THE EFFECT OF LIGHT INTENSITY AND SUCROSE FEEDING ON THE FINE STRUCTURE IN CHLOROPLASTS AND ON THE CHLOROPHYLL CONTENT OF ETIOLATED LEAVES

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ABSTRACT

Changes in the fine structure of proplastids of etiolated leaves exposed to various conditions of light and darkness for 24 and 48 hours were investigated, and the chlorophyll content of the leaves so treated was determined *in vivo*. The light treatments were given while the leaves were floated on tap water or on a 0.2 M sucrose solution. Leaves floated on water under low light intensity (2 foot-candles) were low in chlorophyll and contained plastids with concentric rows of vesicles. Transferring the leaves back to darkness resulted in the disappearance of the concentric rigs and re-formation of vesicular centers together with straight rows of vesicles and tubules, evenly spaced throughout the stroma. Chloroplasts of leaves floated on a sucrose solution under low light showed large vesicular centers together with stacks of rows of elongated tubules. The same chloroplast structure was found in leaves floated on a sucrose solution in the dark, after having been exposed to weak light for 24 hours. Chlorophyll content in these leaves was the same as in leaves floated on water under high light intensity, where the chloroplasts had normal grana and lamellae. The effect of the investigated factors on plastid development is discussed.

Chloroplasts provide an almost unique material for physiological studies on development of fine structures in cell organelles. Their structural development can be markedly influenced by a number of physiological and chemical factors (see *e.g.* 4–7, 9, 10, 13, 17, 25, 26, also for further references), and, in certain chlorophyll mutants, this development is arrested at different stages (22–24). Chloroplasts thus offer obvious opportunities for studies on the correlation between external factors, quantitative and qualitative changes in chemical constituents, and changes in submicroscopic structures. This communication deals with a study on the effect of light intensity and sucrose feeding on the development of fine structure in plastids[1] of detached bean leaves together with the effect of these factors on chlorophyll accumu-

[1] Our increasing knowledge of the continuous and gradual changes in chloroplast structures of etiolated leaves after illumination makes it difficult to use the existing terms for developmental stages, such as "proplastid," "young plastid," "young chloroplast," with any degree of accuracy. In this work the term "proplastid" will be used for plastids containing no detectable amount of chlorophyll. Chlorophyll-containing plastids will be called "chloroplasts" without consideration of their internal structures. When referring to both of these types, the term "plastid" will be used.

lation. Both plastid structure and chlorophyll content were studied in the same leaf under various experimental conditions.

MATERIAL AND METHODS

Bean seedlings of the variety "Bulgarian" were grown in Vermiculite in complete darkness under constant temperature (26°C) for 12 days. The first leaves were then removed and exposed to different weak light. White fluorescent light was used throughout the experiments, which were carried out at 26°C.

Since we wanted to investigate changes in chlorophyll content simultaneously with structural changes in chloroplasts of the same leaves, the chlorophyll content had to be measured *in vivo* in intact leaves. Absorption spectra (550 to 700 mμ) were obtained for a number of individual intact leaves *in vivo* immediately after their removal from the dark-grown plants, and pieces of tissue were fixed for the electron

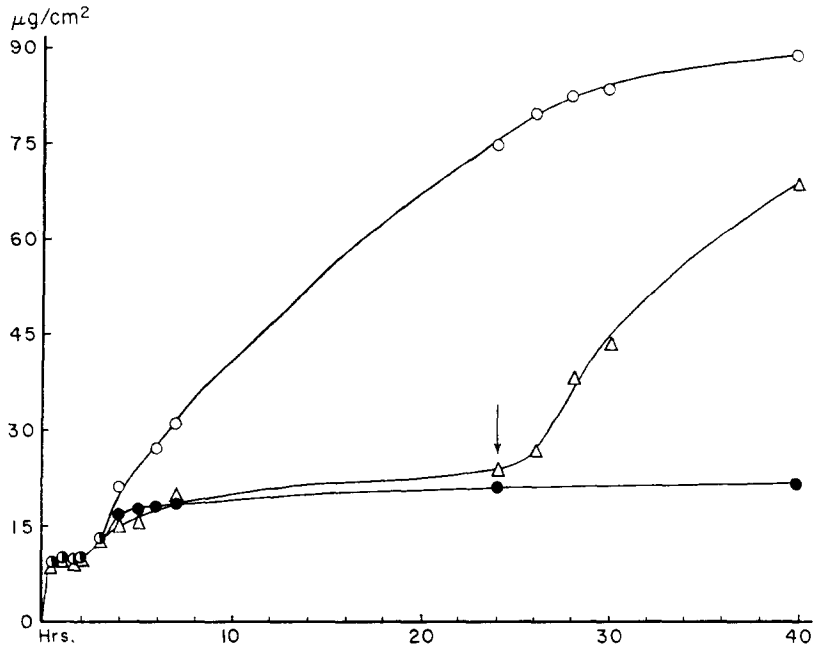


FIGURE 1

Formation of chlorophyll in dark-grown bean leaves under various conditions of light intensity. Chlorophyll formation was followed spectrophotometrically in single leaves. Changes in chlorophyll content are shown in a representative leaf for each treatment. Solid circles, a leaf illuminated with 12 ft-c for 48 hours; open circles, a leaf illuminated with 500 ft-c for 48 hours; Triangles, a leaf illuminated with 12 ft-c for 24 hours followed by illumination with 500 ft-c (arrow) until end of experiment.

treatments. Watering of the plants and removal of leaves were carried out under weak green safety light.

In some of the experiments, leaves were floated on tap water for 48 hours under "strong" white light (400 and 500 ft-c). In others, leaves were floated for 24 hours on tap water under "weak" white light (2 and 12 ft-c), and then floated on tap water for an additional 24 hours under "weak" light, under "strong" light, or in darkness, or were transferred to a 0.2 M sucrose solution in the dark. Leaves were also floated, from the beginning of the experiment, on the sucrose solution for 48 hours under continuous microscopic investigations. These leaves were not used in further experiments. Other leaves were exposed to the various treatments, and for these leaves *in vivo* absorption spectra were obtained after 24 and again after 48 hours. Simultaneously with the spectrum analysis small pieces were cut from the leaves and fixed immediately for electron microscopy. Thus, during the second 24 hours of the experiment, changes occurring in absorption spectrum and in plastid structure could be observed in the same leaf.

Spectrophotometric observations were carried out with a Beckman DK 1 recording spectrophotometer by the method of Shibata (14–16). The leaves were

placed between a piece of opal and a piece of transparent glass. The reference beam passed through two layers of lens paper placed between pieces of the same kinds of glass. In this way, the zero point was shifted down and the noise reduced. The 100 per cent adjustment was kept constant at an arbitrary value during all measurements. For electron microscopy the same procedure as described previously (4)—standard OsO_4 fixation and embedding in methyl-butyl methacrylate—was used.

Leaf samples for electron microscopy were always taken under the light conditions of the experiment or

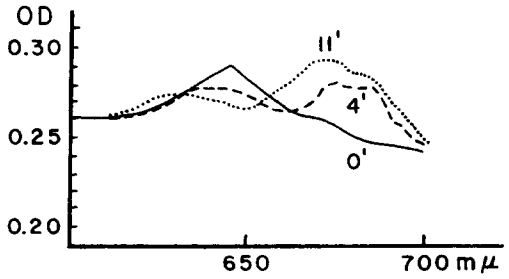


FIGURE 2

The transformation of protochlorophyll to chlorophyll in an etiolated bean leaf exposed to 2 ft-c during 0, 4, and 11 minutes.

under weak green light, and the leaves themselves were transferred to the spectrophotometer in complete darkness.

Total chlorophyll content was estimated from the absorption curves for the intact leaves, and are given in $\mu g/cm^2$ of leaf area. The conversion factor was determined in the following way: Leaves from dark-grown plants were exposed to light for different periods, their surface area was measured, and the absorption spectra were determined *in vivo* for the single intact leaves. These leaves were then separately extracted quantitatively with 80 per cent acetone. From the absorption spectra of the extracts the chlorophyll content per cm^2 leaf area of the various leaves was calculated, using the conversion factors of Mackinney (8). A strong and significant correlation was found between these values for chlorophyll content of the single leaves and the difference (D) between their *in vivo* absorbance at 550 mμ and the absorption maximum ($r = 0.8$, $t = 4.2$, $p < 0.001$). Therefore, D multiplied by a factor, which was found to be 0.015, was used for estimating chlorophyll content of the leaves.

Plants 12 days old were used throughout the experiments, because in older leaves (15 to 17 days) chlorophyll synthesis was less vigorous, while younger leaves (8 to 9 days) were usually too small to allow insertion in the spectrophotometer, after removal of pieces for electron microscopy.

RESULTS

Progress curves for chlorophyll accumulation in some of the detached bean leaves floated on water during illumination with 12 ft-c and 500 ft-c are given in Fig. 1. Similar curves were obtained with other leaves, although the absolute chlorophyll content varied considerably among identically treated leaves. No differences could be found between illumination with 2 ft-c and 12 ft-c or with 400 ft-c and 500 ft-c. These curves are in general agreement with the extensive work done on chlorophyll formation and greening (1). Chlorophyll was formed by transformation of protochlorophyll (Fig. 2) and increased under both low and high light intensity. Under illumination of 400 ft-c the transformation of the existing protochlorophyll took about 1 minute, while about 11 minutes were necessary for this process with 2 ft-c. In light of 400 ft-c and 500 ft-c the chlorophyll content rose for about 30 hours before leveling off. In weak light the chlorophyll content increased only for approximately 5 hours and remained at a low level even after 40 hours of continuous low illumination. When leaves were exposed to 400 ft-c and 500 ft-c after 24 hours

TABLE I

Amount of Chlorophyll (in Micrograms per Square Centimeter) in Etiolated Bean Leaves after Various Treatments

Leaves		$\mu g/cm^2$ chlorophyll after			
		24 hours		48 hours	
Exposed to light intenstity of	Floated on	Range	Mean	Range	Mean
2–12 ft-c	Water	21–45	34±1.2*	21–40	32±4.2
2 ft-c	0.2 M Sucrose	59–69	63±3.0	72–90	80±3.9
400–500 ft-c	Water	54–75	61±3.6	66–90	77±6.0

* Standard error.

under weak light, the chlorophyll content rose sharply and after 15 to 20 additional hours reached the level of the chlorophyll in the leaves exposed to 400 to 500 ft-c for 2 days. Similar results were obtained by Stern *et al.* (19) with *Euglena*, where pigment formation remained low even after 200 hours of illumination with 7 ft-c.

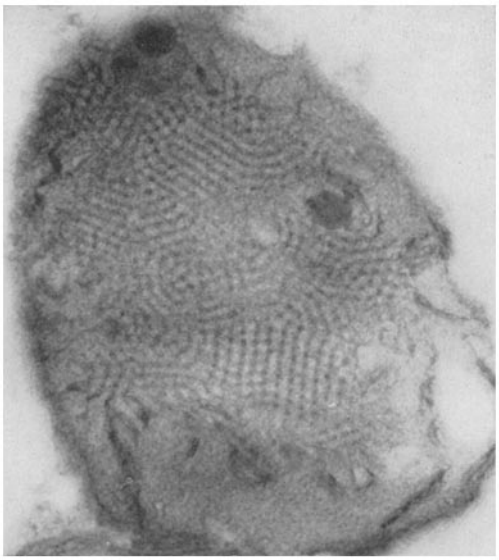


FIGURE 3

Proplastid from a leaf of a 12-day-old dark-grown bean plant. The proplastid contains a well developed vesicular center. × 30,000.

Leaves floated on a 0.2 M sucrose solution had a higher chlorophyll content than leaves floated on water under identical illumination. The amounts of chlorophyll in the leaves after 24 hours and 48 hours are given in Table I.

After short periods of illumination, the duration of which was dependent on the light intensity, a shift of the absorption peak occurred. In agreement with Shibata (14), we found that after 1 minute illumination (500 to 1000 ft-c) the peak was at 680–682 mμ, and it then shifted to 670–673 mμ. No additional shifts could be observed in our material. The same peak, 670–673 mμ, was also found in leaves from plants grown under normal light conditions in a greenhouse. Thus, no difference in the position of the peak was found between the experimental material and "normal" bean plants.

Leaf expansion during the first 24 hours did not exceed 8 per cent of the initial leaf area, except for leaves floated on sucrose, which expanded up to 28 per cent. The amount of chlorophyll in these leaves may thus be even somewhat higher than calculated. Also, during the second day leaf expansion remained less than 8 per cent of the initial area, independently of treatment,

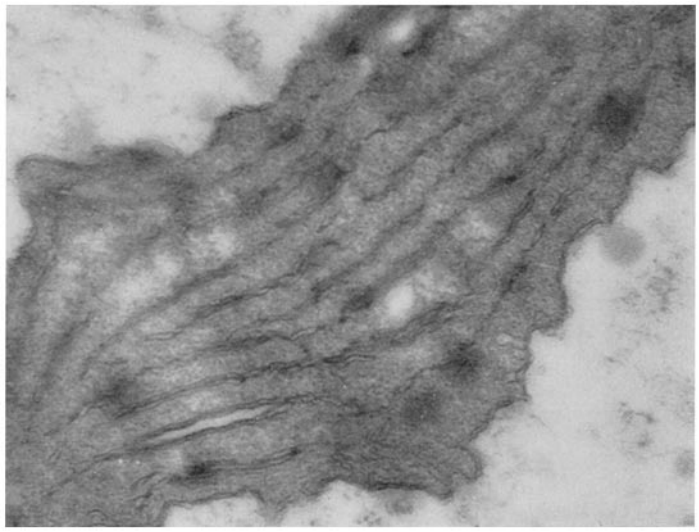


FIGURE 4

Chloroplast from an etiolated bean leaf which was exposed to light of 400 ft-c for 24 hours, while floated on tap water. Chlorophyll content of the leaf, 61 μg/cm². (For explanation of structure see text.) × 40,000.

except for leaves which were transferred from water under light of 2 ft-c to sucrose in darkness. This treatment, however, does not influence the chlorophyll content of the leaves.

STRUCTURAL CHANGES

Plastids in leaves of 12-day-old dark-grown plants showed the well known vesicular centers (prolamellar bodies), with or without fusion of the single particles, as well as a number of rows of vesicles (Fig. 3). The leaves showed a typical absorption spectrum for protochlorophyll with a peak at 648–650 mμ. Since in preliminary experiments the same plastid structures were found in all the etiolated leaves examined, it was not necessary to take samples from all the leaves at the beginning of the experiments.

In leaves treated for 24 hours in strong light, the plastids contained both lamellae and grana of 3 to 6 layers, as well as rows of vesicles (Fig. 4). Occasionally small vesicular centers still remained. In those cases, grana were often arranged around the vesicular centers like wheel spokes round an axis. In the leaf from which the chloroplasts pictured (Fig. 4) were taken, the chlorophyll content was approximately 60 μg/cm². After 24 additional hours under light of 400 ft-c, the chlorophyll content rose to 80 μg/cm². Structurally, there was a tendency toward disappearance of vesicles, straightening out of the lamellae, and a rise in the number of grana, which appeared to have increased both in length and in number of layers (Fig. 5). This is similar to the normal course of development of plastids in leaves attached to the plant. It shows that isolated leaves floated on tap water, given sufficient light intensity and a certain temperature, will develop normal plastid structures, in spite of the physiological differences which are known to exist between detached leaves and leaves *in situ*.

In leaves exposed to 2 ft-c for 24 hours, the prolamellar body is broken up and the vesicles are arranged in more or less concentric rings (Fig. 6). The occurrence of the ring structures is fairly constant in all the cells of the leaf. After an additional 24 hours under the same conditions, no increase in chlorophyll content was found (Table I) and the plastid structure, too, remained approximately the same (Fig. 7). There was, however, a tendency for the vesicles which composed the rings to lengthen and to form tubules. The rings themselves were frequently more elongated and formed ellipsoids. Sometimes the rings were not complete and a beginning of a parallel arrangement of the layers might be seen. Inside the layers the tubules might overlap one another or even occur side by side (Fig. 7*a*, *b*).

When after 24 hours under low light intensity these leaves (containing chloroplasts with ring structures) were transferred to high light intensity for 24 additional hours, the rings no longer appeared and instead perfectly normal lamellae and grana were found (Fig. 8). During this period the chlorophyll content rose to 60–80 μg/cm²,

FIGURE 5

Part of a chloroplast from an etiolated bean leaf which was exposed to light of 400 ft-c for 48 hours, while floated on water. Chlorophyll content of the leaf, 79 μg/cm². × 70,000.

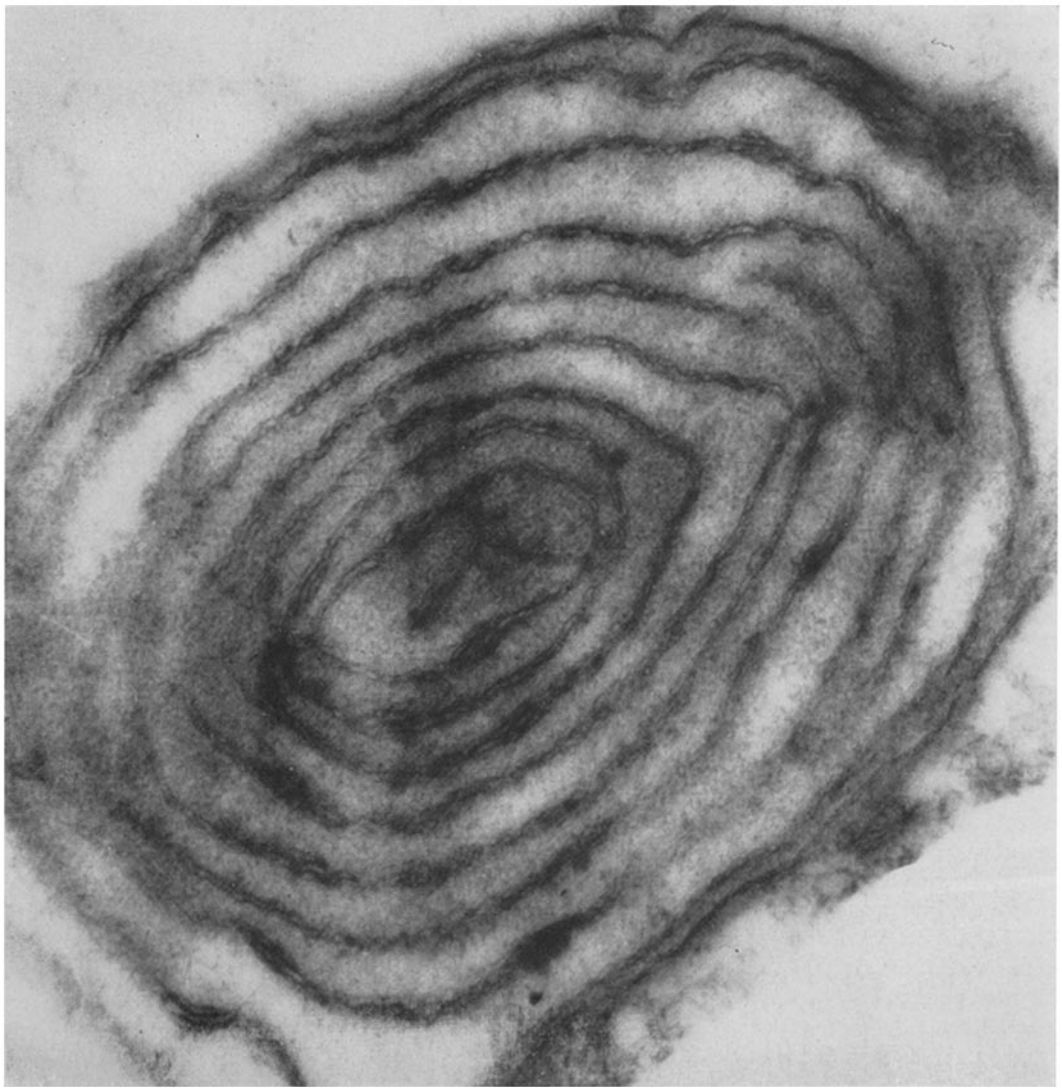


FIGURE 6

Chloroplast from an etiolated bean leaf which was exposed to light of 2 ft-c for 24 hours, while floated on water. Chlorophyll content of the leaf, 33 $\mu g/cm^2$. × 90,000.

reaching approximately the same level as in the leaves which were exposed to 400 ft-c for 2 days (Fig. 5).

The plasticity of the ring structures under changing conditions was also demonstrated by transferring leaves after 24 hours at 2 ft-c back to darkness. Again the submicroscopic structure changed and, instead of ring structures, vesicular centers were found, as well as relatively straight layers of either double lamellae or elongated tubules. These layers were dispersed at more or less regular intervals in the stroma (Fig. 9). As expected, the chlorophyll content did not change in the dark and remained at the same level as after the first 24 hours at 2 ft-c. Leaves, then, containing a "low" amount of chlorophyll—20 to 45 $\mu g/cm^2$—may, depending on light condition, contain plastids with either ring structures or vesicular centers together with rows of lamellae or tubuli.

On the other hand, all the leaves which, during some part of the experiment, were exposed to "high" light intensity and therefore contained higher levels of chlorophyll showed normal lamellar structure, independently of their previous history. Thus, perhaps a certain level of chlorophyll could be necessary and sufficient for normal formation of lamellae. It was therefore attempted to raise the level of chlorophyll in the leaves without exposing them to high light intensity.

Since it is known that carbohydrate feeding increases chlorophyll content in detached leaves (27), the leaves were floated under 2 ft-c for 24 hours on a 0.2 M sucrose solution. This concentration was chosen because it resulted, as found also by Wolf and Price (27), in optimal increase in chlorophyll, without apparent decrease in leaf turgor.

The chlorophyll content of the leaves treated with 0.2 M sucrose under 2 ft-c was equal to that of the leaves floated on water under light of 400 ft-c (Table I). Nevertheless, plastid structure in the sucrose-fed leaves was different: Well developed and extensive vesicular centers remained in them, and in most cases these were accompanied by a small number of rows of elongated tubules. In most of the plastids, quite extensive vacuoles, probably containing starch grains, were found (Fig. 10). After an additional 24 hours under the same conditions, chlorophyll increased up to and even above the level of leaves floated on water under high light intensity for 2 days. Again a different plastid structure was found (Fig. 11): The well developed prolamellar body still remained, but occurred now together with long and straight layers of extremely elongated double-membrane structures. These layers tended

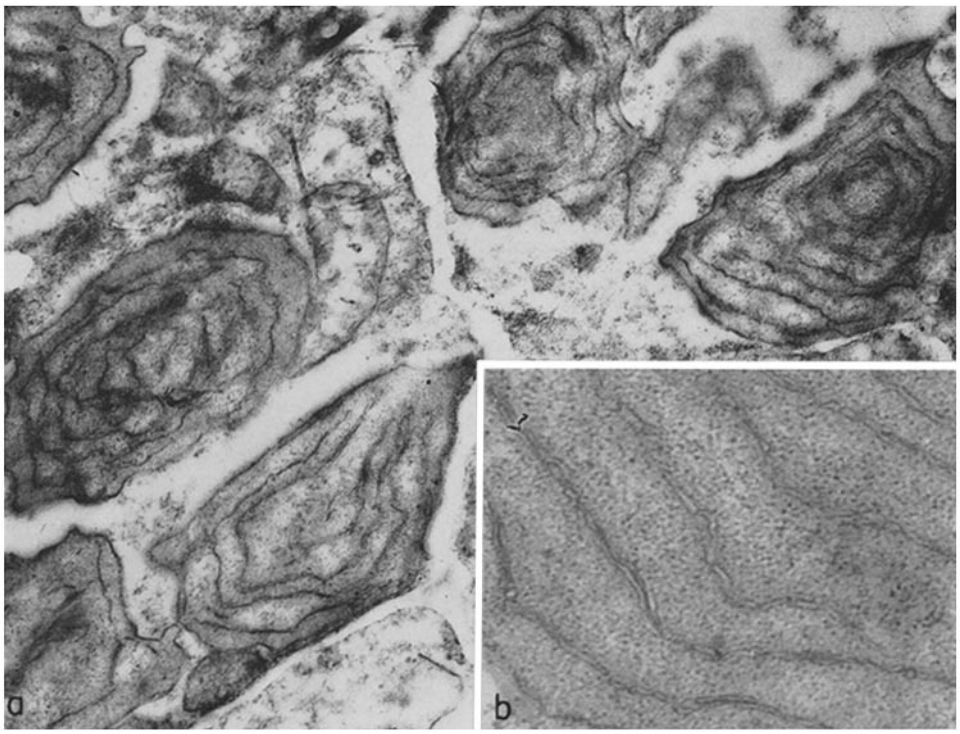


FIGURE 7

a. Cells containing chloroplasts from an etiolated bean leaf which was exposed to light of 2 ft-c for 48 hours, while floated on tap water. Chlorophyll content of the leaf, 37 $\mu g/cm^2$. × 22,000.
b. Detail of a chloroplast from another leaf, treated as described in Fig. 7 *a*. Chlorophyll content of this leaf, 31 $\mu g/cm^2$. × 73,000.

to be concentrated in a few stacks. Extensive "starch" vacuoles existed. Thus, depending on the treatment, leaves containing 60 to 90 $\mu g/cm^2$ chlorophyll may possess plastids with either normal lamellae and grana (when floated on water under 400 ft-c), or vesicular centers together with stacks of layers of elongated double-membrane structures (when floated on sucrose under 2 ft-c).

These differences in plastid structure between leaves having the same "high" chlorophyll content, but having been exposed to different treatments, show that the chlorophyll content may be a necessary, but cannot be a sufficient, factor for *normal* lamellar arrangement and grana formation in plastids.

The effect of feeding sucrose to the detached leaves was also studied in the absence of light. For this purpose, leaves with plastids containing ring structures (after having been floated on water under 2 ft-c for 24 hours) were transferred to a 0.2 M sucrose solution and kept 24 hours in darkness. After this dark period the ring formations disappeared, and the chloroplasts contained one or more vesicular centers and a number of stacks of elongated double-membrane structures (Figs. 12 and 13). These chloroplasts were very similar to those which had received sucrose and light of 2 ft-c for 48 hours (Fig. 11). It should also be remembered that when leaves containing chloroplasts with ring structures were transferred to darkness *on water*, the ring structures disappeared and vesicular centers together with double-membrane structures were seen (Fig. 9). However, under the latter conditions the double-membrane structures were evenly dispersed throughout the stroma and did not occur in stacks. The effect of sucrose seemed to result mostly in a tendency toward stacking of the layers of double-membrane structures.

The markedly different plastid structures which resulted from the various treatments, and the

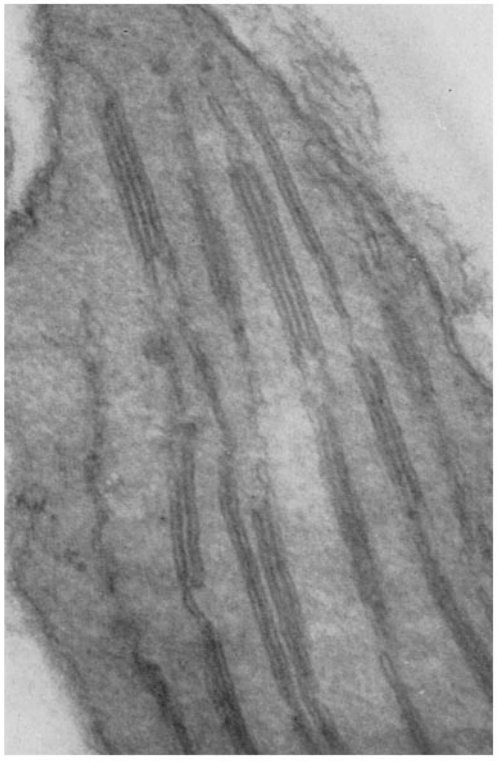


FIGURE 8

Part of a chloroplast from an etiolated leaf which was exposed to light of 2 ft-c for 24 hours and subsequently to light of 400 ft-c for 24 additional hours. During the illumination period the leaf was floated on tap water. Chlorophyll content of the leaf, 61 $\mu g/cm^2$. × 76,000.

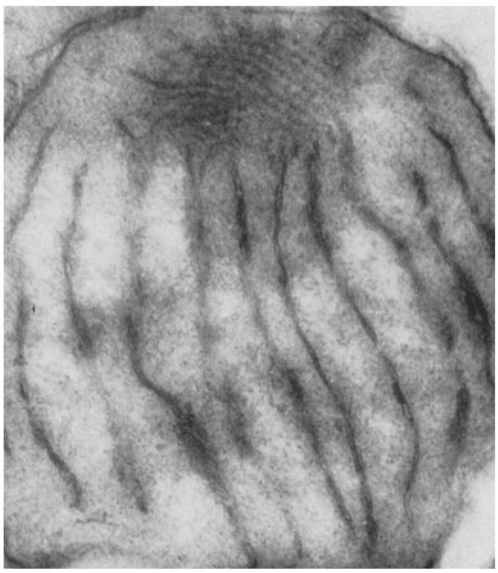


FIGURE 9

Chloroplast from an etiolated leaf which was exposed to light of 2 ft-c for 24 hours and kept for 24 additional hours in darkness. The leaf was floated on tap water. Chlorophyll content of the leaf, 35 $\mu g/cm^2$. × 36,000.

FIGURE 10

Chloroplast from an etiolated leaf which was exposed to light of 2 ft-c for 24 hours, while floated on a 0.2 M sucrose solution. Chlorophyll content of the leaf, 61 $\mu g/cm^2$. × 34,000.

mean chlorophyll content of the leaves, are given schematically in Fig. 14. The changes may be summarized thus:

1. Formation of ring structures in low light.
2. Preservation or re-formation of the vesicular center in low light under the influence of sucrose.
3. Disappearance of the ring structure, occurrence of rows of elongated double-membrane structures, and re-formation of a vesicular center in the dark after previous illumination.
4. Grouping together of the layers of these structures into well defined stacks under the influence of sucrose.

DISCUSSION

Owing to the importance of chlorophyll in photosynthetic processes and to the renewed interest in the structural aspect of physiological systems, the probable connection between chlorophyll accumulation and the development of plastid structure has been considered repeatedly during the past year. Results from a number of studies indicate a correlation between chlorophyll accumulation and the appearance of lamellae and grana structure (see *e.g.* 22, 28). Epstein *et al.* (2), too, found a simultaneous appearance of the first traces of chlorophyll and of structural units in the plastids of dark-grown *Euglena* after light exposure, and a parallel increase in structural complexity and chlorophyll content. On the other hand, among the *Cyanidium caldarium* mutants studied by Nichols and Bogorad (12), lamellar structure still exists in the mutant GGB, which contains no chlorophyll but has phycocyanin and carotenoids. In the mutant GGBI, which contains carotenoids but neither chlorophyll nor phycocyanin, there is still evidence of the existence of lamellar structure (L. Bogorad, F. Mercer, and K. Nichols, private communication by L. Bogorad). So far, then, no definite and general conclusion as to the necessity of chlorophyll for formation of lamellar structure in plastids can be drawn.

von Wettstein and Kahn (3, 25), studying the development of fine structure of plastids in bean leaves in darkness and light, suggested that the development of plastid structure after illumination of etiolated plants may be correlated with the time course of chlorophyll synthesis. These authors found that after a very short light exposure the vesicular centers were replaced by vesicles arranged in concentric layers. A second transformation, the beginning of grana formation, was found to occur after about 3 hours of strong illumination. Thus, the first of these steps, both of which are considered to be light-dependent, would coincide with the period of protochlorophyll-chlorophyll transformation, whereas the second step would be concomitant with the period of steep chlorophyll increase which is known to occur after a lag period. von Wettstein and Kahn also found a re-formation of tubules and vesicular centers when the leaves, after a short illumination, had been exposed to 2 to 6 hours of darkness.

The ring structures described in this paper seem to be identical with the concentric layers described by von Wettstein and Kahn, and with the ones reported from this laboratory after continuous illumination of *Zea mays* leaves at low temperature (4). (The plastid structures in the almost white part of

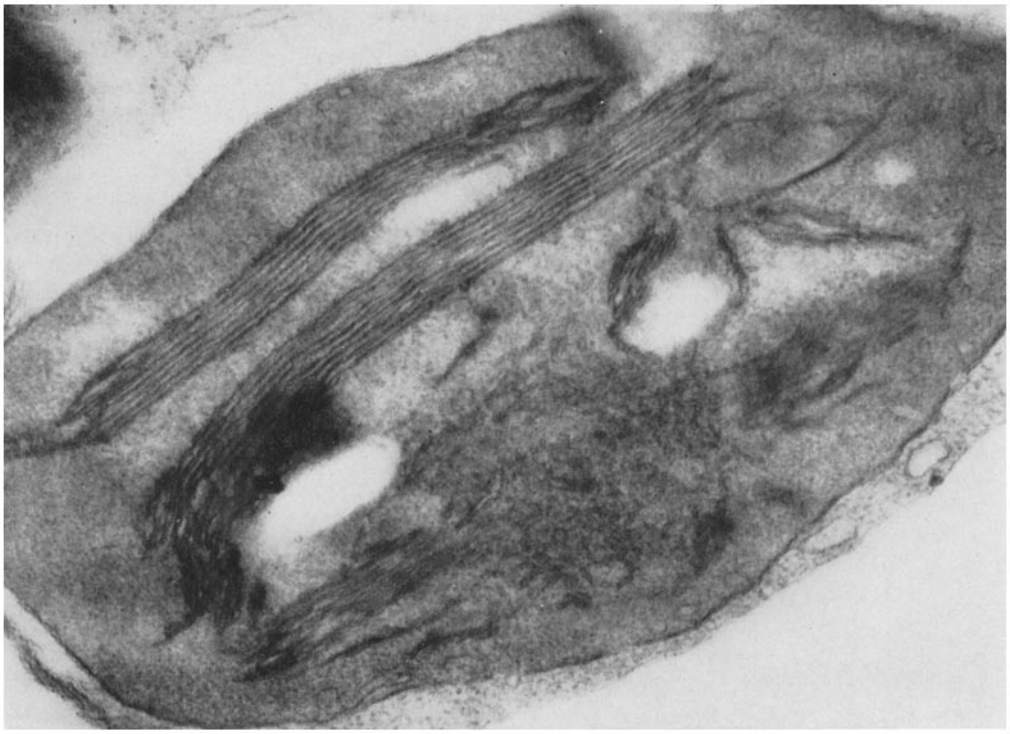


FIGURE 11

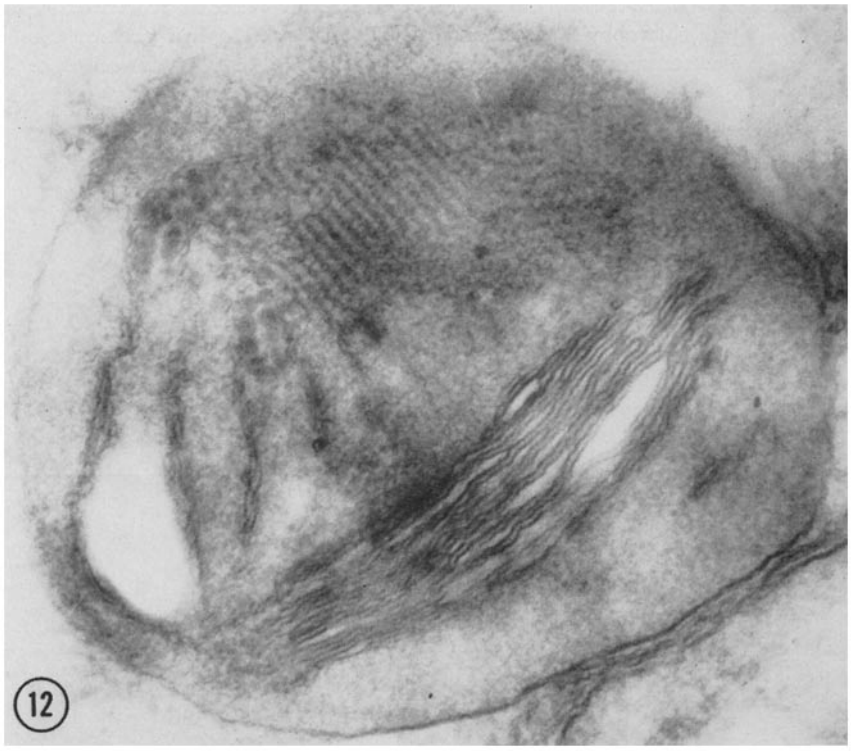
Chloroplast from an etiolated leaf which was exposed to light of 2 ft-c for 48 hours, while floated on a 0.2 M sucrose solution. Chlorophyll content of the leaf, 72 $\mu g/cm^2$. × 50,000.

the variegated leaves of *Liriope platyphylla* (11) seem to be of a similar nature, as well as the rings in albicated plastids in the light yellow part of the albimarginate *Abutilon* leaves described by Sun (21).)

Thus, formation of ring structures can be promoted experimentally by low light intensity at 25°C, as well as by high light intensity at low temperatures, or by short exposure to strong light. This formation, then, occurs under conditions which allow a limited amount of chlorophyll to be formed by photochemical processes, but which are unfavorable for accumulation of chlorophyll precursors and synthetic processes in general. Under any of these conditions, available protochlorophyll would be converted to chlorophyll, but other synthetic processes would be absent or slowed down, owing either to lack of precursors, to slow reaction rates, or to insufficient time. Formation of concentric layers of vesicles under all these conditions tends to confirm the idea that this process is a rearrangement of units already existing in the etiolated plastids (4, 25).

Plastids with vesicular centers appeared under the following conditions: (*a*) in leaves kept in continuous darkness, (*b*) in etiolated leaves which had been illuminated with low light for 24 hours while floated on either water or sucrose solution, and returned for 24 hours to the dark, (*c*) in leaves floated 24 or 48 hours on a sucrose solution under low light.

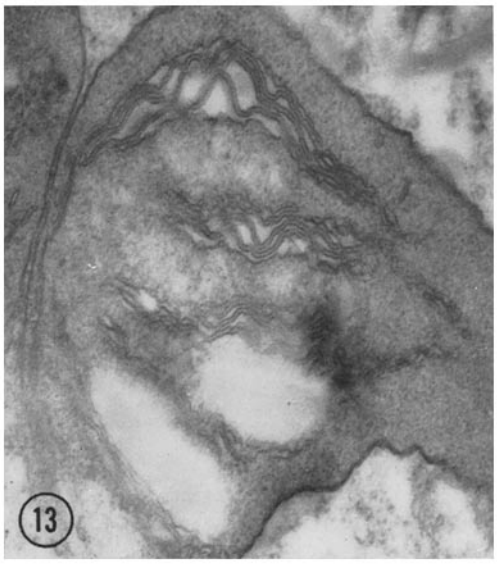
These structures therefore occurred in plastids with varying chlorophyll content, but always under conditions which may be considered favorable to accumulation of chlorophyll precursors. It is known that in dark-grown seedlings that have been illuminated and returned to the dark (see *e.g.* 18), regeneration of protochlorophyll occurs. In regard to the leaves floated on sucrose under low light intensity, the accumulation of protochlorophyll in them, concomitant with formation of chlorophyll, is more problematic. Sucrose feeding increases the amount of protochlorophyll produced (27), but it could be claimed



FIGURES 12 AND 13

Different forms of plastids from etiolated leaves which were exposed to light of 2 ft-c for 24 hours while floated on water, and thereafter transferred to darkness for 24 additional hours while floated on a 0.2 M sucrose solution.

Fig. 12. Chlorophyll content of the leaf, 35 $\mu g/cm^2$. × 52,000.
Fig. 13. Chlorophyll content of the leaf, 40 $\mu g/cm^2$. × 40,000.



that owing to the light given (2 ft-c) it would be transformed immediately to chlorophyll.

We did not attempt to measure spectrophotometrically the amount of protochlorophyll in the leaves which were floated on sucrose under low light, since this is difficult to do in the presence of higher amounts of chlorophyll. Instead, to ascertain whether protochlorophyll accumulates in material so treated, the following experiment was carried out: The chlorophyll content of these leaves was measured *in vivo*, the leaves were exposed to 500 to 600 ft-c for 1 minute, and the chlorophyll content was again determined. Ten leaves were examined and in all of them we found a small rise in chlorophyll content (Fig. 15). In leaves floated on water under the same light conditions, no increase of chlorophyll occurred. Frequently, the chlorophyll peak even decreased somewhat after the 1 minute treatment with 500

to 600 ft-c. Thus, in the leaves floated on sucrose under low light, chlorophyll precursors existed which could be transformed to chlorophyll by a short exposure to strong light. The occurrence of the vesicular centers under conditions favoring protochlorophyll accumulation points toward a possible relation between these phenomena. It may well be that the accumulated protochlorophyll

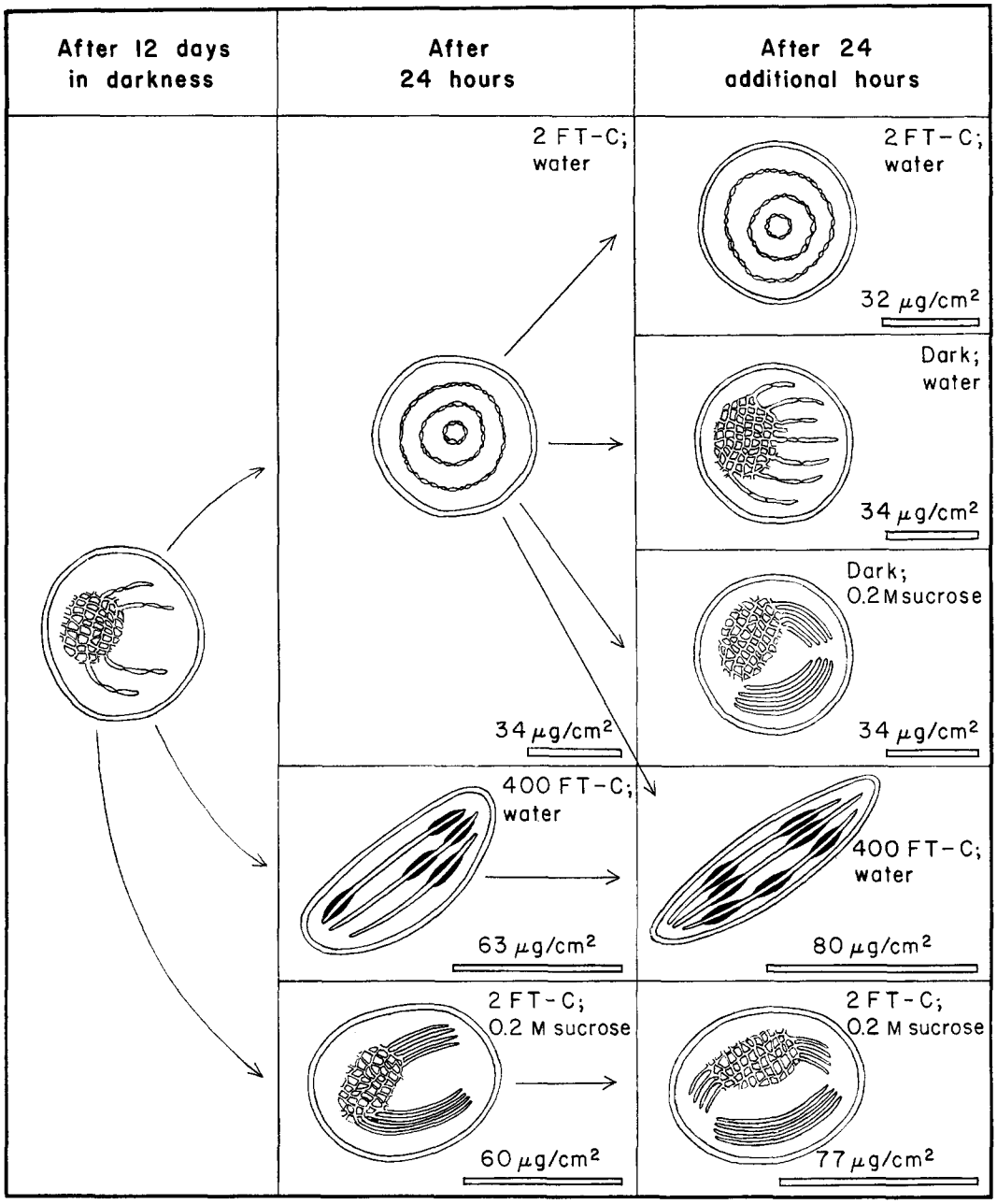


FIGURE 14

A schematic representation of the changes in fine structure of plastids in bean leaves exposed to various experimental conditions. Open bar, chlorophyll content of the leaves per cm^2 area. For explanation see text. No attempt has been made to present the plastid structures accurately.

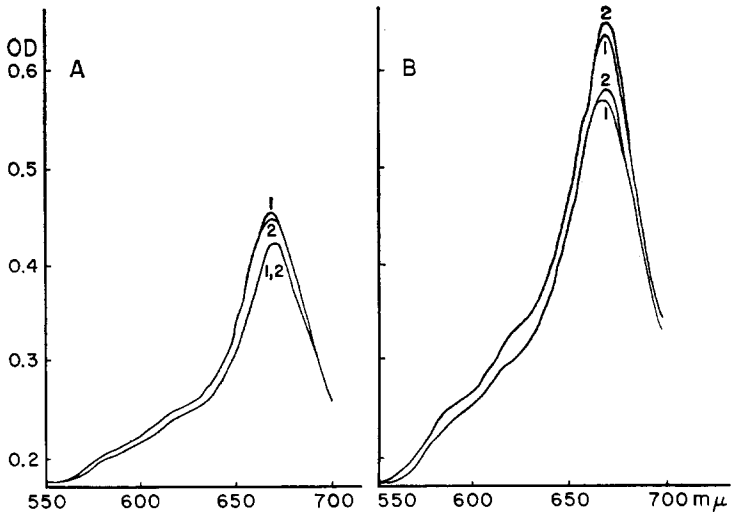


FIGURE 15

Changes in absorption spectra of the etiolated bean leaves, illuminated with light of 2 ft-c for 24 hours, after additional exposure to 500 ft-c for 1 minute. Changes in two leaves are shown for each treatment. *a*, leaves floated on water; *b*, leaves floated on a 0.2 M sucrose solution; *1*, absorption before the short illumination with 500 ft-c; *2*, absorption after this illumination. Note that increase occurs only in leaves floated on sucrose.

is located in the vesicular centers. Strugger and Kriger (20) reported that in proplastids of etiolated leaves of 3- to 4-week-old *Allium cepa*, red fluorescence was restricted to the structures which they consider to be the "grana," but which are probably identical with the vesicular centers.

A pronounced tendency toward stacking of tubules was found not only in leaves exposed to high light intensity, but also in leaves floated on sucrose under low light or in darkness. Under the two latter conditions the stacks resemble, in fact, giant grana. This suggests that stacking of tubules may not be a light-dependent process, but may be influenced by sucrose or its metabolic products. The difference between the normally occurring plastid structures in leaves exposed to high light intensity and those in the leaves treated with sucrose may be due to the fact that in one case the substrates and/or energy-rich compounds necessary for these formations are probably provided by products of photosynthetic processes occurring inside the plastids, whereas in the other case they are breakdown products of externally applied sucrose. The "normal" form of the lamellae and grana, as well as their spacing in the stroma, may depend on the exact nature of the constituent molecules. The latter may differ when derived from externally supplied sucrose rather than from the photosynthetic products formed in the chloroplasts. The finding of von Wettstein and Kahn (25) that the time of grana formation coincides, under their experimental conditions, with the phase of strong chlorophyll increase may be due to the beginning of photosynthetic processes at this stage which lead to the availability of photosynthetic products.

In this work, the effect of light intensity and sucrose feeding on plastid structure has been considered in connection with its effect on chlorophyll formation and accumulation. However, chlorophyll is only one of the chemical constituents that change both quantitatively and qualitatively during the development of chloroplasts. Further studies into these changes are requisite for a fuller understanding of the physiology of chloroplast development.

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