changes during the period of protochlorophyll-chlorophyll transformation, we investigated the next step in fine structure development. Exposure of etiolated leaves to light results in, after a certain time lag, the synthesis of new chlorophyll precursors. Under appropriate illumination, these are transformed to chlorophyll and the leaves become green (6).

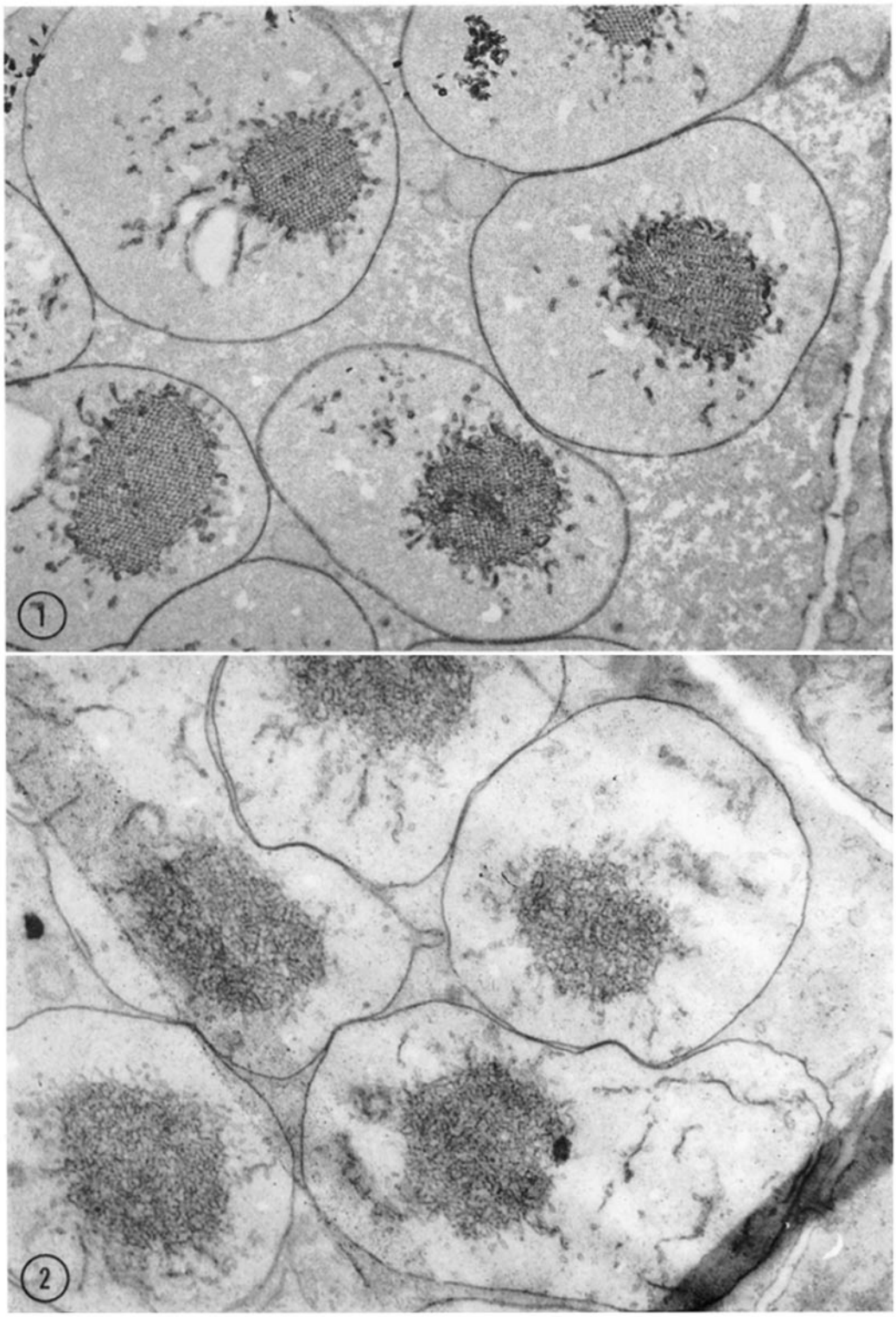
The length of the lag period between the initial protochlorophyll-chlorophyll conversion and the onset of the greening is dependent on a variety of factors and can vary considerably. Effects of leaf age, light intensity, and temperature have been found. Also, the phytochrome system seems to be involved, since a short preillumination with red light at the absorption maximum of phytochrome shortens the lag period (8), and the effect of this pretreatment can be partially reversed by far-red light (16, 21). In preliminary experiments, we found that in detached leaves the duration of the lag phase and the degree of greening also depend considerably upon the pH and ionic strength of the medium with which the leaves are in contact during illumination.

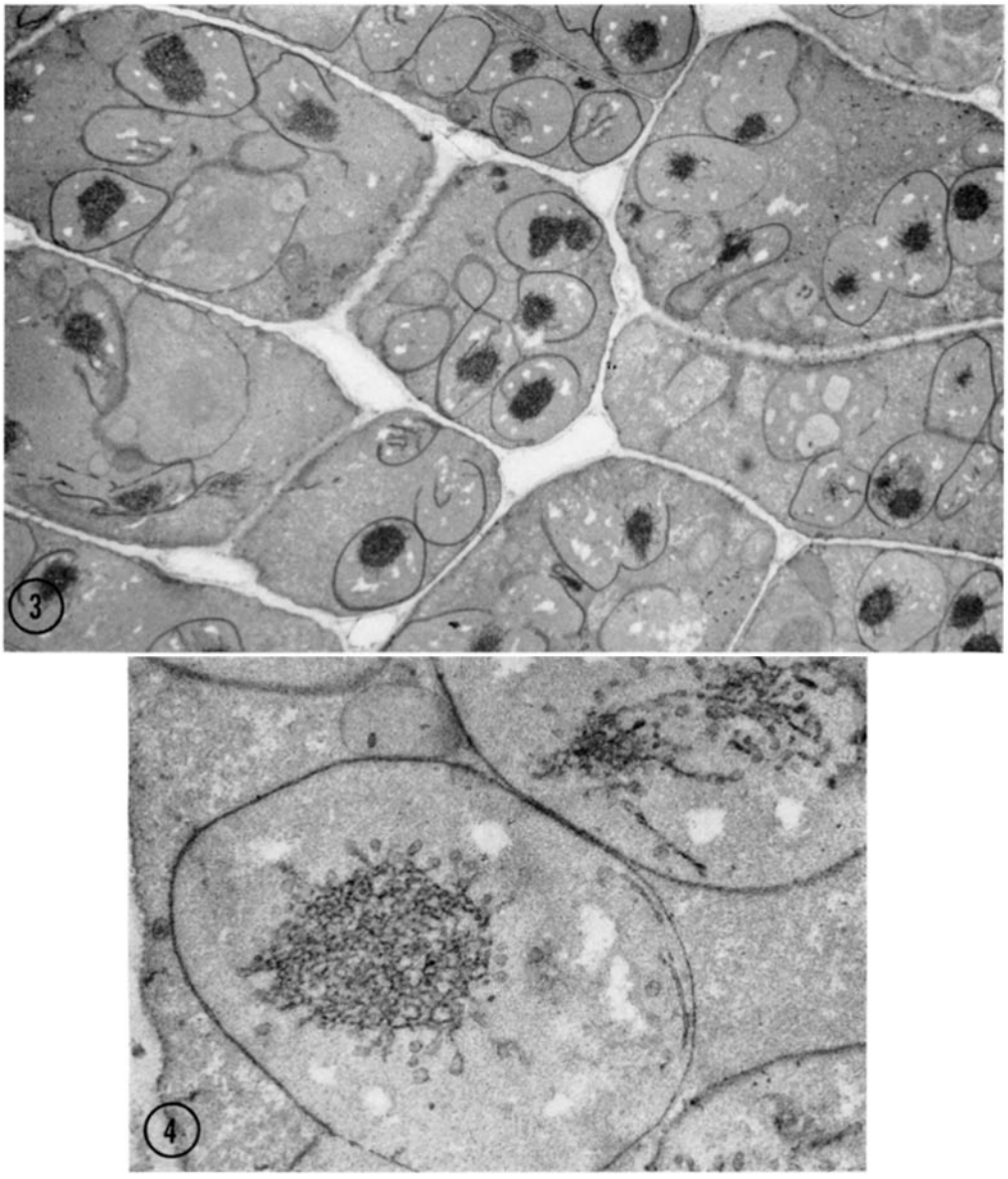
In the experiments reported here, the detached etiolated bean leaves were placed on 2 layers of water-saturated filter paper and exposed to light in a water-saturated atmosphere. Under those conditions, 5 to 5½ hours passed without an increase in chlorophyll content. It was during this lag period, prior to the rapid increase in chlorophyll content, that the occurrence of further changes in plastid structure was investigated.

In all of these experiments, the leaves received 530 erg/mm² of 655 mμ irradiation during 30 seconds, to facilitate the initial pigment conversion. In one series of experiments, this treatment was immediately followed by exposure of the leaves to red light of the same wavelength for 1, 3, and 5 hours. After 1 hour of irradiation with a total of 95.4 kiloerg/mm², essentially no change in chloroplast fine structure could be detected beyond that occurring after the initial 30 seconds. After 3 hours at a light intensity of 60 kiloerg/mm², many of the prolamellar bodies in the chloroplasts were less compact, *i.e.* the vesicular units were somewhat more dispersed, and an increasing number of rows made up of elongated vesicles could be found in sections of the stroma. Very frequently the rows were attached to the centers by both ends and formed loops. After 5 hours in red light, the tendency towards the formation of rows had increased and a large number of chloroplasts contained rows of vesicles, frequently concentrically arranged, while the prolamellar bodies had disappeared completely. A quantitative interpretation of the structural changes, however, is difficult. Whereas the data from the short-time experiments lent themselves well to a quantitative analysis, *i.e.* the ratio of crystalline *versus* non-crystalline prolamellar bodies can be judged quite accurately, a quantitative estimate of fine structure changes after longer light exposure would be less accurate due to the increasing variation and complexity in plastid structure found in the illuminated leaves The estimation of changes in plastid structure from these experiments is qualitative rather than quantitative. Nevertheless, although no clear-cut relationship can be given for energy requirement *versus* structural change during the 5 hours of light exposure, the trend of the changes was obvious. Prolamellar bodies were absent from about 40 to 60 per cent of the plastids in the leaves, which had received a total of 8 or 68 kiloerg/mm² during the

FIGURE 1 Section through part of palisade cell in one of the two first leaves of an etiolated 14-day-old bean plant. No light exposure. Note the regular arrangement of the prolamellar body. × 14,000.

FIGURE 2 Plastids from etiolated bean leaf exposed to red (655 mμ) light for 10 seconds and fixed immediately in the dark. Total light energy received: 280 erg/mm². Note irregular appearance of prolamellar body. × 16,000.


1
2



FIGURES 3 and 4 Plastids from an etiolated bean leaf illuminated with red (655 mμ) light for 20 seconds, receiving a total of 530 erg/mm^2, and kept in the dark for 4 hours before fixation. No structural changes occurred during the dark period. Fig. 3, × 4000; Fig. 4, × 22,000.

5 hour illumination; in leaves irradiated with 480 kiloerg/mm^2, 80 per cent or more of the plastids were without such aggregates of vesicles (Fig. 5). Likewise, length and number of strands of vesicles increased with increasing energy dose.

As mentioned above, 1 hour of exposure to 95 kiloerg/mm^2 of red light did not induce any changes in the plastids beyond those occurring during the initial protochlorophyll-chlorophyll transformation. Also, placing the leaves in the dark for 5 hours after they had received 30 seconds of red light did not affect plastid structure; neither progressive nor regressive changes could be detected (Figs. 3 and 4).

However, 30 seconds of light did enhance structural changes, when a 4 hour dark period was inserted between this pretreatment and 1 hour of red light. Under these conditions more strands were formed and prolamellar bodies were absent from about 20 per cent more plastids than in the leaves exposed to 1 hour of light without pretreatment.

In another set of experiments, a number of leaves were irradiated with red light for 30 seconds (530 erg/mm²/second) and then exposed to the far-red (730 mμ) beam for 5 hours. In the initial experiments the highest available radiant energy of 730 mμ light, 1000 kiloerg/mm² (*i.e.* 55 erg/mm²/second), was applied. As mentioned earlier, however, at this intensity the 730 mμ irradiation was contaminated with a certain amount of visible light which was calculated to contribute about 1 per cent of the total energy. Under these conditions the plastid fine structure changed markedly: strands of vesicles were formed extensively and in 40 to 60 per cent of the plastids the prolamellar bodies disappeared entirely. Because this might have been caused by the visible stray light (in which case a simultaneously supplied 100 times stronger far-red dose was ineffective to prevent it), in further experiments the 730 mμ beam was filtered either through a green filter, or through a 660 mμ interference filter. With these filters the resulting energies at the level of the leaves were 865 kiloerg/mm² and 270 kiloerg/mm², respectively. Although the leaves were now exposed to practically pure far-red light, changes in the plastid structure could still be observed, although to a lesser degree (Fig. 6). The plastids did not reach the developmental stage found after 5 hours in red light, but some strands were formed and about 20 to 30 per cent of the plastids lost their vesicular centers entirely.

Thus, a short illumination with red monochromatic light of 530 erg/mm² is ineffective in causing dispersal of the vesicles and formation of strands. For this development to occur, the leaves had to be exposed to the monochromatic light for a longer time, although a red pretreatment accelerated development somewhat. Prolonged exposure to far-red light also caused a certain amount of dispersion and strand formation, although to a lesser degree than in red light.

DISCUSSION

During the first 5 hours that etiolated leaves were exposed to light, no measurable increase in chlorophyll content occurred after the initial protochlorophyll-chlorophyll transformation; however, significant changes in plastid fine structure could be found during this period. Two different steps seem to be involved. Our results fully confirm the findings of von Wettstein and Kahn (24) and of Eriksson *et al.* (5) that the first of these involves a change in the arrangement of the units of the prolamellar body concomitant with the protochlorophyll-chlorophyll transformation. Also, the energy requirements cited by these authors "zur Transformation der Röhren eines Blattes," 10^4 erg/cm² at a wavelength of 660 mμ or 445 mμ, agree well with our data, which show that at 655 mμ complete transformation can be achieved at an energy level between 1.2 to 2.8 $\times$ 10^4 erg/cm². The finding that no transformations occurred in this energy range at 682 mμ, the absorption maximum of the newly formed chlorophyll(ide) (18), and the ineffectiveness of light in the far-red region of the spectrum confirm the conclusion (5, 22) that the action spectrum for transformation of structure is "compatible with the action spectrum for protochlorophyll-conversion" and that "protochlorophyll conversion and tube transformation are closely related." The action spectrum for protochlorophyll(ide) transformation coincides with the absorption spectrum for protochlorophyll (15). In higher plants the only other known physiologically active light absorber with an absorption maximum in the red region of the spectrum is phytochrome, the pigment responsible for the well known red–far-red reversible light effects on many aspects of plant development (10). However, the action spectrum for phytochrome-mediated effects has a much lower peak in the blue than in the red region, and can be usually reversed by a sufficient dose of far-red light. No such reversion could be found for the structural changes in plastid fine structure, even when a dose of far-red light more than 5 times stronger than the red light was supplied simultaneously. The participation of the phytochrome system in this phase of plastid development seems therefore very unlikely. This makes it almost obligatory to think of some unidentified pigment or more probably of protochlorophyllide as the absorber for the light energy required for the first structural changes in plastids. In etiolated proplastids the red fluorescence is con-

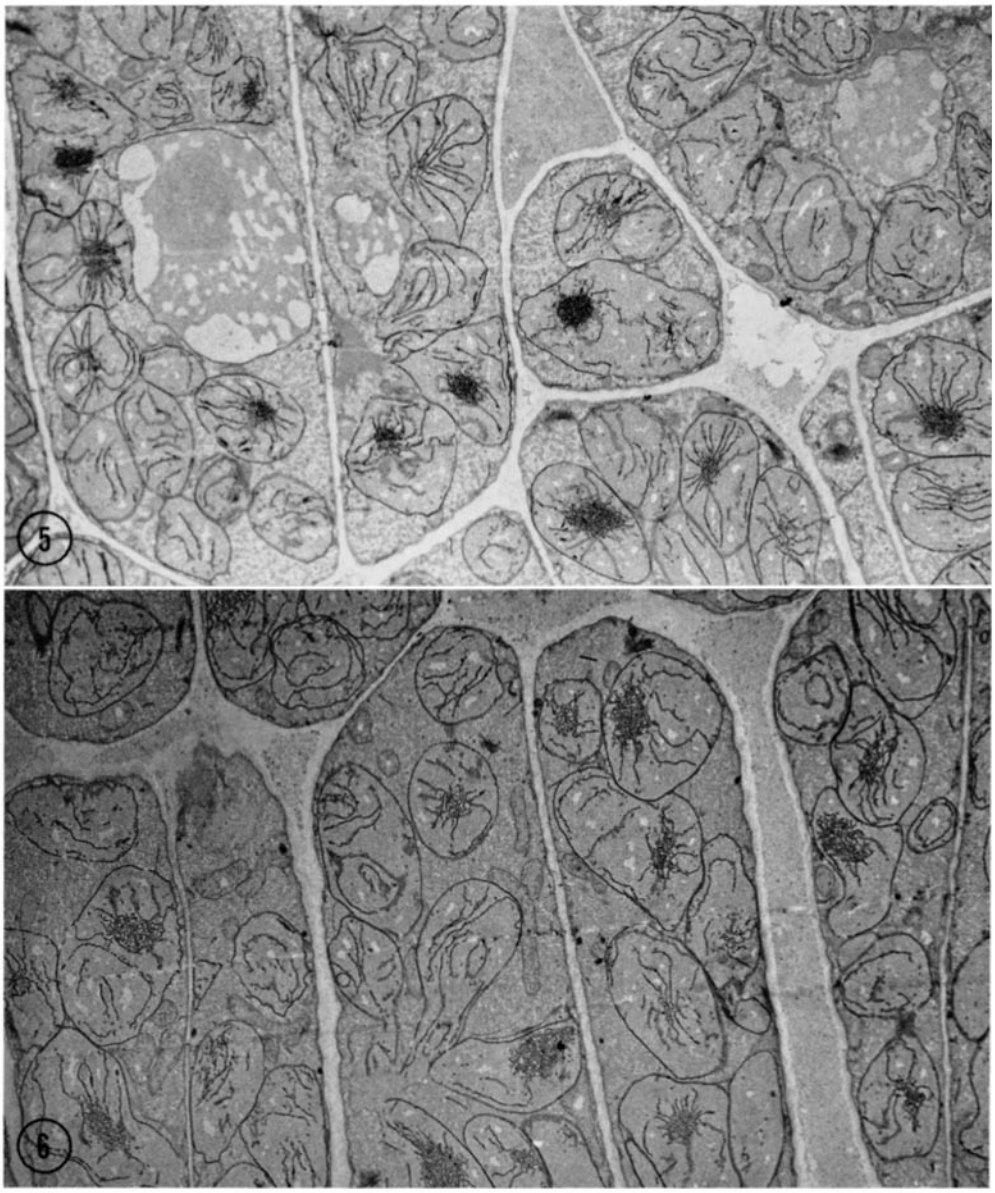


FIGURE 5 Plastids from an etiolated bean leaf after exposure to red (655 mμ) light for 5 hours, having received a total of 480 kiloerg/mm^2. × 5000.

FIGURE 6 Plastids from an etiolated bean leaf exposed to red light (655 mμ; 530 erg/mm^2) for 30 seconds and subsequently irradiated with far-red light (730 mμ) for 5 hours, having received a total of 680 kiloerg/mm^2. × 5000.

centrated in one to three spots (8, 9, 20), which may well be identical with the prolamellar bodies (12). Also, isolated prolamellar bodies purified on a sucrose gradient have a high protochlorophyllide content (Klein, S., data unpublished). There are thus good reasons to assume that the protochlorophyllide holochrome is located in the prolamellar body.

How does the transformation of protochlorophyllide to chlorophyllide affect the structural change of this body? The pigment transformation consists of an hydrogenation of the protochlorophyllide molecule, involving a thus far anonymous donor (19, 21). Although the kinetics indicate that the transformation is second order, an intramolecular hydrogen transfer from the protein moiety of the photochlorophyllide holochrome to the pigment could occur at some stage (21). Thus, it is possible that the pigment transformation may be directly coupled to an oxidation in the protein, thereby changing certain of its properties, which could lead to a structural rearrangement observable at the electron microscopic level.

A prolonged exposure of the leaves to light results in a dispersal of the vesicles in the plastids and their arrangement into "primary layers" (23). This can be achieved with red light of 655 mμ, although at this wavelength no strict reciprocity was found in the energy range applied. A 1 hour irradiation with a total of 95 kiloerg/mm^2 was less effective than illumination with a somewhat lower energy (85 kiloerg/mm^2) for 5 hours, although a pretreatment with red light accelerated the development somewhat. Structural changes were promoted by a 5 hour far-red (730 mμ) irradiation, although less effectively than with red light. It can therefore be assumed that the light mechanism for this step differs from the earlier one. The present data do not necessarily exclude the participation of the phytochrome system in this step.

The effect of the red preillumination on structural development is similar to the one reported on chlorophyll synthesis (25); however, the effect of an inductive red exposure on the lag phase of chlorophyll formation has been shown to be reversible by far-red light (15), while 730 mμ light appears to promote the structural changes described here. On the other hand, in some "high energy requiring" processes far-red light, when given alone, may induce changes similar to those evoked by red light (10).

Changes in plastid fine structure, similar to the ones occurring after 5 hours of illumination, were reported by Klein (11) to occur in etiolated leaves exposed for 24 hours to white light of 400 ft-c at 3°C and by Eilam and Klein (4) after the same period in light of 3 ft-c at 26°C. In both cases the chlorophyll content remained low, and in the latter the pigment was in the non-phytylated form. The duration of this step in development of plastid structure can thus be affected by a variety of environmental controls.

A number of chemical and physiological changes take place between the inductive illumination and the increase in chlorophyll content. Synthesis of protochlorophyll precursors is initiated, probably by formation and/or activation of the δ-aminolevulinic acid synthetase system. The transformed chlorophyllide is phytylated presumably by chlorophyllase (26). The absorption peak of the newly formed chlorophyll holochrome shifts from 682 mμ towards a lower wavelength (18). Solubility of the protein-pigment complex(es) and their spectral properties *in vivo* change (1). Development of energy transport from carotenoid pigments to chlorophyll *a* occurs, although apparent net synthesis of carotenoids starts only later (2), seemingly somewhat after the beginning of the increase in chlorophyll content. TPN-dehydrogenase activity, controlled by the red–far-red system, develops in etiolated leaves after a low energy red irradiation without increase in chlorophyll content (13). An inductive illumination results also in an increase in the size of as well as in the protein and lipid content of the plastids (14). Carotenoid synthesis is also stimulated by this treatment (3). There is however no evidence for any correlation between these changes and the structural development, as observed to date with the electron microscope, prior to the increase in chlorophyll and grana formation. The available evidence rather points in the opposite direction: most of the above-mentioned changes are triggered by light of low energy, but can occur subsequently in the dark, whereas the dispersal of the vesicles and their arrangement into rows is a high energy requiring process, for which more than an inductive illumination is necessary since it cannot proceed in the dark subsequent to a period of irradiation. Furthermore, these structural changes can proceed at low temperatures (23) which would greatly inhibit enzymatic activities. Thus the structural and functional changes during this phase of plastid development seem to occur independently. Whether the structural changes which occur concomitantly with the protochlorophyllide-chlorophyllide conversion are required for the initiation or occurrence of the functional changes remains still to be investigated.

The authors are indebted to Dr. Hewson Swift for his cooperation in providing the use of his electron

microscope facilities in the Whitman Laboratory of the University of Chicago; to Dr. C. F. Ehret for his interest and cooperation; to Mr. Paul W. Ellwanger for his assistance in irradiation of material on the Argonne Spectrograph and with certain aspects of the electron microscopy; and to Mrs. G. Lanier and Miss J. A. Alblinger for their technical assistance.

This work was supported by grants by grants from the National Institutes of Health (A 1010 and GM 10324) and the National Science Foundation (GB-394). It was also supported in part by funds from the C. F. Kettering Foundation and the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Dr. Klein participated in this study while a C. F. Kettering Foundation International Fellow, 1963. His permanent address is the Department of Botany, The Hebrew University, Jerusalem, Israel. Dr. Bogorad is a Research Career Awardee of the Institute of General Medical Science, National Institutes of Health, United States Public Health Service.

Received for publication, October 17, 1963.

BIBLIOGRAPHY

1. BOGORAD, L., and CAPON, B., *Bot. Gaz.*, 1962, **123,** 285.
2. BUTLER, W. R., *Arch. Biochem. et. Biophysics*, 1961, **92,** 287.
3. COHEN, R. Z., and GOODWIN, T. W., *Phytochemistry*, 1962, **1,** 67.
4. EILAM, Y., and KLEIN, S., *J. Cell Biol.*, 1962, **14,** 169.
5. ERIKSSON, G., KAHN, A., WALLES, B., and VON WETTSTEIN, D., *Ber. Bot. Ges.*, 1961, **74,** 221.
6. FRENCH, C. S., *in* Encyclopedia of Plant Physiology, (W. Ruhland, editor), Berlin, Springer Verlag, 1960, **5,** pt. 1, 252.
7. GEROLA, F. M., CHRISTOFORI, F., and DASSU, G., *Caryologia*, 1960, **13,** 164.
8. GRANICK, S., *Internt. Congr. Biochem., 5th, Moscow*, 1961, **6,** 176.
9. HAGEMAN, R., *Biol. Zentr.*, 1960, **79,** 393.
10. HENDRICKS, S. B., *in* Comparative Biochemistry of Photoreactive Systems, (M. B. Allen, editor), New York, Academic Press, Inc., 1960, 303.
11. KLEIN, S., *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 529.
12. KLEIN, S., and POLJAKOFF-MAYBER, A., *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 433.
13. MARCUS, A., *Plant Physiol.*, 1960, **35,** 126.
14. MEGO, J. L., and JAGENDORF, A. T., *Biochim. et Biophysica Acta*, 1961, **53,** 237.
15. MITRAKOS, K., *Physiol. Plantarum*, 1961, **14,** 497.
16. MONK, G. S., and EHRET, C. F., *Radiation Research*, 1956, **5,** 88.
17. MÜHLETHALER, K., and FREY-WYSSLING, A., *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 507.
18. SHIBATA, K., *J. Biochem., (Tokyo)*, 1957, **44,** 147.
19. SMITH, J. H. C., *in* Comparative Biochemistry of Photoreactive Systems, (M. B. Allen, editor), New York, Academic Press, Inc., 1960, 251.
20. STRUGGER, S., *Naturwissenschaften*, 1954, **41,** 286.
21. VIRGIN, H. I., *Physiol. Plantarum*, 1961, **14,** 439.
22. VIRGIN, I., KAHN, A., and VON WETTSTEIN, D., *Photochem. and Photobiol.*, 1963, **2,** 83.
23. VON WETTSTEIN, D., *Brookhaven Symp. Biol.*, 1958, **11,** 138.
24. VON WETTSTEIN, D., and KAHN, A., *Proc. European Regional Conf. Electron Microscopy, Delft, 1960*, 1961, **2,** 1051.
25. WITHROW, R. B., WOLFF, J. B., and PRICE, L., *Plant Physiol.*, 1956, **31,** Suppl, xiii.
26. WOLFF, J. B., and PRICE, L., *Arch. Biochem. and Biophysics*, 1957, **72,** 293.