MACROMOLECULAR PHYSIOLOGY OF PLASTIDS

VIII. Pigment and Membrane Formation in Plastids of Barley Greening under Low Light Intensity

K. W. HENNINGSEN and J. E. BOYNTON

From the Institute of Genetics, University of Copenhagen, Copenhagen, 1353, Denmark. Dr. Boynton's present address is Department of Botany, Duke University, Durham, North Carolina 27706

ABSTRACT

Sequential changes occurring in the etioplasts of the primary leaf of 7-day-old dark-grown barley seedlings upon continuous illumination with 20 lux have been investigated by electron microscopy, in vivo spectrophotometry, and thin-layer chromatography. Following photoconversion of the protochlorophyllide pigment to chlorophyllide and the structural transformation of the crystalline prolamellar bodies, the tubules of the prolamellar bodies are dispersed into the primary lamellar layers. As both chlorophyll *a* and *b* accumulate, extensive formation of grana takes place. After 4 hr of greening, protochlorophyllide starts to reaccumulate, and concomitantly both large and small crystalline prolamellar bodies are formed. This protochlorophyllide is rapidly photoconverted upon exposure of the leaves to high light intensity, which also effects a rapid reorganization of the recrystallized prolamellar bodies into primary lamellar layers.

INTRODUCTION

The formation of crystalline prolamellar bodies in etioplasts of dark-grown angiosperms is dependent on the synthesis of protochlorophyll(-ide) (6, 15, and K. W. Henningsen, J. E. Boynton, and D. von Wettstein, data in preparation). Upon illumination, the protochlorophyllide is rapidly converted into chlorophyllide *a* (1, 2, 5, 10, 25, 26, 43, 50). Along with the pigment conversion, a concomitant series of structural rearrangements of the membrane material in the prolamellar body is initiated (3, 5, 8, 11, 14, 16, 17, 23, 44, 47, 49). The time course for these developmental steps—tube transformation, dispersal, and grana formation—has been described. While tube transformation and dispersal can proceed in darkness following a brief illumination, formation of grana requires illumination over a prolonged period, either as continuous illumination or as a series of light flashes (40). The onset of formation of grana parallels the rapid increase in chlorophyll synthesis, and the area of the paired regions of the discs has been shown to increase in direct proportion to the amount of chlorophyll accumulated (A. Kahn and D. von Wettstein, unpublished data).

Under continuous illumination with 2000 lux

or higher intensity, etioplasts differentiate into chloroplasts which have a lamellar system consisting only of membrane discs, either cemented together into grana or interconnecting the grana.

Prolamellar body material completely dispersed into primary lamellar layers can undergo recrystallization in darkness (11, 16, 49). This recrystallization of the prolamellar bodies parallels the resynthesis of protochlorophyllide, the rate of which is dependent on the age of the plants (1, 14, 16). The high rate of resynthesis found in younger plants promotes a very rapid recrystallization of the prolamellar body material from the transformed state, possibly even preventing the initial dispersal reaction. In dark-grown plants having maximal amounts of protochlorophyll(-ide), a lag in the resynthesis of protochlorophyllide follows the photoconversion of the pigment (1, 14, 16), during which time dispersal of the prolamellar body material takes place in the dark (14, 16). At the end of this lag period, a reformation of crystalline prolamellar bodies is initiated parallel to the resynthesis of protochlorophyllide (16).

In plastids of light-grown plants, crystalline prolamellar bodies have been observed in addition to grana when the plants have been placed in darkness for long periods prior to fixation (13, 24, 27–29, 31, 32, 39, and K. W. Henningsen, unpublished data). Under continuous illumination with 300 lux or lower intensity, etioplasts develop into chloroplasts with a membrane system consisting of one or several large crystalline prolamellar bodies, from which numerous primary lamellar layers and grana stacks radiate (7, 9, 30). The major leaf pigment under these conditions is chlorophyll *a*, but some evidence suggests that also small amounts of protochlorophyll are present in these plastids (7, 30). The prolamellar bodies present in plants kept under low light intensity for a long period disappear rapidly when the plants are exposed to high light intensity (52).

The present investigation was undertaken to study tube transformation and dispersal of the prolamellar bodies under continuous illumination with low light intensity. We were interested in determining whether the reformation of the crystalline prolamellar bodies in the light is paralleled by the resynthesis of protochlorophyllide. It is also important to determine whether the protochlorophyllide accumulated under 20 lux can be photoconverted to chlorophyllide *a* by exposure of the leaves to bright light and what effect this would have on the recrystallized prolamellar bodies present in the plastids.

MATERIALS AND METHODS

Seeds of barley, *Hordeum vulgare*, cultivar Svalöfs Bonus, were germinated in a dark room at 23°C and 80% relative humidity, with use of plastic trays containing vermiculite. The seedlings were watered at intervals with tap water. Handling of the seedlings was carried out under a dim green safelight. 7 days after planting, the trays containing the seedlings were placed in light of either 20 lux (67.5 erg/cm^2 sec) or 3200 lux (2160 erg/cm^2 sec) obtained from white fluorescent tubes. A xenon light source was used for the illuminations with high intensity white light. During the illuminations, the air temperature was 23°C, and a humid atmosphere was maintained. At intervals seedlings were analyzed for pigment content and ultrastructure. All handling of the leaf material and pigment extracts was carried out under a dim green safelight. For in vivo spectrophotometry and fixation, the upper 10 mm of the primary leaf was discarded, and the next 15 mm was analyzed. For extraction of leaf pigments the upper 50 mm of the primary leaf was used.

In vivo absorption spectra were obtained from a single layer of the leaf pieces by use of a Zeiss RPQ-20A recording spectrophotometer (Carl Zeiss Inc., New York) equipped with a modified integrating sphere attachment. The optical density at the absorption maximum for chlorophyll and protochlorophyll in vivo was used as a measure of the amount of chlorophyllous pigments present per unit area of the leaves at the various stages of greening.

Leaf pigments were extracted in cold 80% acetone containing small amounts of $Ca(CO_3)_2$ by grinding in a Sorvall omnimixer (Ivan Sorvall Inc., Norwalk, Conn.) and then centrifuging. The extracts were stored in the dark at 4°C under nitrogen until analyzed by thin-layer chromatography on cellulose MN 300 in a solvent system of methanol, dichloromethane and water (100:18:20, v/v/v) (36, 37). The chlorophyllous pigments were located by their fluorescence. The protochlorophyllide region on the chromatograms was eluted and the protochlorophyllide was identified by its fluorescence spectrum yielding a maximum at 627 mμ (cf. 10).

Leaf tissue was fixed in 4.2% glutaraldehyde in phosphate buffer (0.067 M, pH 7.2) for 2 hr at 0–4°C and kept dark until termination of the fixation. After several changes in phosphate buffer, postfixation with 2% OsO_4 was carried out for 2 hr at room temperature. Following dehydration in a graded ethanol series, the tissue was embedded in an epoxy resin formulation (42). Thin sections cut with a diamond knife on a Cambridge ultramicrotome

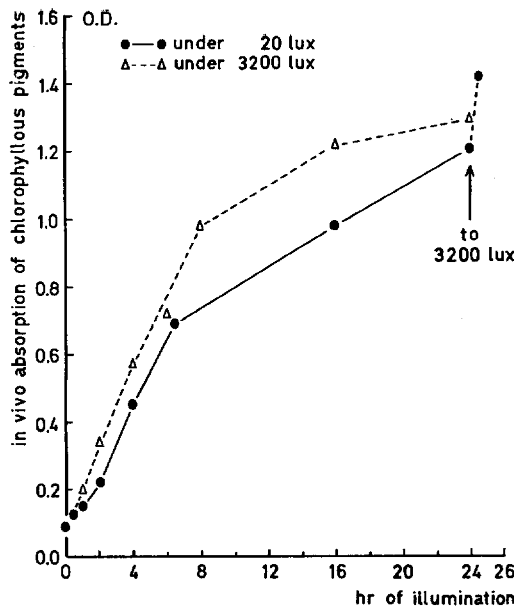


FIGURE 1 Content of chlorophyllous pigments in primary leaves of barley seedlings greening under 20 and 3200 lux. Peak height at the maximum in the 640–685 mμ region of the in vivo absorption spectrum is used as a measure for the pigments. A large increase in chlorophyll absorption takes place within 5 min after the light intensity has been increased to 3200 lux.

(Cambridge Instrument Co., Inc., Ossining, N. Y.) were contrasted with uranyl acetate and lead citrate. Quantitative estimation of the plastid membrane configurations was carried out on micrographs of nonserial sections obtained with a Siemens Elmiskop I (Siemens America, Inc., New York). Between 20 and 200 plastid sections were examined for each treatment.

RESULTS

Pigment Changes

Greening of barley seedlings grown in darkness for 7 days takes place rapidly without any lag in chlorophyll synthesis (Fig. 1). While the amount of chlorophyllous pigments accumulated at any one stage of greening is only slightly lower in seedlings illuminated under 20 lux, as compared to seedlings illuminated under 3200 lux, the composition of the pigments shows qualitative differences (Fig. 2). Photoconversion of the protochlorophyllide accumulated in the dark is complete within 5 min of illumination under either 20 or 3200 lux. The protochlorophyll present in dark-grown leaves is not photoconverted within this period of illumination but disappears after 30 min of illumination, either as a result of a slower photoconversion than is observed for the nonphytylated pigment or as a result of destruction by photooxidation.

After 4 hr of illumination under 20 lux, and possibly sooner, protochlorophyllide again begins to accumulate in detectable amounts. At all later stages of greening under 20 lux, protochlorophyllide and chlorophyllide *a* are present. These pigments are either absent or exist only in minute quantities in seedlings greened for the same length of time under 3200 lux. Chlorophyllide *a* is present in the leaves immediately after illumination with 20 lux, whereas it is not detectable in leaves shortly after illumination with 3200 lux. This may indicate that the phytylation of the chlorophyllide *a* proceeds at a higher rate in the leaves illuminated with high light intensity. Under both high and low light intensity, chlorophyll *b* and protochlorophyll are present after 8 hr of illumination.

Seedlings greened for either 8 or 24 hr under 20 lux show a marked increase in in vivo absorption of the chlorophyll when exposed for 1 min to 5×10^6 erg/cm^2 of white light (cf. Fig. 1). Extracts of leaves from seedlings greened under 20 lux for 8 or 24 hr followed by a 5 min exposure to 3200 lux (6.5×10^5 erg/cm^2) do not contain any detectable protochlorophyllide (Fig. 2). By difference spectrophotometry it can be established that the protochlorophyllide present in leaves greening under low light intensity is rapidly photoconverted into chlorophyllide *a* if sufficient light energy is provided.

A difference spectrum obtained between a leaf sample from seedlings greened for 24 hr under 20 lux and a leaf sample which, following the 24 hr under 20 lux, has been exposed to 3200 lux for 2 min shows a maximum at around 680 mμ (Fig. 3), the expected peak for newly converted chlorophyllide *a*. When similar leaves exposed to 3200 lux for 30 min are measured against leaves which have received only 20 lux for 24 hr, the difference spectrum shows a maximum at 676 mμ (Fig. 3). Thus the spectral shift of the newly formed chlorophyllide *a* from 685 to 672 mμ, observed in vivo in leaves from dark-grown plants shortly after they have been exposed to light, also takes place in leaves of barley seedlings greened under 20 lux after they have been ex-

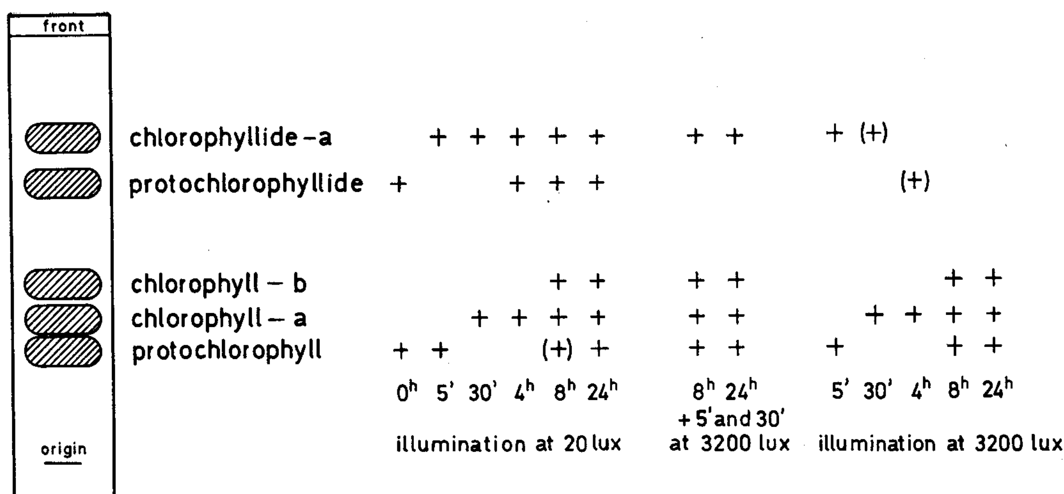


FIGURE 2 Schematic presentation of the chromatographic separation of chlorophyllous pigments present in barley leaves at various stages of greening under 20 and 3200 lux.

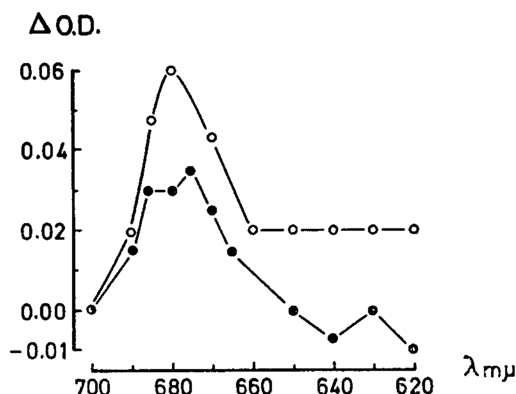


FIGURE 3 Difference spectra calculated from the in vivo spectrum of leaf samples, demonstrating the spectral shift of chlorophyllide *a* after conversion of protochlorophyllide accumulated under low light intensity. *Open circles*: difference spectrum between leaf samples, illuminated for 24 hr under 20 lux and followed by a 2 min exposure to 3200 lux. *Solid circles*: difference spectrum, leaf samples 24 hr at 20 lux and followed by a 30 min exposure to 3200 lux (see text for further explanation).

posed to a light intensity which converts the accumulated protochlorophyllide.

Structural Changes

ETIOPLASTS: Barley seedlings grown for 7 days in the dark (23°C) have a maximum amount of protochlorophyllide and crystalline prolamellar body material (16). In a random sample of plastid sections from the primary leaves of 7-day-old barley seedlings, prolamellar bodies are observed in 68% of the sections whereas only primary lamellar layers are seen in the remaining sections.

TRANSFORMATION AND DISPERSAL: After 30 min of illumination under 20 lux (1.2×10^5 erg/cm^2) all the tubules in the prolamellar bodies are completely transformed (Figs. 4, below; 5 and 6). The number of plastid sections containing a prolamellar body is slightly lower than that found for the dark sample, indicating that a reduction in the volume of the prolamellar bodies has already taken place via the process of membrane dispersal. A gradual dispersal of the transformed prolamellar bodies continues during illumination of the seedlings for 2 hr (4.9×10^5 erg/cm^2). At this point the number of plastid sections containing a prolamellar body has decreased to about 50% of the dark value. The remaining prolamellar bodies are all in the transformed stage, and the majority have decreased considerably in volume. Primary lamellar layers arising from the dispersal of the tubular membrane material of the prolamellar bodies are, when cut obliquely and in surface view, clearly identified as membrane sheets with numerous perforations (16). The equidistant spacing of these perforations can be observed in surface views of areas of primary lamellar layers (Fig. 6). As grana formation takes place, the perforations in the newly dis-

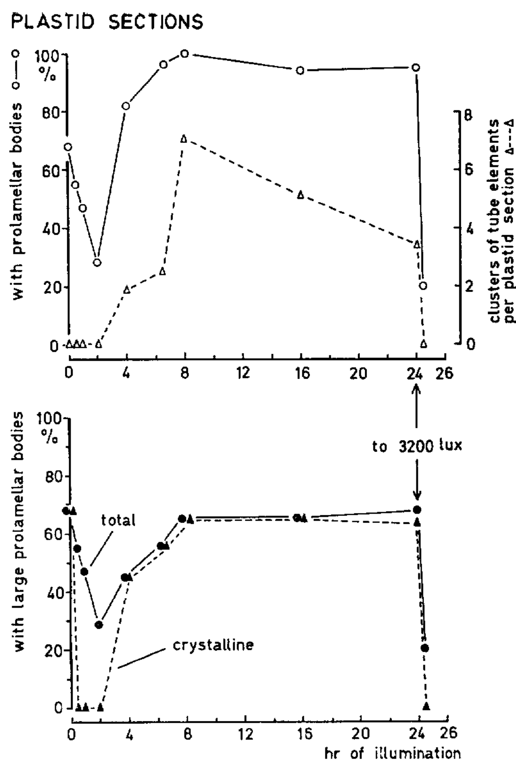


FIGURE 4 Distribution of different types of prolamellar body material in plastid sections from the primary leaf of barley seedlings greening under 20 lux. The effect of a subsequent exposure to 3200 lux is also depicted. Each point represents a value determined from examination of 20–200 plastid sections.

persed, primary lamellar layers gradually disappear (cf. Fig. 7).

REFORMATION OF CRYSTALLINE PROLAMELLAR BODIES: In the period between 2 and 4 hr of illumination under 20 lux a considerable amount of chlorophyll *a* is accumulated in the primary leaf of the seedlings, and at 4 hr several paired regions of discs have formed along the primary lamellar layers in many plastid sections. In addition to the presence of these first two-disc-grana, an increasing number of the plastid sections contain a prolamellar body. In marked contrast to the transformed prolamellar bodies observed at 2 hr, all prolamellar bodies are fully crystalline after 4 hr (Fig. 4). The number of plastid sections containing a prolamellar body increases gradually from 4 to 8 hr of illumination, at which time the values have reached a level close to those found for unilluminated samples. In the period from 4 to 16 hr of illumination under 20 lux, all the prolamellar bodies are of the crystalline configuration (Figs. 4, 7, 10, 11). At 24 hr a minor fraction of the prolamellar bodies appears to have lost their crystalline character (Fig. 4), although the rest show a clearly crystalline narrow or wide spacing of the tubular membrane system (Figs. 10, 11).

The majority of the large crystalline prolamellar bodies formed between 2 and 4 hr of illumination appear to originate from a recrystallization of the not completely dispersed prolamellar bodies still present at 2 hr. With the occurrence of the large crystalline prolamellar bodies at 4 hr of illumination, simultaneously many new small prolamellar bodies are found in connection with the primary lamellar layers (Figs. 4, above; 8, 9). Since the tubules in these small prolamellar bodies often show a lower degree of interconnection than those in the larger crystalline prolamellar bodies and frequently appear as three-armed modules, they are designated *tube elements*. These tube elements seem to form clusters located close to the few remaining perforations of the primary lamellar layers. The tube elements show no tendency to be found in connection with the larger prolamellar bodies or the plastid envelope.

Between 4 and 8 hr of illumination, when recrystallization of prolamellar bodies gradually takes place, the average number of tube element clusters increases from about two per plastid section at 4–6 ½ hr to a maximum of seven at 8 hr. During this period, reformation of the large prolamellar bodies approaches completion. We suspect that these clusters of tube elements are synthesized *de novo* since reformation of the large prolamellar bodies and the formation of grana have consumed the originally dispersed lamellar material.

Fine tubules, with a smaller diameter and different staining properties than the tubules in the prolamellar body, are found more or less frequently in the plastid sections at all stages of development. The diameter of the fine tubules is approximately 120 A as compared to about 210 A for the tubules in the prolamellar body. These fine tubules are usually oriented with their long axis parallel to the primary lamellar layers, although, in the vicinity of the tube element clusters and the remaining perforations in the primary lamellar layers, they are sometimes oriented at an angle to the lamellar surface (Fig.

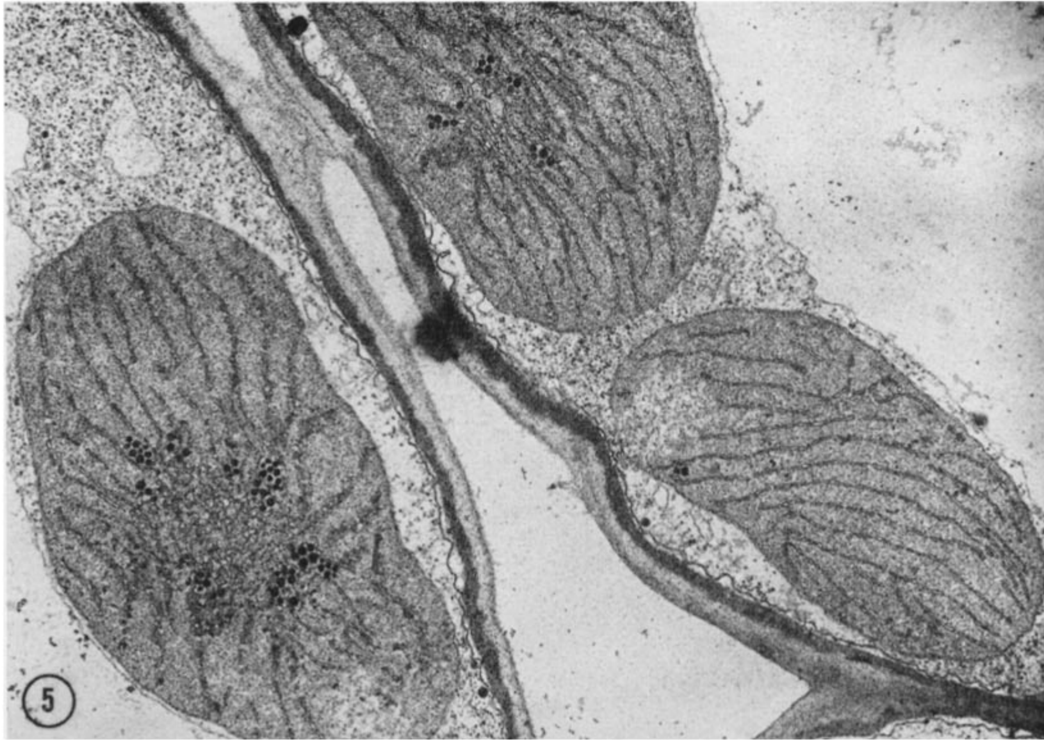


FIGURE 5 Sections through plastids from the primary leaf of 7-day-old dark-grown barley seedlings illuminated under 20 lux for 60 min. From left to right the plastid sections show the progressing dispersal of the tubular membrane system of the transformed prolamellar body into primary lamellar layers. × 19,000.

9) and occasionally appear to connect to the tube elements.

During the period between the 8th and 24th hr of illumination, a gradual decrease in the number of tube element clusters is observed (Fig. 4). It is reasonable to assume that the tube elements either are consumed in the process of formation of grana or are incorporated into the larger prolamellar bodies. The latter possibility seems less likely since the large prolamellar bodies do not increase significantly in size.

FORMATION OF GRANA UNDER 20 LUX ILLUMINATION: Up to 1 hr after the seedlings have been illuminated with 20 lux, the paired regions of the primary lamellar layers do not increase significantly in area and number beyond the level observed in unilluminated leaf samples. At 2 hr, many areas occur in which two discs are paired, and a few grana with three to four discs are found. These paired regions are generally of short length, in contrast to the few, rather long, paired regions already present in the unilluminated plastids. Between 2 and 4 hr, no new regions of pairing are observed along the primary lamellar layers, but the paired regions formed at 2 hr increase considerably in size. After 6½ hr of illumination, the area of the discs has increased further and also there seems to be an increase in the number of grana per plastid section. Grana consisting of four discs are the most prevalent type. In the 6½–8 hr period during which the amount of prolamellar body material of the crystalline configuration increases considerably, no drastic increase in the number and size of the paired discs is observed. From 8 to 16 hr of illumination, the diameter and the number of the paired discs continue to increase gradually, and a further increase in the diameter of the discs is observed at 24 hr. Between 8 and 24 hr no obvious change in size of the prolamellar body sections is

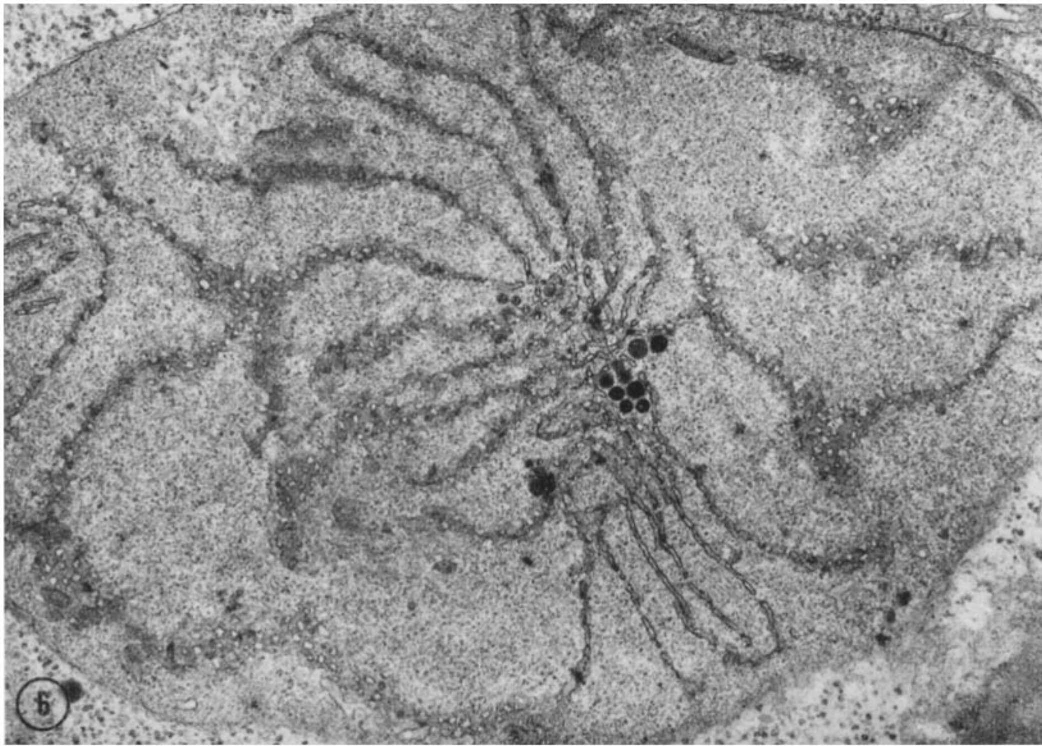


FIGURE 6 Plastid section from the same material as Fig. 5. The dispersal of a transformed prolamellar body is almost completed. Radiating from the remaining prolamellar body are seen several primary lamellar layers in cross-section and surface view. At a few spots the perforations form a regular pattern. × 39,000.

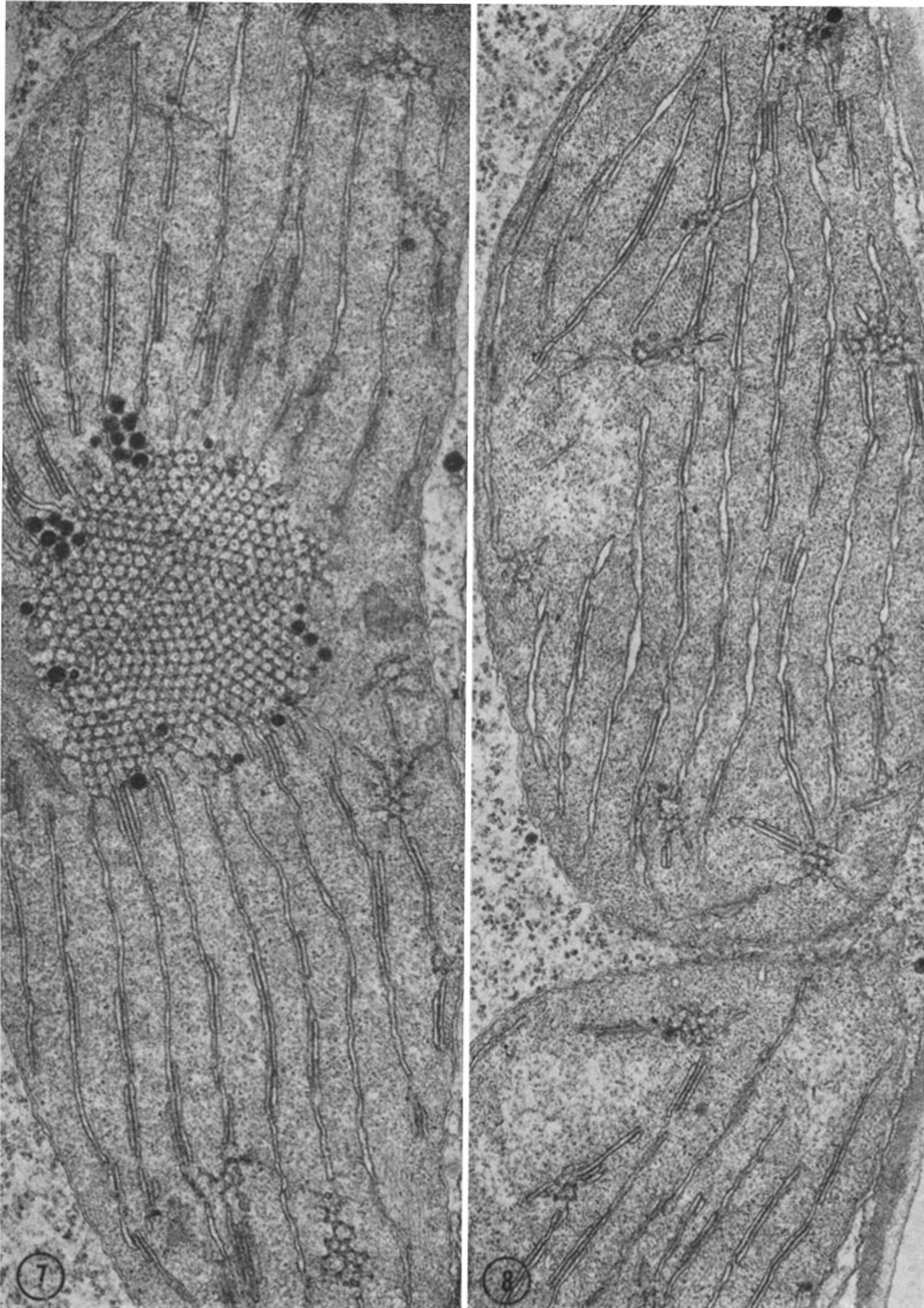
observed. Small grana appear within some of the prolamellar bodies between 16 and 24 hr (Fig. 11).

DISPERSAL OF CRYSTALLINE PROLAMELLAR BODIES FORMED UNDER ILLUMINATION WITH LOW LIGHT INTENSITY: The protochlorophyllide and the tubular material in the crystalline prolamellar bodies formed in the primary leaves of barley seedlings illuminated under 20 lux for 24 hr undergo rapid changes when the seedlings are exposed to higher light intensities. As previously mentioned, a 1 min exposure to white light (5×10^6 erg/cm^2) or a 5 min illumination with 3200 lux from fluorescent tubes (6.5×10^5 erg/cm^2) completes the conversion of the protochlorophyllide present. Within a period of 30 min of illumination with 3200 lux (3.9×10^6 erg/cm^2), all the prolamellar bodies have lost their crystalline configuration. During the same period, the dispersal of the prolamellar bodies is almost completed. The remaining transformed prolamellar bodies are, in all cases, reduced considerably in volume.

In the majority of the plastid sections the result of the dispersal process is clearly identified as primary lamellar layers connected to the lamellar system with the grana (Figs. 12 and 13). These newly organized membrane discs have numerous perforations and are largely oriented parallel to each other and to the long axis of the plastids. The osmiophilic globuli present as inclusions in the tubular membrane system of the crystalline prolamellar bodies formed under low light intensity are distributed over the area of the newly formed lamellae after completion of the dispersal process and are often trapped between neighboring primary lamellar layers.

DISCUSSION

Gymnosperms grown in darkness are able to form large amounts of chlorophyll, in addition to accumulating protochlorophyll(-ide) (cf. 46). Grana and crystalline prolamellar bodies have been observed within the same plastid in cotyledons of dark-grown seedlings of spruce (45, 46).



FIGURES 7 and 8 Plastid sections from the primary leaf of barley seedlings illuminated for 8 hr under 20 lux. Along the primary lamellar layers, paired regions of the newly formed grana are evident, consisting of 2–4 discs. Scattered along the primary lamellar layers tube elements and small crystalline prolamellar bodies with a wide spacing of the tubular membrane system are found. Small diameter tubules cut in cross-section and longitudinally are found at several spots in the stroma. In Fig. 7: a large crystalline prolamellar body formed by recrystallization of a partially dispersed prolamellar body is depicted. Both × 38,000.

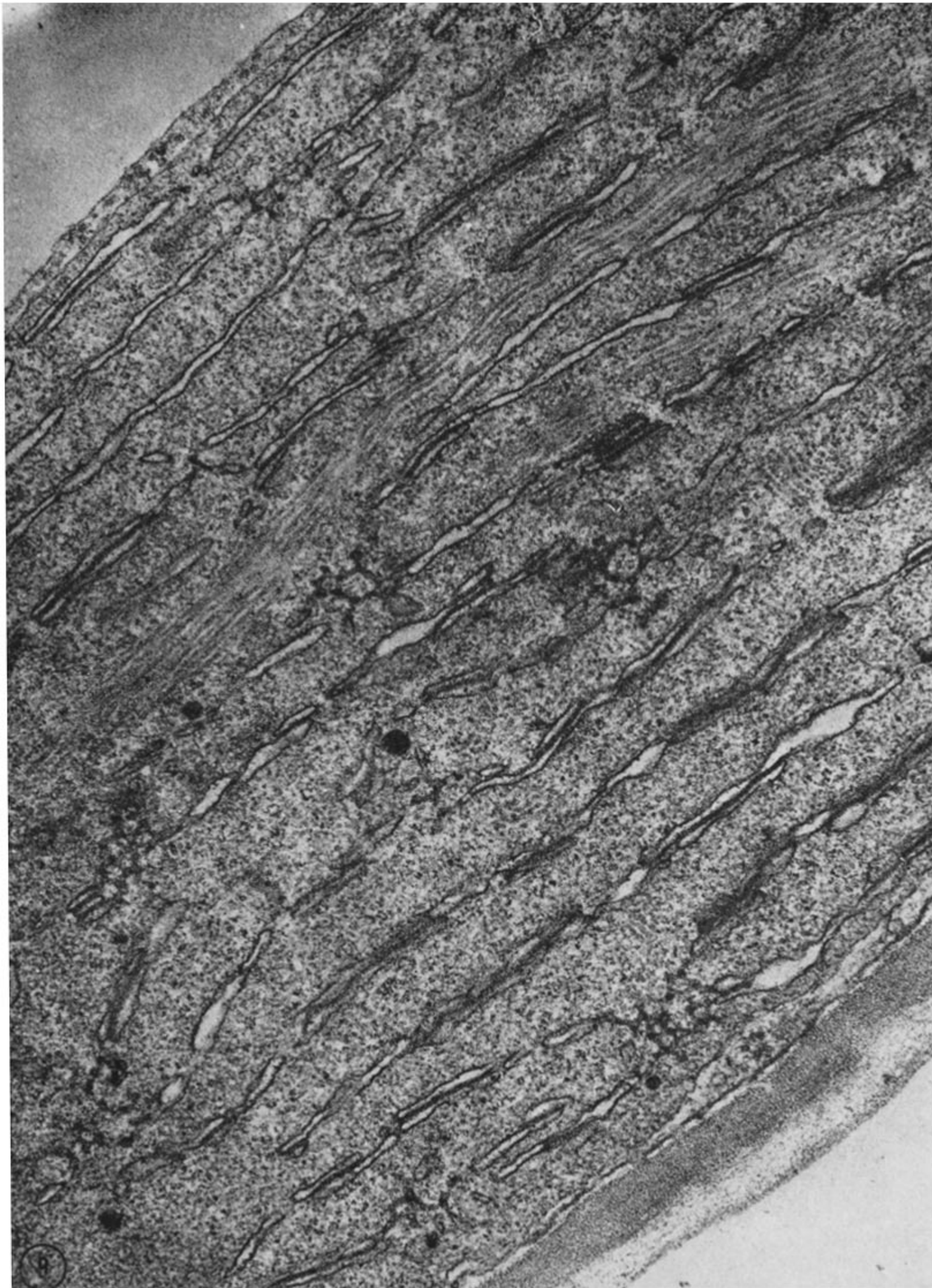
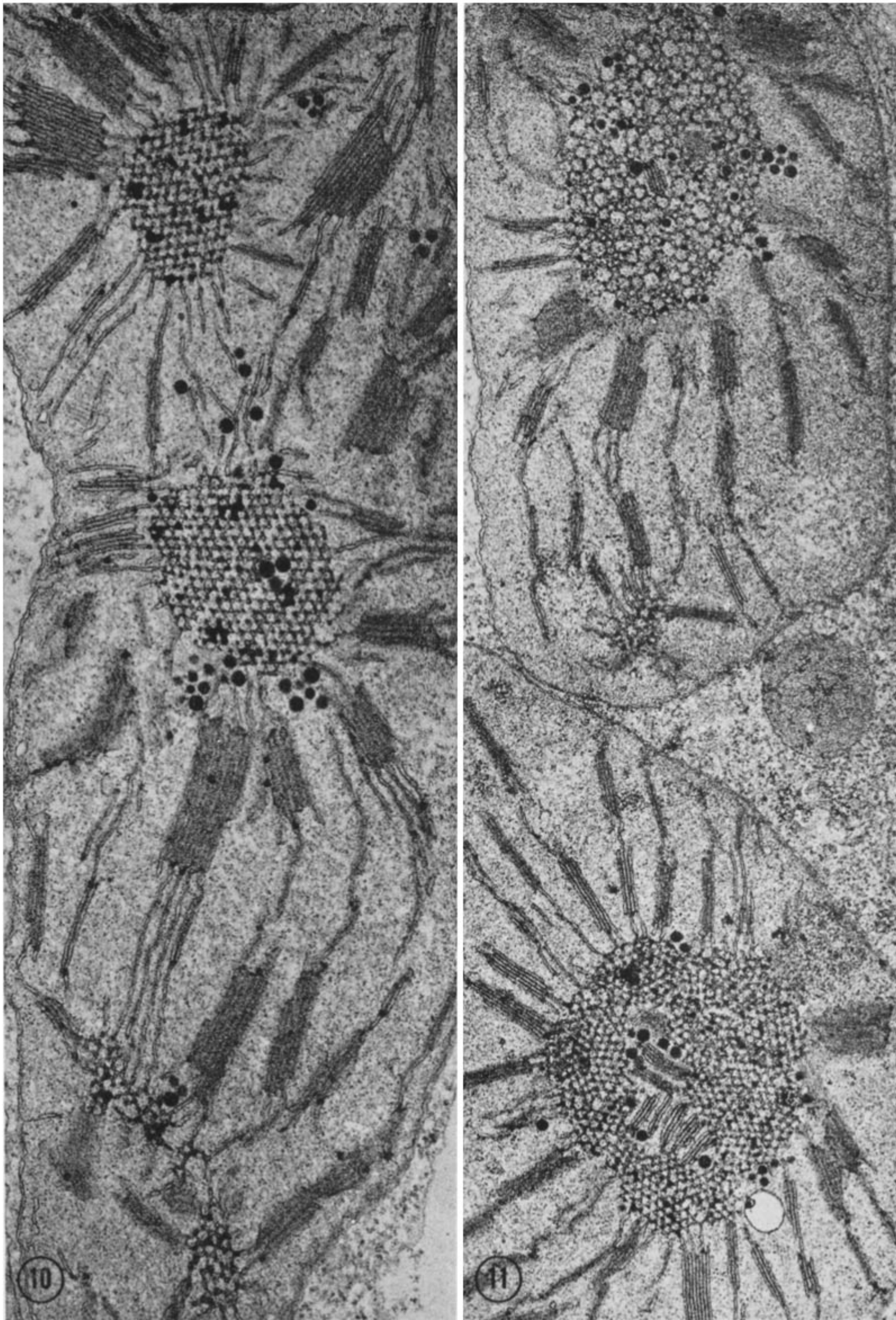
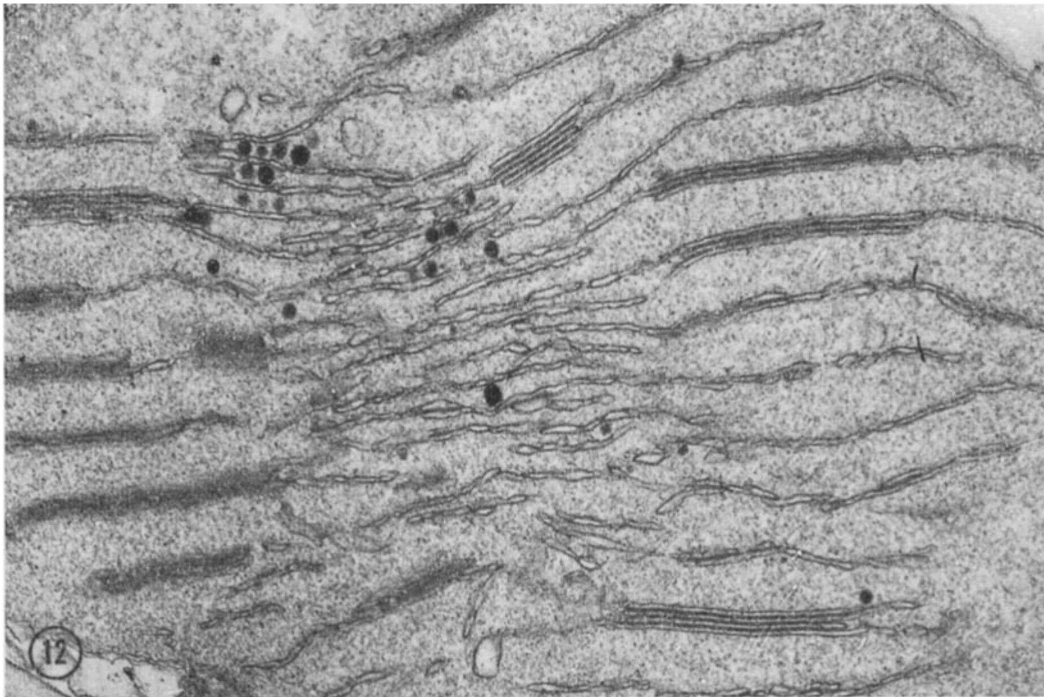
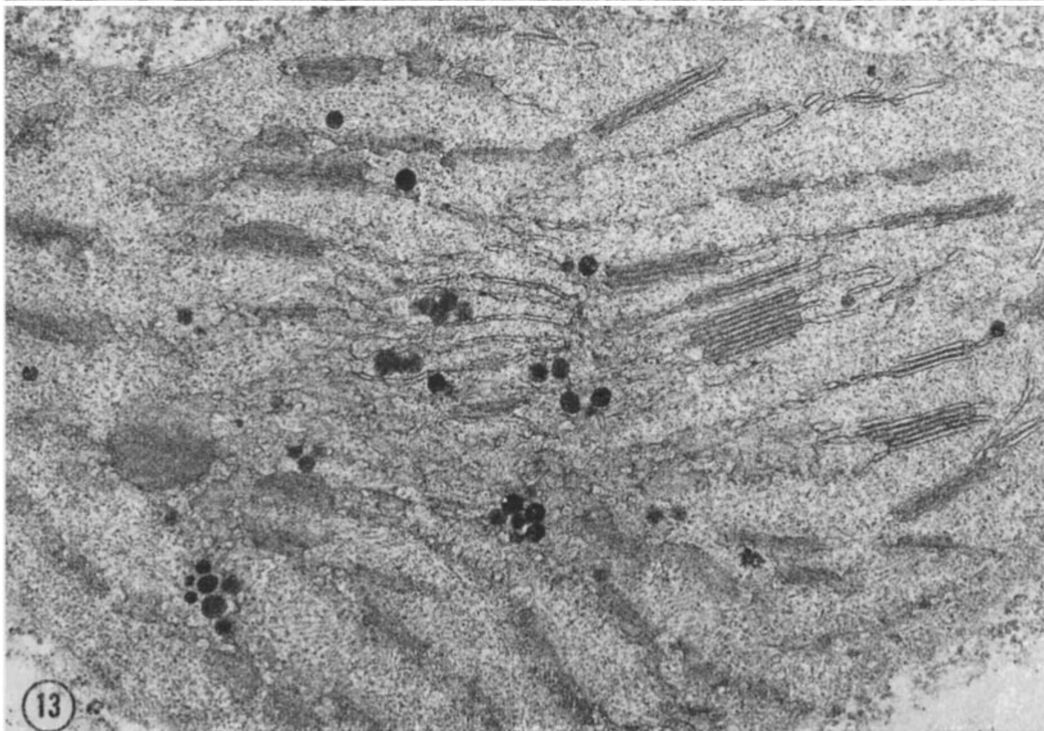


FIGURE 9 Plastid section from the same material as Figs. 7 and 8. At several points along the primary lamellar layers grana and clusters of tube elements are found. The tube elements are formed around the perforations in the lamellar layers, and clusters of tube elements form connections between adjacent layers. In the stroma, bundles of small diameter tubules are found. Many of the tubular bundles are oriented parallel to the lamellar layers and are observed as continuous structures over considerable distances. Facing the lamellar perforations and the tube element clusters, bundles of small diameter tubules are oriented at an angle to the primary lamellar layers and seem to be connected to the tube elements. × 67,000.



FIGURES 10 and 11 Plastid sections from the primary leaf of barley seedlings illuminated for 24 hr under 20 lux. Numerous grana with up to 8–10 discs per stack and large crystalline prolamellar bodies with narrow (Fig. 10) and wide (Fig. 11) spacing of the tubular material are seen. Small diameter grana are present as inclusions in some of the prolamellar bodies (Fig. 11). Fig. 10, × 38,000. Fig. 11, × 29,000.





FIGURES 12 and 13 Plastid sections from the primary leaf of barley seedlings illuminated for 24 hr under 20 lux followed by a 30 min exposure to 3200 lux. The crystalline prolamellar body material present prior to the period of high light intensity has been dispersed into primary lamellar layers, consisting of large membrane discs with numerous perforations, shown in cross-section (Fig. 12) and surface view (Fig. 13). Discs in the grana are connected to the primary lamellar layers. Osmiophilic globuli and small diameter tubules are present in the stroma. Fig. 12, × 45,000. Fig. 13, × 39,000.

In the angiosperms, plastids containing both grana and crystalline prolamellar bodies occur in the meristematic tissue of plants grown in the light and subsequently placed in darkness for a prolonged period prior to the analysis (13, 24, 27–29, 31, 32, 38).

Chloroplasts in mesophyll cells of sugar cane leaves, either grown under a 14 hr photoperiod of 20,000 lux or grown initially in darkness and subsequently greened at 5000 lux, contain crystalline prolamellar bodies together with an extensive grana-stroma lamellar system (22). In contrast, plastids in the adjacent bundle sheath cells of the same leaves lack crystalline prolamellar bodies and may or may not have a few grana, depending on the stage of plastid development.

In younger leaves of maize seedlings growing under a light-dark cycle, crystalline prolamellar bodies formed during a dark period disappear in the following light period (39). Plastids of the primary leaf of dark-grown barley seedlings which have formed grana during a 6 hr illumination (3200 lux) produce numerous small crystalline prolamellar bodies after the seedlings are returned to darkness for 6 hr (16).

Crystalline prolamellar bodies have been observed in excised leaves and whole seedlings of beans greened or kept under low light intensity and controlled temperature (7, 30, 52). Plastids containing both crystalline prolamellar bodies and grana also occur in leaves of chloroplast mutants in maize (9, 35) and barley (15, and K. W. Henningsen, J. E. Boynton, and D. von Wettstein, data in preparation) when the seedlings are grown under low light intensity to prevent bleaching of the leaf pigments. The presence of protochlorophyllide in leaves greened under low light intensity has been inferred from the absorption spectrum of total extracts of leaf pigments (7) and from the increase in the in vivo absorption in the chlorophyll region upon exposure of the leaves to higher light intensity (30).

As shown in the present investigation the sequence of pigment and structural changes in etioplasts of dark-grown barley seedlings upon continuous illumination with low light intensity results in plastids that contain large amounts of chlorophyll *a* and *b* in a lamellar system with grana as well as a small amount of protochlorophyllide and crystalline prolamellar bodies.

Crystalline prolamellar bodies formed in the plastids during growth in darkness are transformed and largely dispersed into primary lamellar layers by the time the seedlings have been illuminated for 2 hr with 20 lux. The rate of membrane dispersal is lower under illumination with 20 lux than is observed in similar leaves illuminated with 3200 lux (J. E. Boynton and K. W. Henningsen, data in preparation) where the dispersal process is almost completed after illumination for 1 hr. Whether the slower rate of dispersal is a direct effect of light intensity on the dispersal reaction or an indirect effect resulting from a slower tube transformation under low light intensity will have to be analyzed in further experiments.

The observation that the protochlorophyllide and not the phytylated pigment is rapidly converted agrees with earlier investigations (1, 2, 19, 25, 43, 50). The protochlorophyll formed during growth in darkness disappears within 30 min after onset of illumination under high as well as low light intensity, due to either a slow conversion or photooxidation. It remains absent during the first few hours of illumination, but reappears in small amounts at all later stages of development. Chlorophyllide *a* is present at all stages of greening under low light intensity. Under high light intensity, chlorophyllide *a* is detectable only shortly after the onset of illumination, in spite of the fact that chlorophyll *a* is rapidly accumulated. This observation, that phytylation of the newly formed chlorophyllide *a* takes place faster under high light intensity, indicates that the rate of phytylation may be dependent on the rate of the energy metabolism in the leaves, as has been observed previously (50, 51). Furthermore, it may mean that the chlorophyllide *a* cannot be phytylated when it is associated with the tubular material of the prolamellar body in either the crystalline or transformed configuration. If so, phytylation of the chlorophyllide would become possible only when dispersal of the prolamellar body tubules has relocated the pigment on, or detached it from, the membrane system (14, 19).

The reappearance of protochlorophyllide in leaves greened under 20 lux coincides with the reformation of crystalline prolamellar bodies from the dispersed membrane material. In every case studied thus far, the presence of protochlorophyllide is associated with the presence of crystalline prolamellar bodies. These observations in connection with the aforementioned studies on reformation of protochlorophyllide and the simultaneous recrystallization of the prolamellar body

material (16) and the reported absence of crystalline prolamellar bodies in mutants of barley completely blocked in protochlorophyll synthesis (6, 15, 48, and K. W. Henningsen, J. E. Boynton, and D. von Wettstein, data in preparation) strongly indicate that protochlorophyllide is an indispensable component for the formation of prolamellar bodies with crystalline tube configuration.

Crystalline prolamellar bodies formed during greening under low light intensity have the same structural and physiological characteristics as the prolamellar bodies usually formed in dark-grown leaves. The wide and narrow spaced tubules in the crystalline prolamellar bodies formed under low light intensity are rapidly transformed and dispersed into primary lamellar layers when the leaves are exposed to sufficiently high light intensity. As the structural rearrangements of the plastid membrane system take place, the absorption maximum of the newly formed chlorophyllide *a* shifts from 683 to 672 mμ as is usually observed for the chlorophyllide shortly after illumination of dark-grown plants (1, 14, 16, 47).

Fine tubules located in the stroma of the plastids (4, 41) at all stages of development may be related to the stroma centers found in wheat and beans (11, 12). The occasionally observed association of the fine tubules with the tube element clusters suggests that a certain functional class of these fine tubules may serve as structural precursors of the tubules of the prolamellar body.

The transformation and dispersal of the crystalline prolamellar bodies reformed under dim light conditions take place at a higher rate than is found for the prolamellar bodies in dark-grown seedlings placed under illumination with either 20 or 3200 lux (J. E. Boynton and K. W. Henningsen, data in preparation). This sensitized transformation and dispersal observed in plastids preilluminated under low light intensity resembles the sensitized transformation and dispersal observed in leaves of 5-day-old dark-grown barley seedlings where the resynthesis of protochlorophyllide and the concomitant recrystallization of the prolamellar body material have been completed in darkness following a brief illumination (16). This sensitivity of the prolamellar bodies formed in dim light may be due to the fact that the pigment content in the tubes is higher than that present in prolamellar body tubes made during prolonged growth in darkness.

It could be that the cause for the formation of crystalline prolamellar bodies under dim light conditions is a difference in the rate of the formation of plastid proteins and/or lipids. Although dark-grown barley seedlings green at essentially a normal rate at 20 lux, their photosynthetic performance is greatly impaired under the same environment. It has been recently found that such seedlings examined at low light intensity do not show any detectable carbon dioxide fixation, measured as bicarbonate-^{14}C uptake, or any Hill reaction, whereas under high light intensity they show a high capacity for both reactions (18). The photosynthetic activity of the seedlings under dim light conditions is most likely too low to compensate for respiration, particularly if under these conditions light in the blue region constitutes a major part of the energy absorbed and thus effects a blue light stimulation of respiration as described previously (20, 21, 33, 34).

A high rate of protochlorophyllide synthesis relative to the rate of photoconversion could explain the reformation of crystalline prolamellar bodies under dim light conditions. One can argue that shading from the chlorophyll and carotenoids serves to diminish the intensity of the light reaching the protochlorophyllide and thereby causes a slow rate of photoconversion. However, since the reformation of protochlorophyllide is completed at a point where the amount of chlorophyll accumulated is still rather low, this explanation seems not very plausible. More probably, the protochlorophyllide accumulation is in some way related to the altered metabolism of these green leaves and their inability to photosynthesize under 20 lux.

In leaves placed in darkness following a brief illumination the resynthesis of protochlorophyllide stops at the same time that recrystallization of the prolamellar bodies is completed. In such leaves, then, all the protochlorophyllide sites are rapidly occupied, which leads to an inhibition of protochlorophyllide synthesis. If this picture is correct, the dim light must be able to support the synthesis of new protochlorophyllide carrying sites. During the time crystalline prolamellar bodies are accumulating in the plastids in addition to the grana, the carrier sites are synthesized in excess to those necessary for formation of grana and at a faster rate than protochlorophyll.

The authors wish to express their appreciation to

Professor D. von Wettstein for his interest and support. Our thanks are also due to Miss Karen B. Pauli for her skillful technical assistance.

This work was supported by grant GM 10819 from the National Institutes of Health and by grants from the Carlsberg Foundation and the Danish Natural Science Research Council to Professor D. von Wettstein.

Received for publication 4 June 1969, and in revised form 16 September 1969.

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