THE EFFECT OF LOW TEMPERATURE ON THE DEVELOPMENT OF THE LAMELLAR SYSTEM IN CHLOROPLASTS

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ABSTRACT

The influence of low temperature (3°C.) on development of submicroscopic structure in plastids of *Zea m.* leaves was studied. Leaves from 8-day old etiolated plants, with plastids showing the prolamellar body and few lamellae, were floated for 1 day on tap water both in the dark and in the light, at 26° and at 3°C. The structures remain unchanged in the dark, independent of temperature. Whereas in the light at 26°C., normal development of parallel compound lamellae and formation of grana occurs, in light at 3°C. ring structures are formed. Under the latter conditions protochlorophyll is converted to chlorophyll, although the *in situ* absorption maximum is different from the one for chlorophyll in plants grown in light at 26°C. When leaves were transferred from light at 3°C. to light at 26°C., ring structures in the plastids disappeared and normal development occurred. The possibility is discussed that development of parallel-arranged compound lamellae is due both to photochemical and synthetic processes, involving not only accumulation of chlorophyll, but also synthesis of other compounds.

Investigations of the morphology of the photosynthetic apparatus have been taken almost to the molecular level. Not only is the structure of the lamellar system in chloroplasts fairly well known, but as well the development of this fine structure has been described (for reviews see 3, 6, 12, 26). However, the physiological factors, which influence and determine this development, are not yet sufficiently known and only a limited amount of electron microscopic work dealing with this aspect has thus far been reported. In the physiological approach to chloroplast development the main interest has been centered in the concomitant appearance of chlorophyll and the lamellar system. This has been studied by examining chlorophyll content and plastid structure; (*a*) in algae after dark adaptation (25); (*b*) by comparing them in plants at different stages of development (21); (*c*) by examining chlorophyll mutants (14, 15, 22). The lamellar system develops either from a prolamellar crystalline body (4, 9) or directly from a more primitive stage (11). Von Wettstein, and later Mühlethaler and Frey-Wyssling have shown that in barley the prolamellar body accumulates only in etiolated plants, whereas this stage of development is bypassed when plants are grown in sufficient light intensities (13, 23). Appearance of the prolamellar body does not, therefore, seem to be a necessary step in lamellae formation. However, the investiga-

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tion of the development of the lamellar system in plastids from etiolated plants after illumination is of advantage, particularly because the environmental conditions, leading to formation of these lamellae, can easily be controlled (5).

The influence of external factors and of chemical substances on chloroplast development has been studied only using the light microscope (10, 24, also for references), the single exception being the work of Bogorad *et al.*, who showed an effect of iron deficiency on the chloroplast structure in *Xanthium* leaves (1). The effect of such external conditions on development of submicroscopic structures in general and of the submicroscopic structure of chloroplast in particular has not yet been described.

The present paper, which is a part of a broader investigation in progress, is concerned with the influence of low temperature on the development of the lamellar system from the prolamellar body.

MATERIALS AND METHODS

Seeds of *Zea mays*, var. Nave-Yaar 1958, were germinated in vermiculite and kept in a dark room at 26°C. for 8 days. The plants were watered two to three times under weak green light. After 8 days, plants in which the first leaf was about to pierce the coleoptile or just protruded through it, were selected. Only plants with coleoptiles 5 to 6 cm. long were used. The coleoptiles and the leaves were cut from the plants with a razor blade, the leaves freed from the coleoptile and floated in petri dishes on tap water. This operation was also carried out under weak green light. Detached leaves were used because of convenience in handling the material. Control experiments showed that the development of the plastids in these leaves during the experimental period was similar to that in whole plants. Leaves were exposed to the following conditions:

1. Darkness at 26°C. for 24 hours
2. " at 3°C. "
3. Light at 26°C. "
4. " at 3°C. "

The leaves were transferred to the cold room in light-tight containers and remained there in the dark for ½ hour. The boxes were then opened and the leaves exposed to the light. As light source a fluorescent light and an additional 40 W incandescent lamp were used giving an intensity of 200 FC at the site of the leaves.

In a few experiments, whole plants were exposed to the light in the cold room for 24 hours and then transferred to light at 26°C. for an additional 24 hours. Sections were cut from the leaves under the conditions of the treatments and immediately immersed in a veronal-buffered OsO_4 solution of pH. 7,4. Fixation was carried out for ¾ to 1½ hours in the cold. The sections were dehydrated in graded alcohols, embedded in a methyl-butyl-methacrylate mixture 1:4, and cut with a diamond knife using a Porter-Blum ultra-microtome. The sections were examined in an RCA EMU C3 electron microscope.

RESULTS

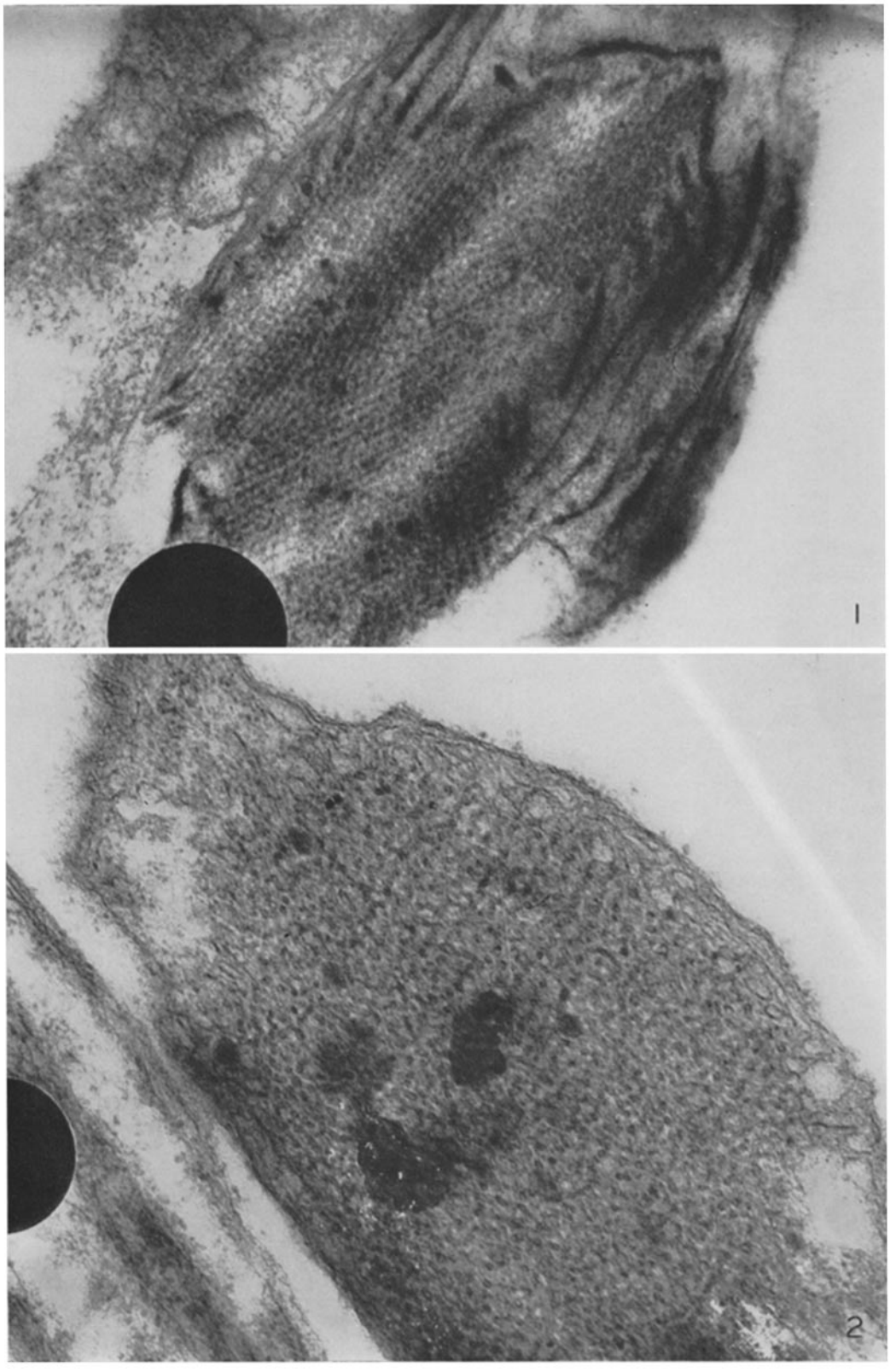
At the beginning of the experiment, the leaves were curled, yellow and, except when transferred to light at 26°C., remained in this state. Only in light at 26°C. did the leaf sheath expand, flatten out, and turn visibly green. Although no visible change to green occurred in the light at 3°C., nevertheless protochlorophyll in the examined leaves was converted to chlorophyll, as found by determining the absorption spectrum of whole leaves using the opal glass method described by Shibata (16). While the absorption spectrum of leaves kept in darkness showed a maximum at

FIGURE 1

Plastid from etiolated 8-day-old corn plant. The crystalline arrangement of the "prolamellar body" and lamellae protruding out from it are visible. Size of polystyrene particle 0,34 μ. × 82,000.

FIGURE 2

Plastid from 8-day-old etiolated corn plant after the detached leaf was floated for 1 day on tap water at 26°C. in the dark. Note connection between prolamellar body and outer plastid membranes. Size of polystyrene particle 0,34 μ. × 70,000.



647 mμ, indicating protochlorophyll, the maximum for leaves kept in the light at 3°C. was at 671 mμ. In leaves grown in light at 26°C., the maximum was at 675 mμ and absorption was much stronger than in the earlier cases. From the data of Koski *et al.* (8), Smith (19), Koski and Smith (7), Smith and Young (18), it is evident that at low temperatures up to 80 per cent of the protochlorophyll may be converted to chlorophyll during the first minutes of illumination, while little or no increase occurs during the subsequent illumination.

Blackening of the tissues in the OsO_4 fixative was dependent on the temperature to which the plants or leaves had previously been exposed. All those kept for 1 day at 26°C., either in light or darkness, blackened during fixation, whereas none of those kept at 3°C. showed darkening and the tissues remained pale brown. The blackening of the tissues was independent of illumination, and only depended on temperature. It can be assumed that the variation in blackening was not due to different penetration of the fixative through closed or open stomata but to compositional differences brought about by the treatment with the different temperatures.

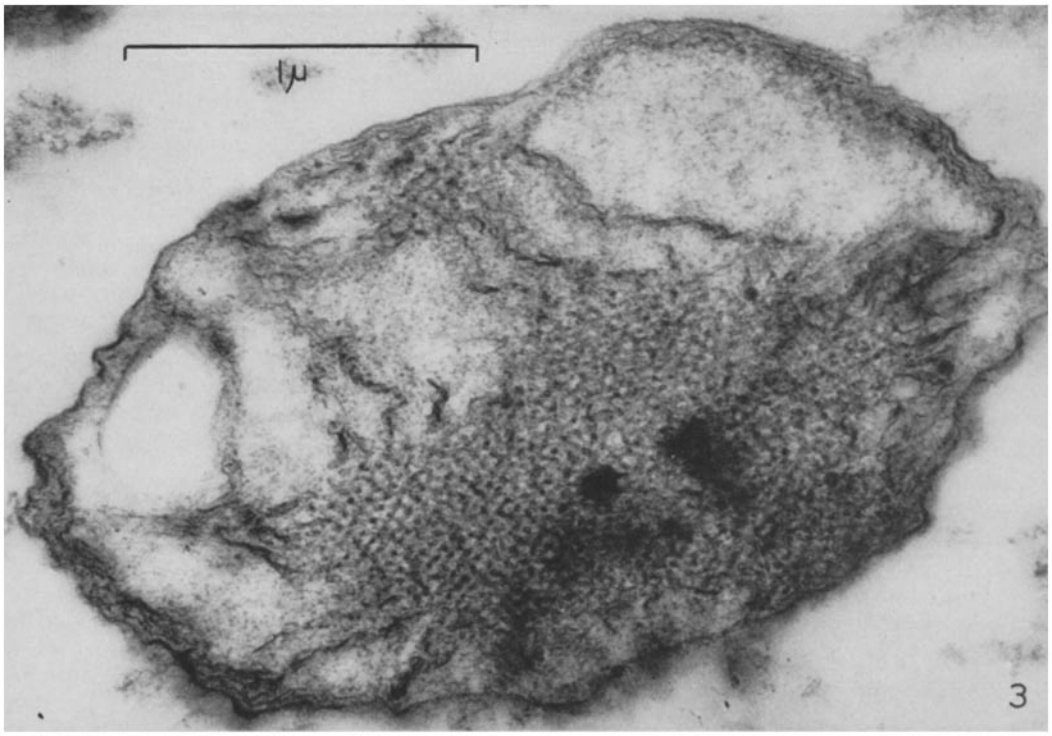
Chloroplast Structure:

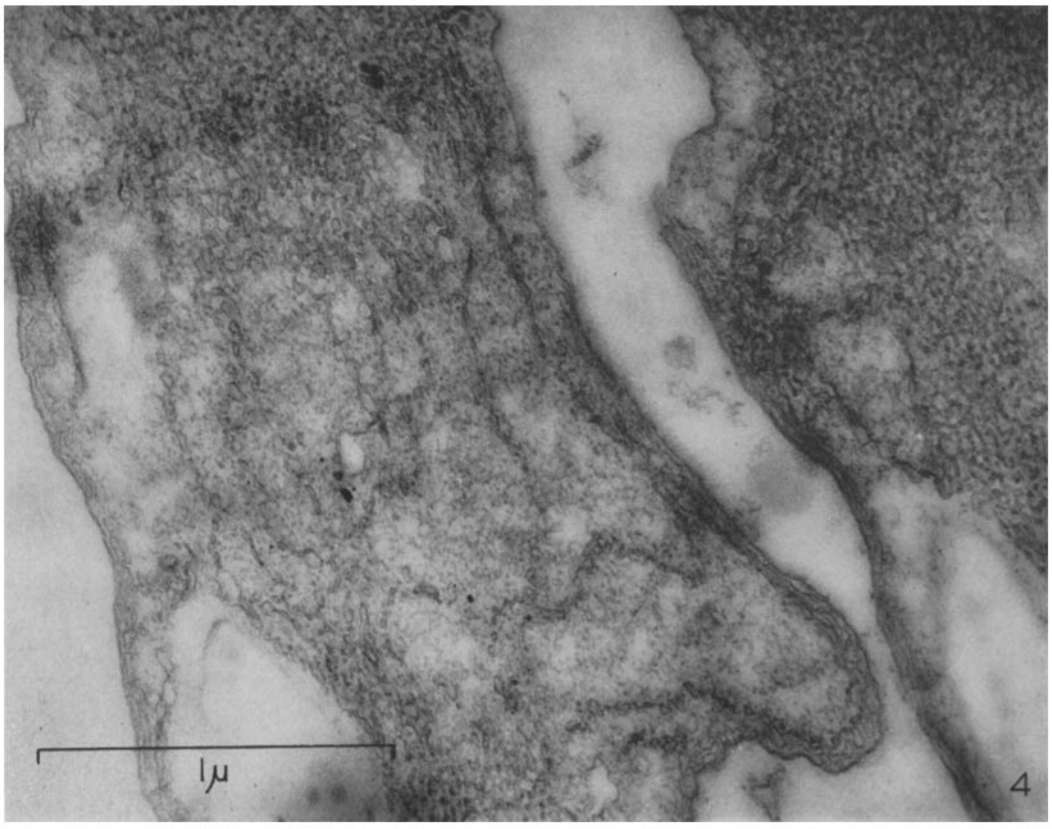
The starting material (8-day-old etiolated leaves) contained plastids as shown in Fig. 1. The "prolamellar" body is clearly visible and so are a number of lamellae radiating out from the structure. Whereas the prolamellar body was found in all the plastids, the number of lamellae varied from plastid to plastid. According to von Wettstein (21), protrusion of vesicles from the prolamellar body and formation of primary layers occur in barley even in the dark. In our experiments the corn plants were exposed occasionally to doses of very weak, green light, which is known to be weakly active in chlorophyll transformation. It cannot, therefore, be stated with certainty whether or not these lamellae were formed in the darkness or under the influence of this light. Vacuoles with a light inner core were frequently visible. These cores have usually been described as starch vacuoles. However, the leaves gave very poor iodine-potassium-iodide reactions and it seems to be far from certain that these structures actually contain starch grains. Occasionally different vacuoles, probably resulting from swelling of the plastids, were formed between the border membranes and the prolamellar body, which itself remained intact. Plastids in excised leaves that were kept an additional 24 hours in the dark at 26°C. did not change significantly (Fig. 2). It may be noted that the prolamellar body is not set apart from the plastid membranes, but it is connected to the latter by a complicated system of canals and vesicles. Certain differences appeared at 3°C. in the dark (Figs. 3 and 4), resulting mainly in the more frequent appearance of a network of small vesicles instead of the more compact prolamellar body in plants kept at the higher temperature. In these pictures it can be clearly seen that the "strands" protruding from the prolamellar body are the result of fusion of vesicles (Fig. 4).

The most striking differences were found between the light treatments at 3° and 26°C., respectively. Whereas the plastids developed normally at 26°C. and showed well developed lamellar systems with grana, the structures found after 24 hours, in the light at 3°C., were quite abnormal (Figs. 5 to 7). Instead of parallel-stacked lamellae a kind of "concentric ring system" could be seen. In most cases nothing remained of the "crystalline" prolamellar body itself. The concentric rings, however, are not completely comparable with the compound lamellae which developed in the light after 24 hours. They seemed still to be partly made up of small vesicles which resembled those found in plastids in the dark at 3°C. The light, therefore, did not seem to change the

FIGURES 3 and 4

Plastids from 8-day-old etiolated corn plant, the detached leaf of which was floated for 1 day on tap water at 3°C. in the dark. In Fig. 3 the crystalline arrangement of the prolamellar body is visible; in Fig. 4 it is seen to consist of a network of elongated vesicles. Fig. 3, × 47,000. Fig. 4, × 47,000.





structures themselves as much as it influenced their arrangement.

It was of interest to investigate whether these abnormal plastids could develop further at higher temperatures. Plants from which samples had been taken after 1 day at 3°C. in light were transferred to 26°C. and illuminated for another 24 hours, during which time they greened visibly. (At the end of this period nothing of the ring structure could be found in the plastids which now contained normal lamellae and grana (Figs. 9 and 10).) No differences between them and plastids from plants grown for 2 days in light at 26°C. could be found.

The changes induced by low temperature and light are not unique for the condition described. In preliminary experiments we found that the same changes occurred when etiolated leaves were exposed for 1 day to light at 26°C., while floating on 10^{-4} DNP (dinitrophenol) solutions. Under these conditions uneven greening of the leaves occurred, some areas remaining yellow. Plastids from these areas showed ring configurations (Fig. 8) similar to those kept in the light at 3°C.

DISCUSSION

A number of differences could be observed between plants kept for 1 day in light at 3°C. and those kept at 26°C. besides structural differences in plastids; at 3°C. the leaves remained curled, no expansion of leaf surface occurred, and protochlorophyll was converted to chlorophyll without or with only a very limited further accumulation of chlorophyll. At 26°C. the leaf turns green and the leaf sheath expands. Granick has found that during leaf expansion there is an increase in total nitrogen and protein both in the protoplasma and in the chloroplasts (2). This has been substantiated by the findings of Stäubli, who detected greater amounts of RNA in isolated "young" chloroplasts than in mature ones (20). Development of the lamellar system has frequently been associated with changes in chlorophyll content. Von Wettstein found an increasing amount of chlorophyll in developing plastids (21) and correlation between chlorophyll content and lamellar structure in barley mutants (22). Wolken (25) has shown that in *Euglena* upon dark adaptation both chlorophyll and lamellar structure are lost and that whenever chlorophyll is detected, lamellar structure is found.

The change from the prolamellar body to a system of lamellae may, therefore, be assumed to be dependent on a variety of factors, including synthesis of chlorophyll, protein, and other compounds. At 3°C., however, most of the synthetic processes are very much slowed down. Under this condition protochlorophyll conversion due to photochemical activity occurs with few or no other accompanying metabolic changes.

The same holds for leaves floated on DNP solutions. The concentration used is known to inhibit oxidative phosphorylation and does not allow ATP requiring processes to proceed.

It is impossible to tell whether the ring formations in the plastids, as seen in the electron microscope, are artefacts due to fixation or to the procedures following fixation, or whether these forms actually occur in the plants. However, since they were found only under the conditions stated and not in the other experiments, they are indicative of changes in the prolamellar body.

The formation of ring structure has been

FIGURES 5 and 6

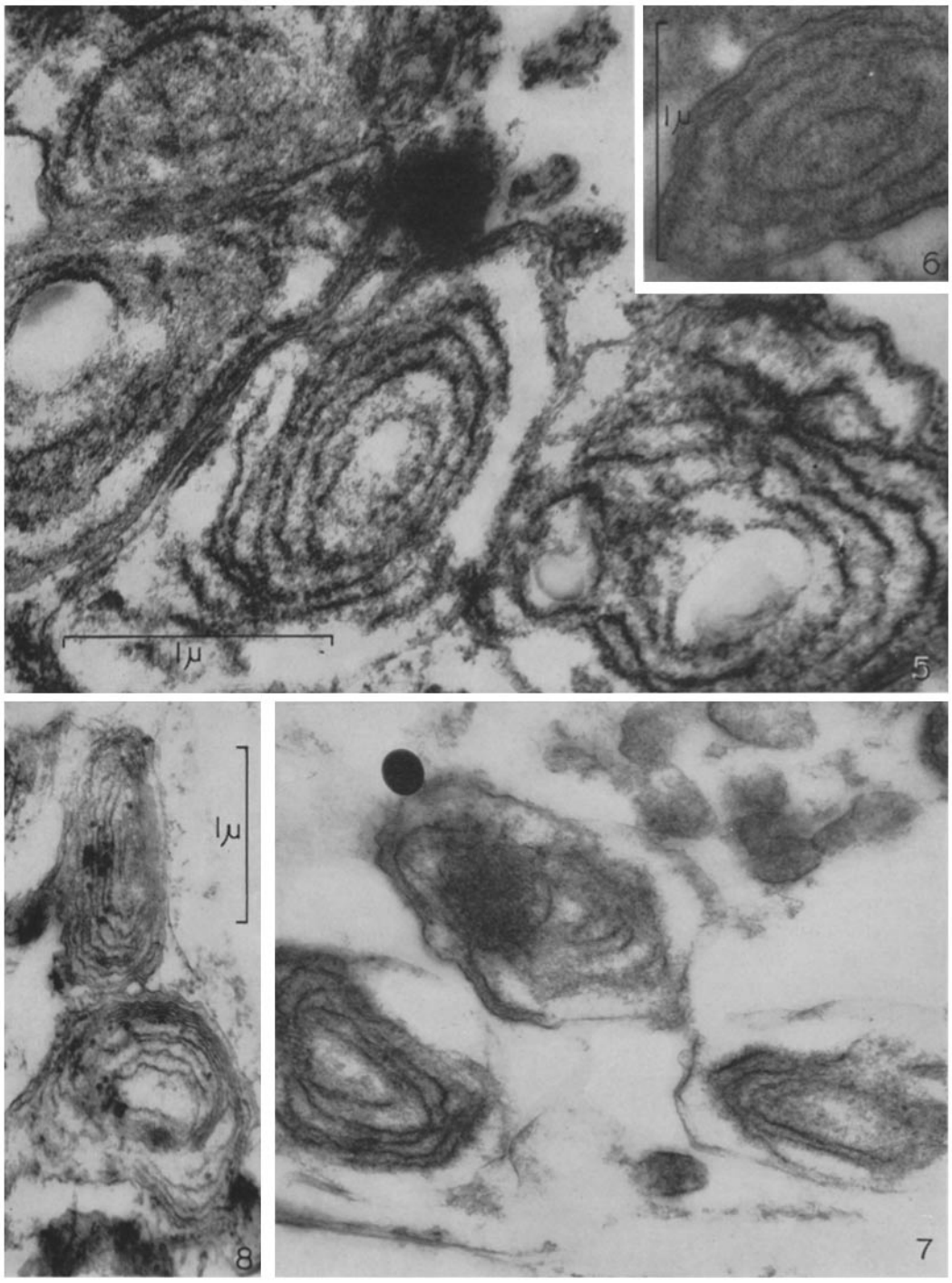
Plastids with "ring formations" from 8-day-old etiolated corn plants, the detached leaves of which were floated for 1 day on tap water at 3°C. in the light. Parts of the rings are built up by vesicles. Fig. 5, × 39,000. Fig. 6, × 35,000.

FIGURE 7

Plastids with ring formation from 8-day-old etiolated plants, after the whole plant was exposed to light for 1 day at 3°C. Size of polystyrene particle 0,34 μ. × 17,000.

FIGURE 8

Plastids in 8-day-old etiolated corn plants, the leaves of which were floated for 1 day on a 10^{-4} M DNP solution in the light at 26°C. × 26,000.



previously noticed in plant cells. Von Wettstein (23) found them after prolonged etiolation (20-day) in corn and also in the barley mutant *Xantha* 10, which is said to be devoid of both protochlorophyll and chlorophyll. Von Wettstein draws the conclusion that (*a*) chlorophyll is not essential for the formation of the lamellae proper, but rather for their arrangement and stacking and (*b*) that the parallel arrangement of lamellar discs is dependent on a photochemical reaction, leaving open the question whether the presence of chlorophyll is sufficient to secure the further development of chloroplast lamellae. It is difficult to ascertain whether the ring structures found in our experiments are identical with those described by von Wettstein. However our results show that at least with 8-day-old etiolated corn plants light is necessary, not only for the parallel arrangement of the lamellae but even for the formation of ring structures. This 8-day-old material is probably less deranged than the older material used by von Wettstein. Our results also indicate that the amount of chlorophyll accruing from the conversion of protochlorophyll, existing in the plastids of etiolated leaves, is not sufficient to secure normal development and parallel arrangement of lamellae.

It appears, therefore, that the prolamellar body in the absence of synthetic processes, but in presence of (and perhaps under the influence of) small amounts of chlorophyll is broken up into more or less concentric layers or spheres.

The actual build-up of straight compound lamellae could well be the result of increased chlorophyll production and synthesis of other compounds. This is indicated by the appearance of normal lamellae and grana, when plants containing plastids with ring formation were transferred to light at 26°C., *i.e.* to conditions favouring synthetic processes. It may also be noticed that the absorption peak for chlorophyll in illuminated plants grown at 3°C. is at a different wave length than that for plants grown at 26°C., when measured in intact leaves (671 versus 675 mμ). No such differences have been reported for extracts. It is of interest that Shibata (17) when studying the transformation of protochlorophyll to chlorophyll in intact leaves at normal temperatures, found this process to consist of 3 steps, the first occurring in the light, the other two in darkness. Absorption maxima for the steps occurring in darkness were 671 to 672, and 674 to 676 A, respectively. Since he found differences in the spectra only in intact leaves, but identical spectra in ether extracts, he maintained that "it is probable that the change from one (form of chlorophyll) to the other is a change *in vivo* in the nature of the combination of chlorophyll a with other substances, presumably protein." Whereas the peak in the case of those of our leaves which have been kept at 26°C. in light coincides with the peak for the "finished" chlorophyll in Shibata's material, the plants kept in light in the cold show a peak identical with his after the first dark reaction. These findings too make it plausible that the difference between plastids with ring formation and those containing normal lamellae is not due to different amounts of chlorophyll alone, but also to differences in other components, presumably proteins.

It may be concluded, therefore, that the formation of lamellae from the prolamellar body is tantamount not only to a physical rearrangement of already existing substances under the influence of chlorophyll but that additional synthetic processes are involved.

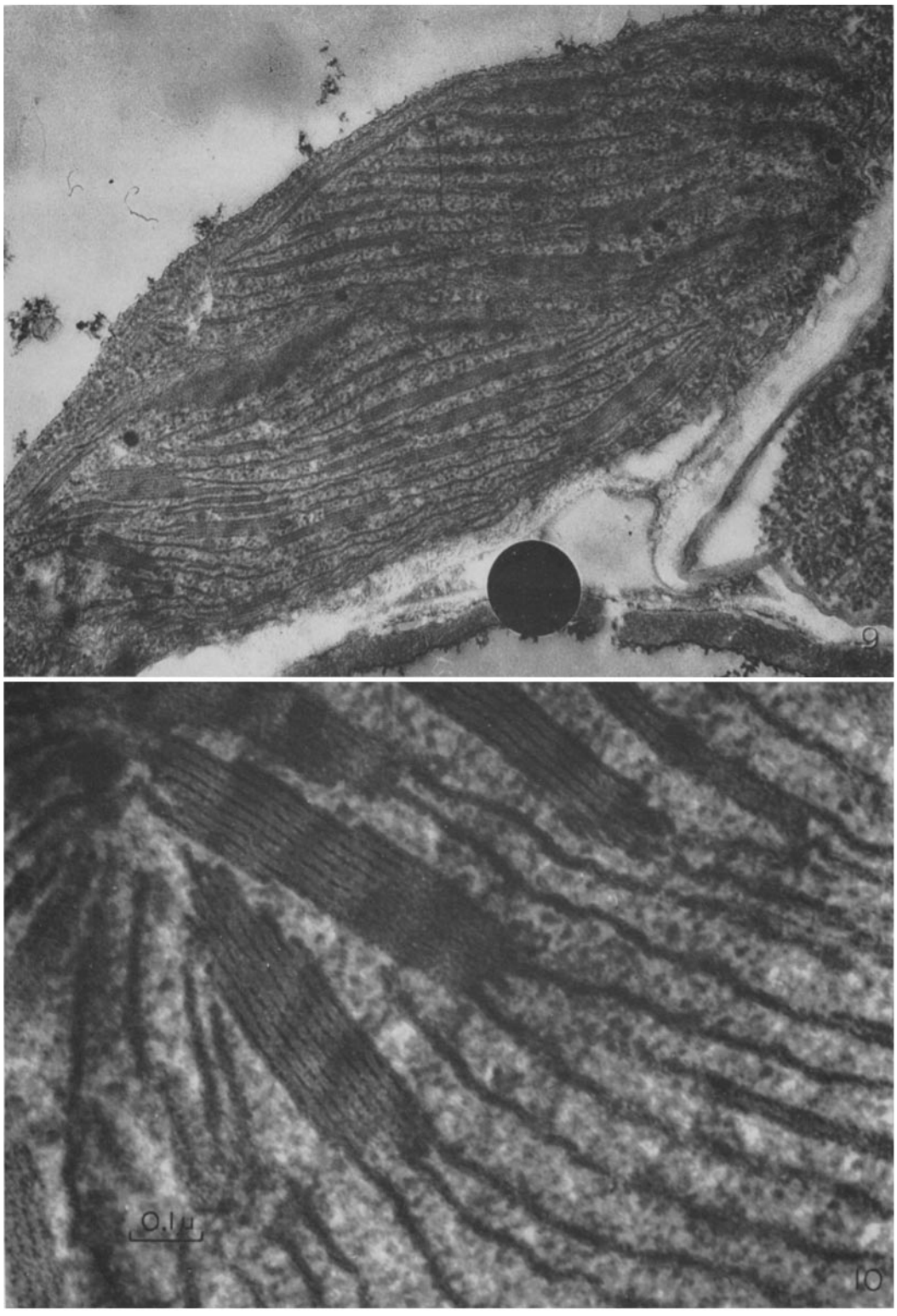
The author wishes to thank Mrs. Y. Ilam for help with the spectrophotometric measurements and Miss N. Krishmaro for technical assistance. Thanks are

FIGURE 9

Plastid from 8-day etiolated corn plant, which was exposed to light for 1 day at 3°C. and then an additional day at 26°C. The rings structure has disappeared and the normal chloroplast structure is visible. Size of polystyrene particle 0,34 μ. × 41,000.

FIGURE 10

Detail from a plastid in material treated as for Fig. 9, showing the normal arrangement of more or less parallel inter-grana lamellae and grana. × 110,000.



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