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Fine Structure of Lamellar Systems as Illustrated by Chloroplasts*

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THE fine structure of specialized organelles such as mitochondria, which are known to be concerned with the metabolic side of the carbon cycle, has been discussed in the three foregoing chapters (Bennett, p. 297; Sjöstrand, p. 301; Fernández-Morán, p. 319). The structures concerned with the other half of this cycle—namely, the photosynthetic aspect—are considered here.

It is of interest, in terms of the evolution of specialized photosynthetic organelles, to examine the fine structure of certain primitive types of cells, since these are presumably representative of organisms arising very early in the evolutionary process. Such a group of organisms are the blue-green algae. Examination in the electron microscope shows that, in certain types such as *Nostoc* (Fig. 1), there are no specialized membrane-bounded organelles such as mitochondria, nuclei, and chloroplasts; rather there exists a generalized membrane structure ramifying throughout the cytoplasm. It seems reasonable to conclude that, in such cells, the various metabolic functions such as oxidative phosphorylation and photosynthesis are carried on in relation to an apparently undifferentiated membrane system (or perhaps in specialized patches of the membrane), rather than in well-defined organelles. In other blue-greens (e.g., *Anabena*), the photosynthetic material is present in a more specialized and segregated state, but the primitive organelles lack limiting membranes. It is of interest to note that, in higher organisms, an intracellular membrane system (the endoplasmic reticulum)[1] appears to be universally present, and to have clear continuity with the membranous envelope of the nucleus (and possibly with the limiting membranes of other organelles). It seems likely, therefore, that comparative study of such primitive cells might yield important clues concerning the origin and interrelationship of the endoplasmic reticulum and specialized organelles such as mitochondria, chloroplasts, and nuclei in the cells of higher organisms.

Proceeding a little higher in the evolutionary scale to a typical green alga, such as *Nitella* (Fig. 2), one observes typical membranous elements of the endoplasmic reticulum, the characteristically structured mitochondria found in all higher organisms, and chloroplasts, bounded by a well-defined, double limiting membrane and containing a number of relatively well-ordered dense lamella within a finely granular matrix material.[2] Chloroplasts of this structural type are highly characteristic of the lower forms of plant cells.[3–6]

In the higher plants, of which corn (*Zea mays L.*) is chosen as an example, the structure of the chloroplasts[7–10] usually differs from the more primitive pattern already described. Figure 3 shows in transverse section the leaf of a three-week-old corn plant. Around each vascular bundle are a number of parenchyma sheath cells, the chloroplasts of which are specialized for the formation and storage of starch. Most of the photosynthetic activity, however, is carried out in the chloroplasts of the mesophyll cells. Even at the light-microscopical level, there are obvious differences in appearance between the chloroplasts of the parenchyma-sheath cells and those of the mesophyll cells. In the electron microscope (Fig. 4), the parenchyma-sheath chloroplasts of *Zea*[7] resemble algal chloroplasts (cf. *Nitella*, Fig. 2) in their over-all plan of organization. Within a double external limiting membrane, a number of densely staining lamellae (each about 130 A thick) are set in a finely granular matrix which presumably contains most of the soluble enzymes of the chloroplast.

The mesophyll chloroplasts are similarly lamellated but, in addition, possess well-defined regions (the grana) in which the lamellae are more densely and regularly packed (Fig. 4). If the section is in the plane of the lamellae, the grana appear as circular profiles. If, on the other hand, the plane of the section is normal to the lamellar plane, the grana appear as regular rectangular-shaped regions. The concentration of lamellar surface within the grana is about twice as high as in the intervening (intergrana) regions, and it can be seen (Fig. 5) that this comes about because of a pairing or bifurcation of the lamellae at the edges of the grana. This type of chloroplast with well-defined grana is characteristic of higher plants, most of which do not possess plastids of the type found in the parenchyma-sheath cells of *Zea*.

In both types of chloroplasts, the individual lamellae (each 130 A thick) of both the grana and intergrana regions exhibit a compound-layer structure (Figs. 6 and 7), consisting of a central dense line (the *P* zone, about 40 A thick and often resolvable as a doublet) on both sides of which are less dense layers (the *L* zones). Finally, the entire compound layer structure is edged by very thin dense lines, the *C* zones. Such differences in density arise in part from differences in reactivity

* Most of the work described herein was carried out in the Chemical Physics Section, C. S. I. R. O., Melbourne, Australia, in collaboration with Dr. F. V. Mercer and Dr. J. D. McLean of the Botany Department, University of Sydney, N. S. W.

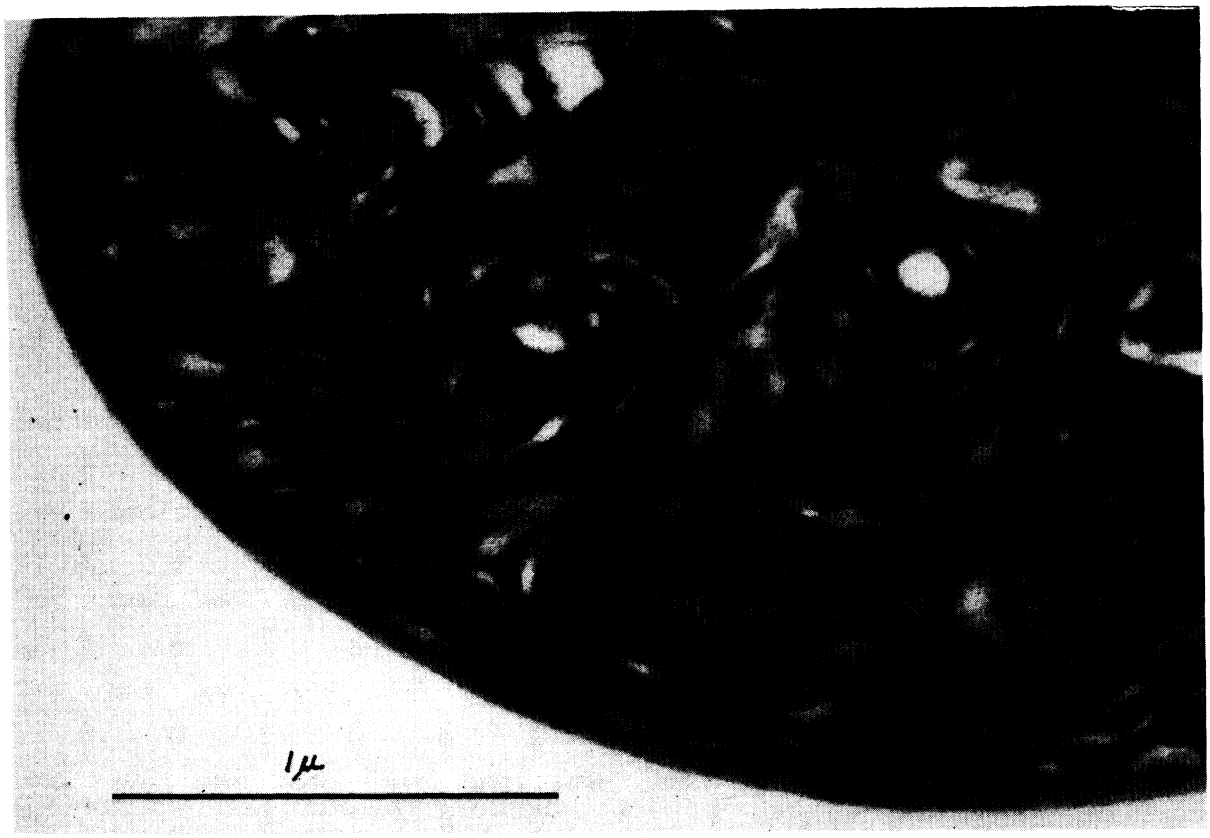

FIG. 1. Vegetative cell of a *Nostoc* spp., showing the generalized whorl-like lamellar system and the absence of specialized organelles such as chloroplasts and mitochondria. ×60 000. (Unless otherwise indicated, all illustrations are electron micrographs of thin sections of plant material fixed in osmium-tetroxide solutions of appropriate *p*H and tonicity, and embedded in methacrylate. Details are given in references 2, 7, 13, and 14.)

with osmium tetroxide as well as from intrinsic differences in electron density within the various components of the structure. Within the grana, the close packing of such compound lamellae results in close apposition of *C* zones and, thus, gives rise to a layer structure (Figs. 8 and 9) with a repeat period of 130 A, bearing a remarkable resemblance to that found in the myelin sheath of nerve fibers. The central dense lines of the compound lamellae correspond to the major dense lines of the periodic structure within the grana and are often observed as doublets (Fig. 9), each line of the pair being about 15 A thick. It is clear, therefore, that the symmetrical structure of the individual compound lamellae arises from the close apposition of two structurally asymmetric "unit membranes" (each about 70 A thick) as shown schematically in Fig. 11. The *I* zones, which occur midway between *P* zones in the grana, arise by apposition of the *C* zones of the unit membranes. The work of a number of people, notably Robertson,[11] indicates that the cell membranes and intracellular membrane systems of most, if not all, cells consist of such unit membranes in single, double, or compound array. This also appears to be true of plant cells. In Fig. 10, the tonoplast is seen as a single membrane with a pair of dense edges, and the double limiting membrane of the chloroplast consists of two such unit membranes. The intrinsic asymmetry of these unit membranes is not usually evident in the electron microscope except where they are stacked in double or multiple array, as in the lamellae and grana of chloroplasts and in myelin sheath. In both cases, this stacking results in a set of major dense lines with less-dense intermediate lines in between. As already indicated by Fernández-Morán (p. 319), there is good reason to believe that each 70-A membrane comprises a double layer of mixed lipids (the *L* zones) sandwiched between two thin monolayers of protein which stain densely with osmium tetroxide, with a probable contribution to this latter density arising from reaction of the hydrophilic groups of the phospholipids with osmium tetroxide. In the case of myelin sheath, a very satisfactory correlation between electron-microscopic and x-ray diffraction data has been achieved[12] and the chemical composition is reasonably well known. It seems fairly certain, therefore, that this type of structure is essentially correct for most cellular membranes, with only such minor differences in detail arising as are demanded by differences in function.

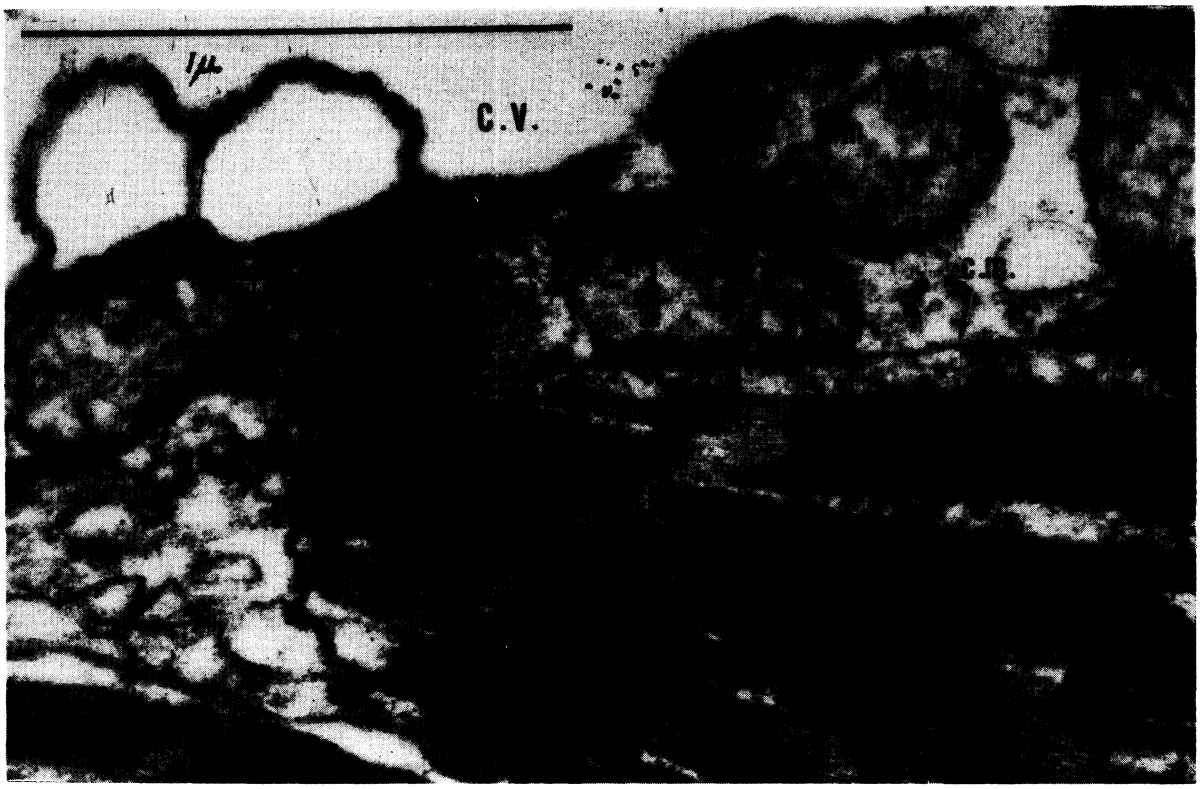

FIG. 2. Transverse section through the cytoplasm of a *Nitella* cell, showing the lamellated structure of a chloroplast surrounded by its limiting membrane *c. m.*, mitochondria *m*, various membranous elements within the cytoplasm, and the tonoplast lining the lumen of the central vacuole *C. V.* ×75 000.

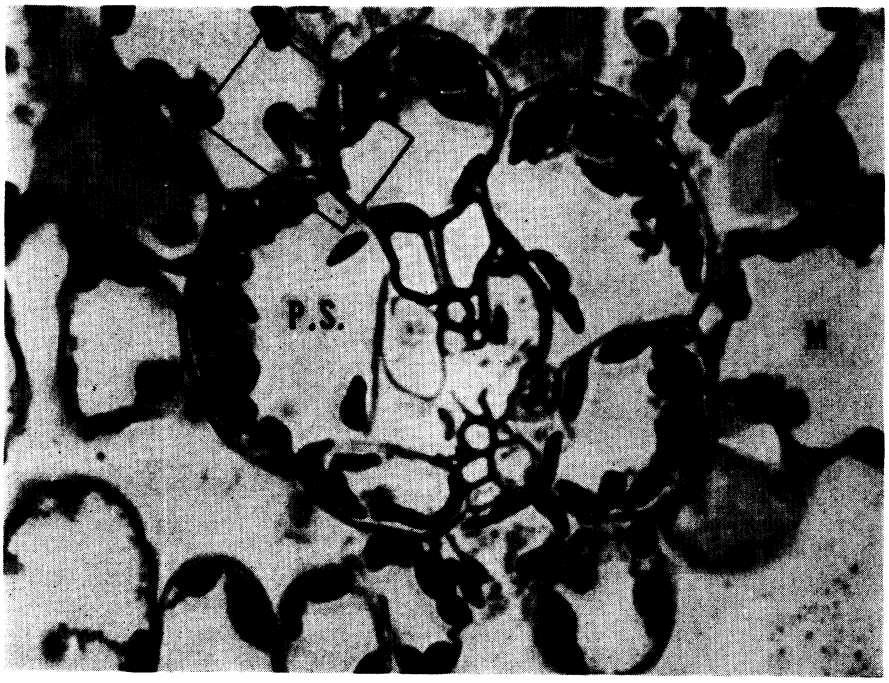

FIG. 3. Phase-contrast micrograph of a relatively thick transverse section through the leaf of a three-week-old plant of *Zea mays L.*, illustrating the difference in appearance between the chloroplasts of the parenchyma-sheath cells *P. S.* and those of the mesophyll cells *M.* A vascular bundle lies in the center of the field.

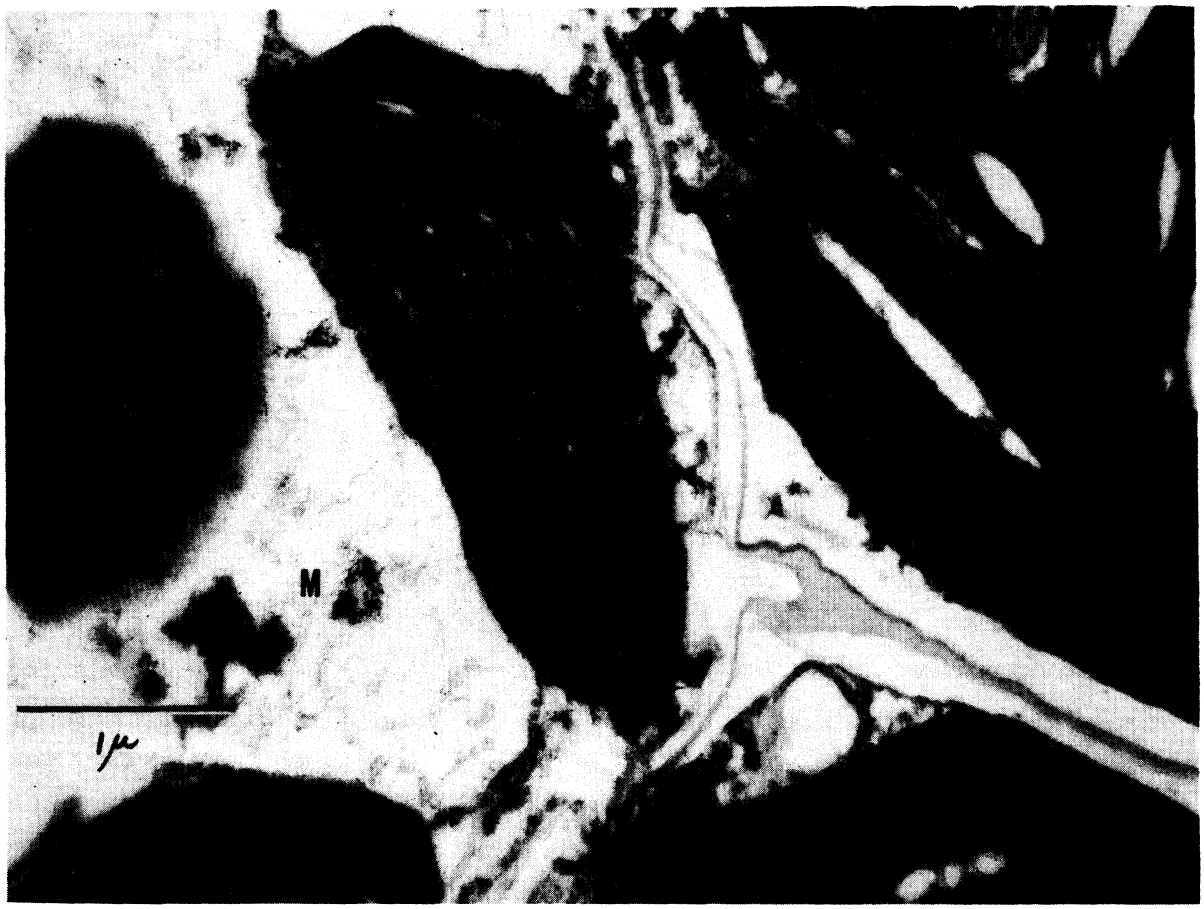

FIG. 4. Electron micrograph of a region similar to that outlined in Fig. 3, illustrating the lamellar structure both of parenchyma-sheath cells *P. S.* and mesophyll *M* chloroplasts of *Zea*. In the mesophyll chloroplasts, the grana appear as dense rectangular regions when the section is normal to the lamellar plane, and as circular regions when the plane of section is parallel to the lamellar plane. ×30 000.

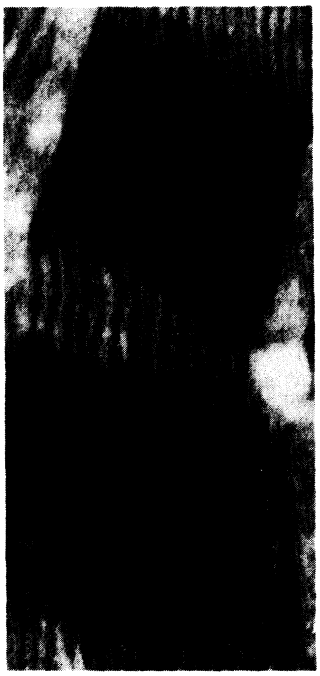

FIG. 5. Two grana in a mesophyll chloroplast of *Zea*, showing the type of connection between the grana lamellae and those in the intervening (intergrana) regions. (Cf. Fig. 11.) ×85 000.

In the case of the chloroplast, the situation is less well documented, but certain conclusions can be drawn from the available structural and chemical data, and by extrapolation from the better characterized myelin system. It can be seen from Fig. 10 that the membranes making up the lamellae and grana of the chloroplast are considerably more dense than the external limiting membranes of the chloroplast and the tonoplast, which exhibit densities more characteristic of the usual cellular membrane systems. In particular, the *L*-zones of the chloroplast lamellae exhibit higher densities than are found in cell membranes, endoplasmic reticulum, etc. A possible explanation of this may lie in differences of chemical composition. It is known that the chloroplast is deficient in phospholipids as compared with myelin sheath.[7] This deficiency is counterbalanced by the presence of considerable amounts of carotenoids and chlorophyll. Furthermore, as is shown later, there is good evidence to suggest that chlorophyll is an integral component of the chloroplast lamellae and that sufficient is present for its incorporation as a monolayer over the entire lamellar area of the chloroplast as estimated from electron micrographs. Thus, the general conclusion

FIG. 6. Mesophyll chloroplast of *Zea*, illustrating the high degree of order within the grana, and the compound-layer structure of the intergrana lamellae (arrow). ×160 000.

seems justified that the photosynthetic lamellar elements of the chloroplast consist of structurally asymmetric unit membranes (Fig. 11) in which chlorophyll and carotenoids are incorporated in orderly array. It is of interest to note that the exciton-migration theory of energy transfer proposed by Calvin appears to require the presence of such a structural asymmetry. The type of model suggested by Calvin (p. 157) has the necessary asymmetry (Fig. 12) and appears to be consistent with the presently available chemical and structural evidence. Such a staggered, partially overlapping configuration of the chlorophyll molecules has the further advantage over other models (which usually involve coplanarity of the porphyrin "heads" of the chlorophyll) in that it offers a plausible explanation for the low values of dichroic effects so far observed in chloroplasts. Furthermore, it would allow a greater degree of π-electron interaction than allowed by the coplanar type of model, thus facilitating energy transfer, and is consistent with the type of packing found in crystals of polycyclic organic compounds by x-ray diffraction. As has been seen the chloroplasts of higher plants characteristically contain grana in which the unit membranes are arranged in a highly ordered and close-packed array. The significance of this specialization is unknown at the present time, but it is tempting to speculate that the high degree of order might lead to more efficient trap-

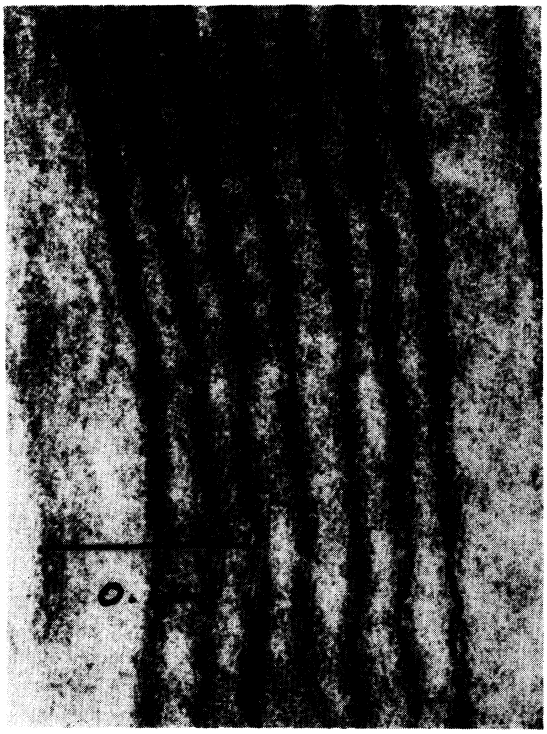

FIG. 7. Compound layer structure of the lamellae in a parenchyma-sheath chloroplast of *Zea*. ×270 000.

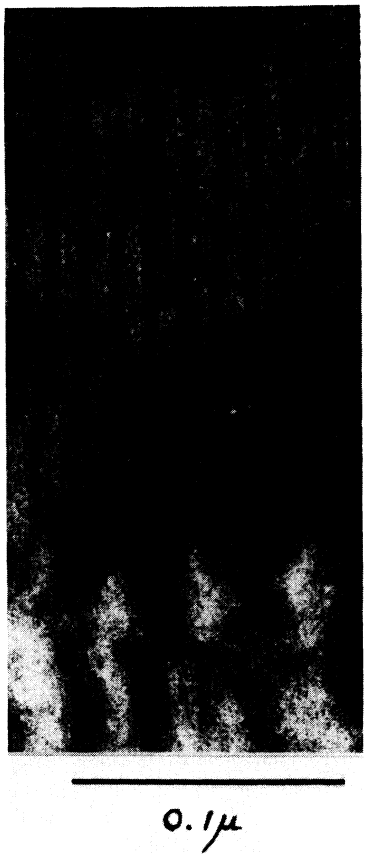

FIG. 8. Mesophyll chloroplast of *Zea* to illustrate the myelin-like structure within a granum. Note the main periodicity of about 130 A and the presence of fainter intermediate lines. ×370 000.

ping of photons, or perhaps result in facilitation of exciton migration as a result of a stabilization of the layer structures which possess the conduction bands necessary for energy transfer.

SWELLING PROPERTIES OF THE CHLOROPLAST

The swelling characteristics of chloroplasts offer a striking demonstration of the "plasticity" of such lipoprotein layer systems. As already mentioned the swelling of mitochondria can be largely prevented by active expenditure of energy from ATP hydrolysis. A similar phenomenon exists in the case of chloroplasts. Klein (unpublished work) has shown that the swelling response of isolated chloroplasts depends on whether the suspension of plastids is illuminated or is kept in the dark.

When isolated *Nitella* chloroplasts are placed in a hypotonic medium, swelling results first in a separation of the lamellae to distances many times that characteristic of the intact chloroplast.[2] In highly hypotonic media, the lamellae break up. The hydrophobic hydrocarbon chains of the lipids thus are exposed at the free edges of the membranes, giving rise to an unstable situation, with the result that such free edges "zip" together to form membrane-bounded vesicles. Similar results have been obtained for isolated *Zea* chloroplasts (Fig. 13), and the characteristically vesicular structures found in so-called microsome fractions isolated by conventional procedures arise in similar fashion (e.g., Hodge[13]) from the endoplasmic reticulum. This plastic behavior is understandable in terms of what is known of membrane structure. In the type of layer structure under consideration here, it seems likely that the oriented lipid molecules possess a high degree of rotational freedom and considerable translational freedom within the plane of the membrane. Both properties are characteristic of the smectic fluid-crystal state and confer a remarkable capacity for changes in the topographical configuration of such membrane systems.

CHLOROPLAST DEVELOPMENT

The development of the chloroplast is of interest in that it appears to take place by a process which is essentially the reverse of that involved in the swelling phenomena just discussed. In brief, the compound- and extended-layer structures of the lamellae and grana appear to be formed by a process involving the fusion of small vesicles or micelles one with another to form extended cisternae or "double-membrane structures."

The development of a leaf or of a plant is a complicated sequence of events, the time and spatial relationships of which lead to difficulty in following the process of chloroplast development. The etiolated plant (one grown from seed in total darkness) is a much more

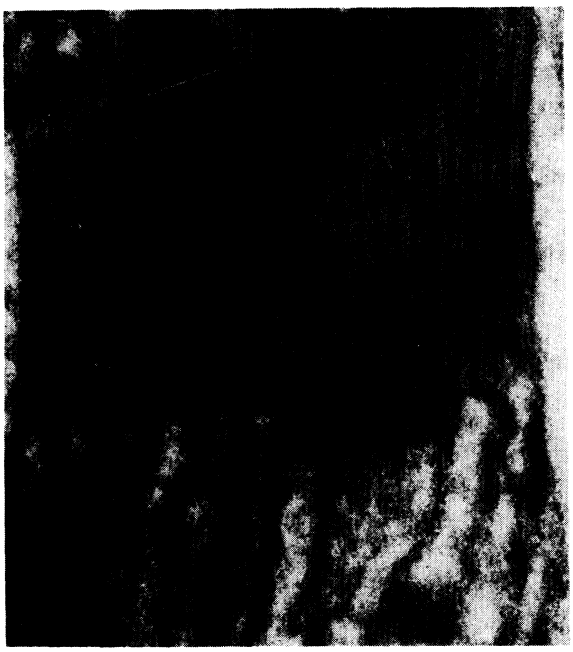

FIG. 9. A granum in a mesophyll chloroplast of *Zea*, illustrating the splitting of the main dense lines (arrow). The central dense lines (*P* zones) of the intergrana lamellae are also occasionally resolvable as doublets, thus showing that the compound lamellae consist of two closely apposed asymmetric unit membranes. ×220 000.

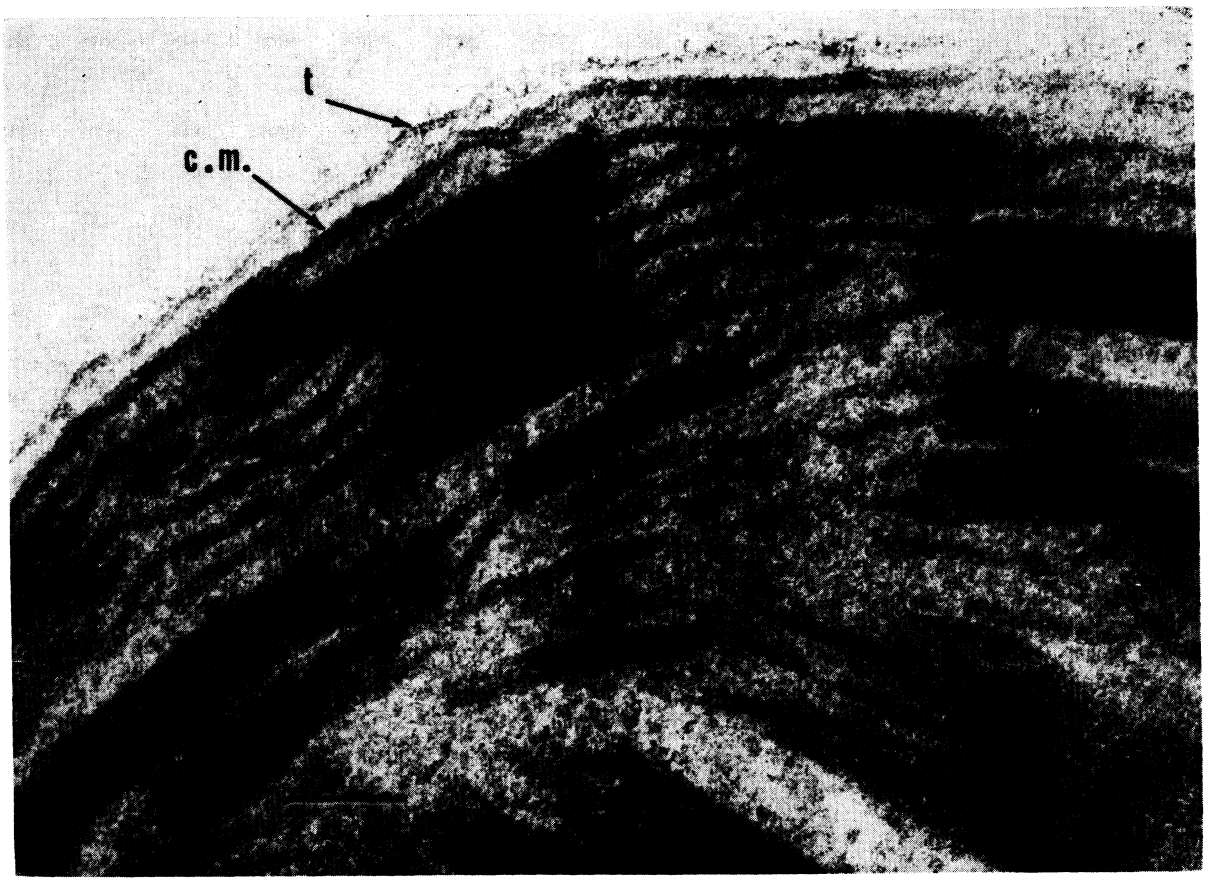

FIG. 10. Chloroplast in a three-week-old wheat plant, showing lamellar structure very similar to that in *Zea mays*. The tonoplast *t* appears as a single membrane, and where suitably oriented with respect to the plane of the section appears as two fine dense lines with a less dense layer between them, the over-all thickness being about 70 A. The chloroplast envelope *c. m.* comprises two unit membranes spaced about 100 A apart. ×160 000.

favorable system for such a study, since such seedlings can be exposed to light for various periods and the effects on chloroplast structure noted at definite time intervals following such exposure. This system depends on the fact that light is essential for chlorophyll synthesis in higher plants.

Figure 14 shows typical plastids in an etiolated leaf of *Zea*.[14] In the absence of chlorophyll, the plastids, although recognizable as well-defined organelles with external limiting membranes, fail to develop lamellae. Instead, the interior of each plastid is partially filled with a mass of small vesicles (the prolamellar body).

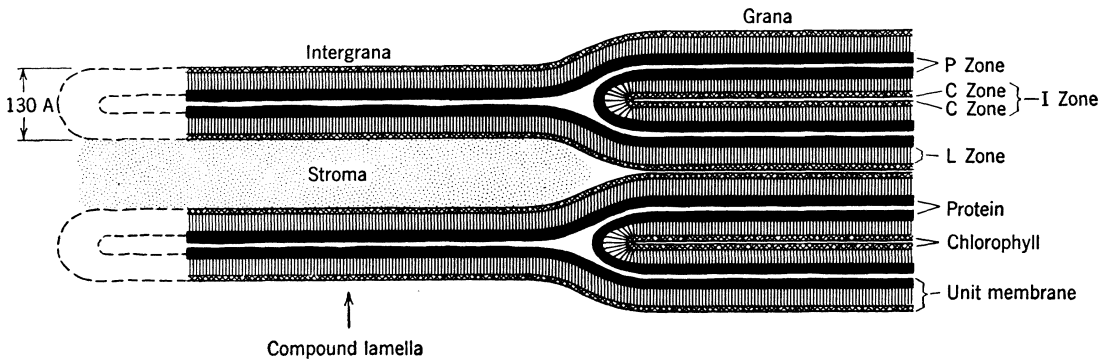

FIG. 11. Diagram to illustrate the densities observed in electron micrographs of osmium-fixed chloroplast lamellae and grana, and the structureal relationships involved in the formation of the compound lamellae (and grana) from structurally asymmetric unit membranes.

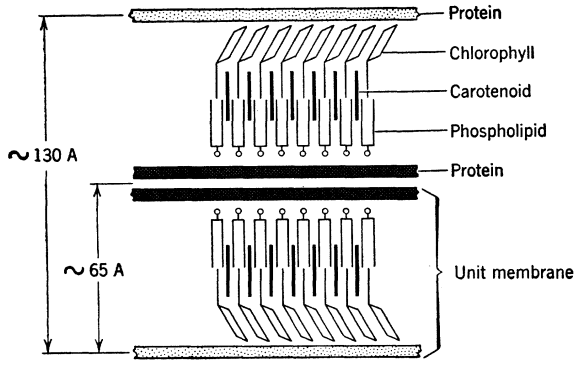

FIG. 12. Diagram to illustrate one way in which the compound lamellae observed in electron micrographs of chloroplasts could be built up from structurally asymmetric unit membranes having a molecular structure of the type proposed by Calvin (p. 157).

On placing the plant in daylight, chlorophyll is synthesized (as evidenced by a progressive greening of the leaves), and definite structural changes are observed in the plastids. Double membranes are observed (Fig. 15) apparently emerging from the prolamellar body.[14] At a later stage, typical chloroplast lamellae are seen, usually in a pattern radiating from a progressively decreasing prolamellar body (Fig. 16). It is of interest to note that in certain *Zea* mutants which are unable to synthesize chlorophyll, the plastids are similar to those of etiolated normal *Zea* in that they lack formed lamellar elements and contain only small vesicular structures. The evidence thus strongly suggests that chlorophyll is at least essential for the formation of typical chloroplast lamellae and is probably an integral component of these membrane structures.

At a later stage, rudimentary grana with a fine structure indistinguishable from that of the more mature grana already described may be observed (Fig. 17) within the mesophyll chloroplasts. After two days, the developing plastids closely resemble normal chloroplasts and no prolamellar bodies can be discerned, presumably as a result of complete conversion of the small vesicles into formed lamellar elements. At this stage and in earlier stages of recovery from etiolation, however, and in young chloroplasts of normal plants, one commonly observes immediately under the chloroplast limiting membrane a number of vesicles and sacs (Fig. 18) with membrane densities considerably lower than those characteristic of the already differentiated lamellae and grana. These observations suggest the possibility that this region, or the limiting membrane of the chloroplast itself, may be responsible for the formation of the small vesicles, perhaps by outpocketing and pinching-off from the limiting membrane, or by some direct synthetic process.

The concept of vesicle fusion either with other vesicles or with already existing extended membranes is attractive in relation to (1) the formation and maintenance of structures of higher order, such as the

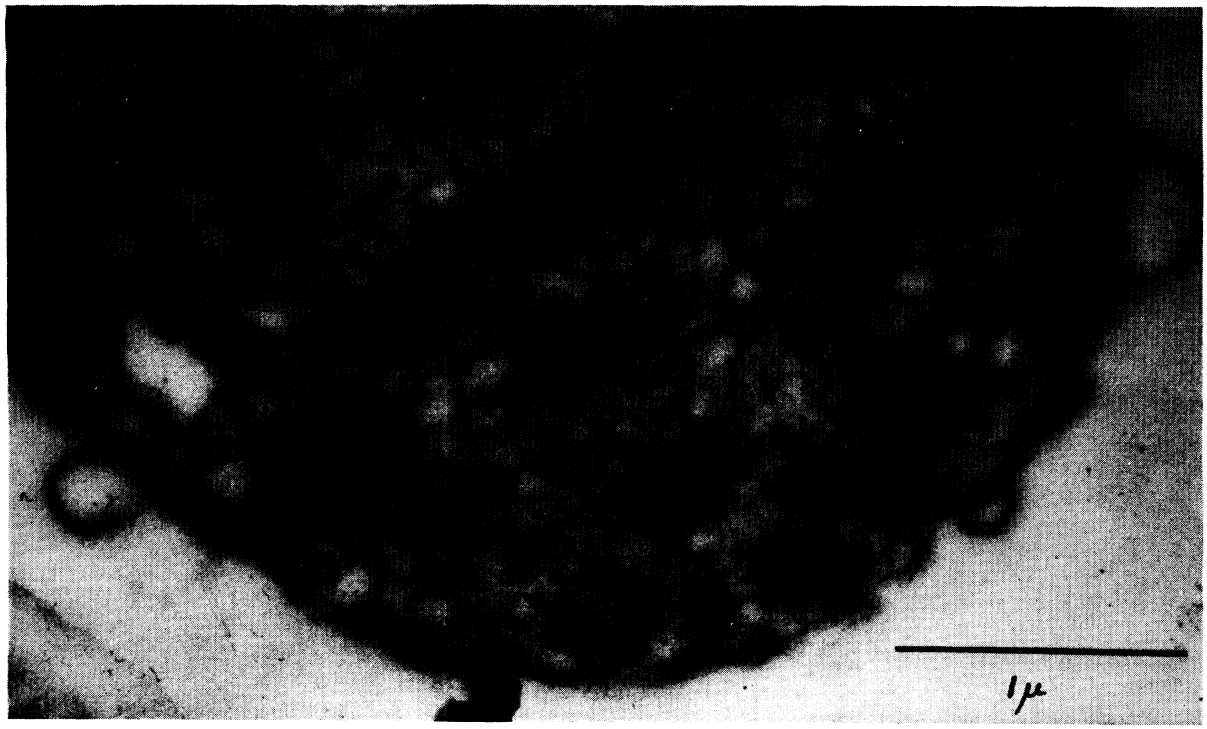

FIG. 13. Mesophyll chloroplast of *Zea* isolated in 0.5 M glucose in neutral phosphate buffer, showing a marked swelling reaction. (Compare with Figs. 4 and 6.) Note that the intergrana lamellae have broken up and formed large numbers of vesicles. × 40 000.

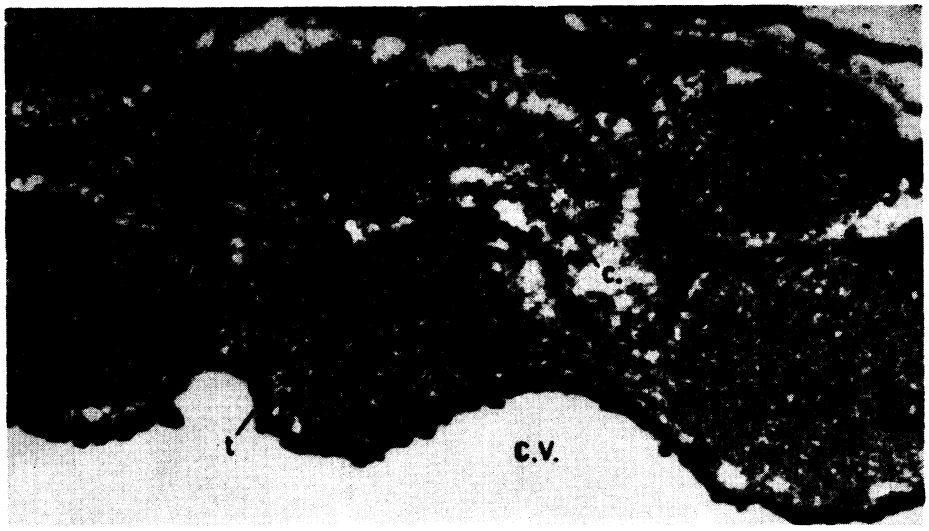

FIG. 14. Plastids in an etiolated leaf of *Zea*. The plastids contain masses of minute vesicles, but are devoid of lamellae, presumably because the absence of chlorophyll prevents their formation.

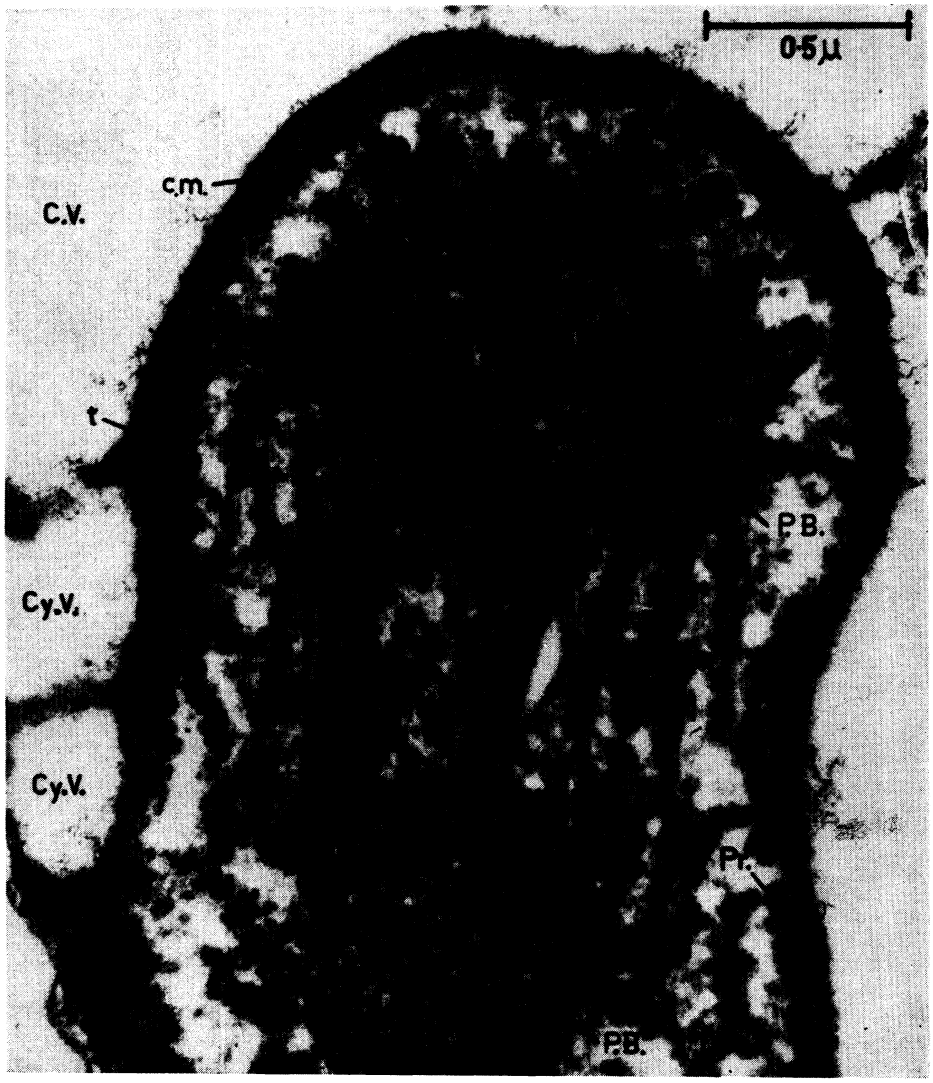

FIG. 15. Plastid from an etiolated leaf of *Zea* after several hours exposure to daylight showing an early stage in the formation of lamellae. Note that the lamellae appear to arise initially as double membranes, apparently by a process involving an orderly fusion of the small vesicles comprising the prolamellar body *P. B.* ×55 000.

FIG. 16 (upper left). A somewhat later stage in recovery from etiolation than that shown in Fig. 15, illustrating the progressive reduction in size of the prolamellar body *P. B.* and formation of typically dense chloroplast lamellae.

FIG. 17 (upper right and lower area). Mesophyll plastid of etiolated *Zea* after 20-hr exposure to daylight showing the beginning of grana formation. ×210 000.

endoplasmic reticulum and the various organelles of the cell (the vast increase in membrane area required for the formation of the myelin sheath of nerve fibers could be accounted for by fusion of vesicles with the Schwann-cell membrane); and (2) the transport both into and out of the cell of various substances. Such a

FIG. 18. Mesophyll plastid of etiolated *Zea* after 48-hr exposure to daylight. Typically dense chloroplast lamellae and grana are present. Note the presence of less-dense vesicles and cisternae in the region immediately beneath the chloroplast envelope *c. m.* ×85 000.

mechanism has been proposed for the extrusion of acetylcholine in "quantized" amounts from the nerve endings in myoneural junctions, and for the passage of zymogen granules from the apical regions of acinar pancreas cells through the limiting membrane into the lumen of the acinus. Similarly, the occurrence of the reverse process (i.e., pinocytosis) seems to be well documented. While mechanisms of this general type are widely supported by indirect experimental evidence, and appear to be thermodynamically feasible in terms of current knowledge concerning the structure of lipoprotein layer systems, the crucial problem of energy coupling in the physical control of such membrane systems remains unelucidated.

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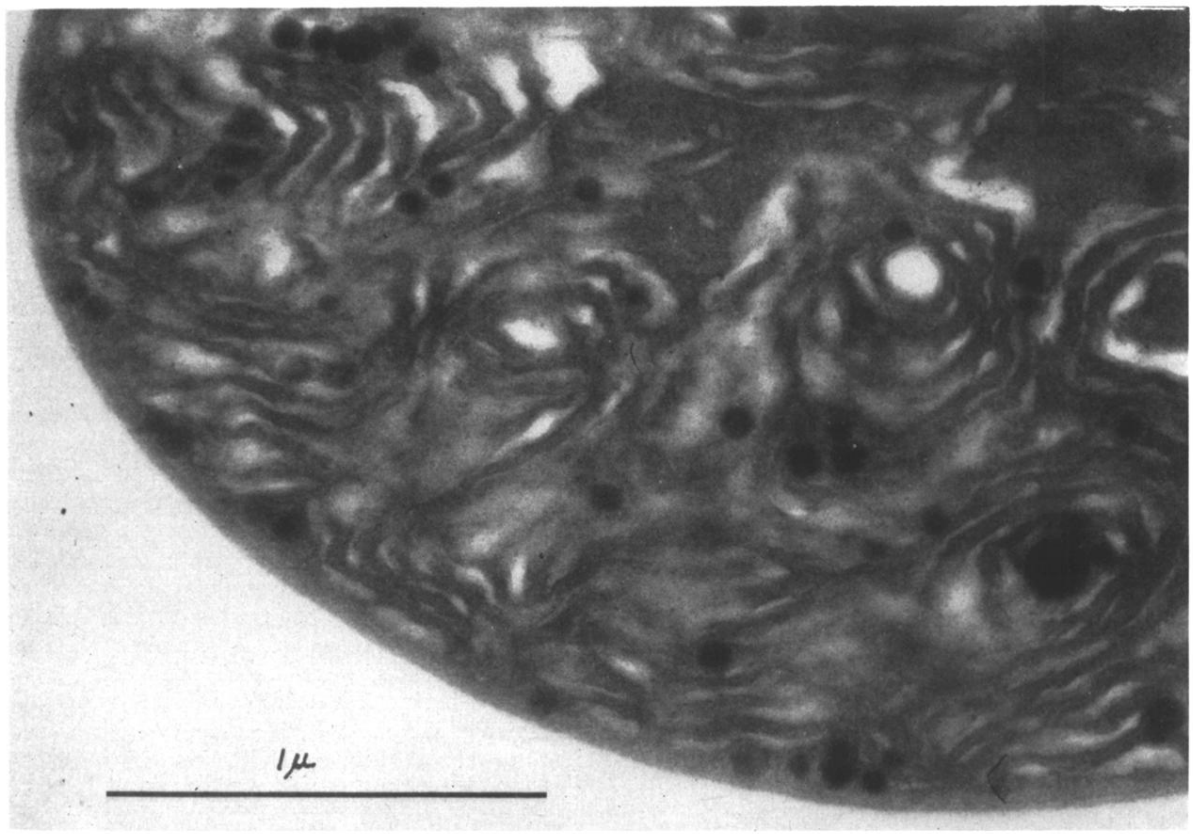

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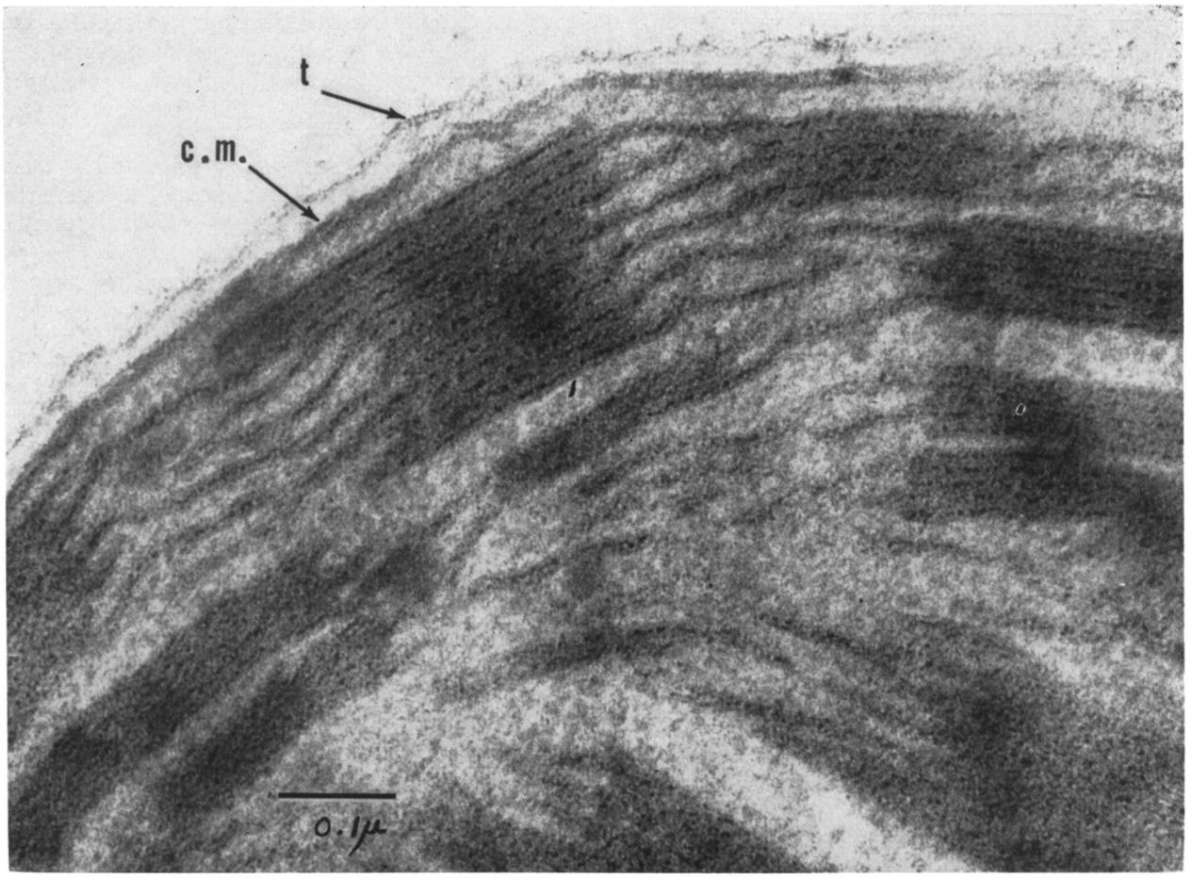

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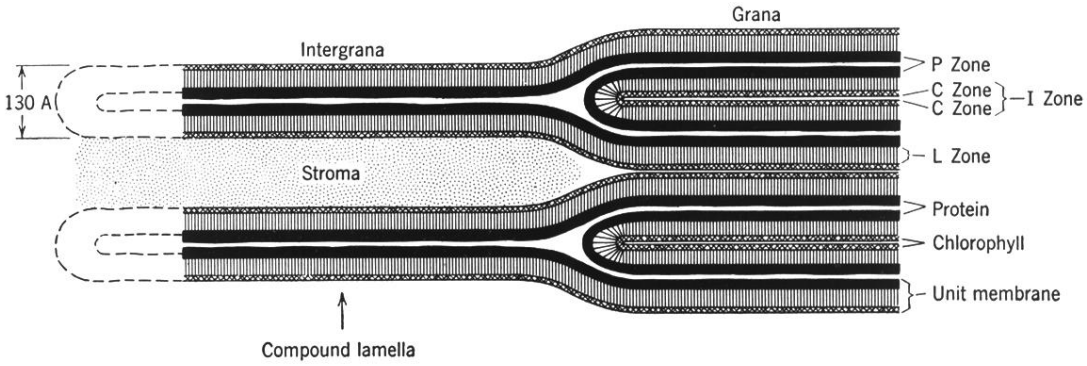

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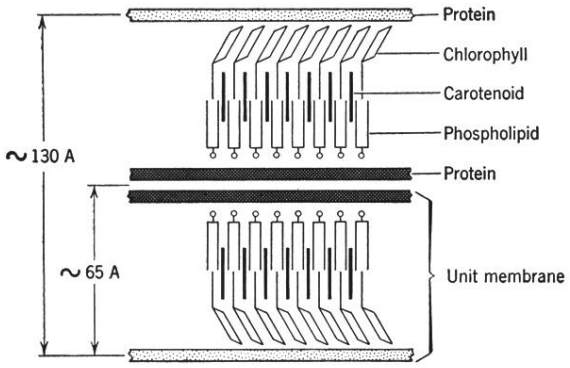

FIG. 12. Diagram to illustrate one way in which the compound lamellae observed in electron micrographs of chloroplasts could be built up from structurally asymmetric unit membranes having a molecular structure of the type proposed by Calvin (p. 157).

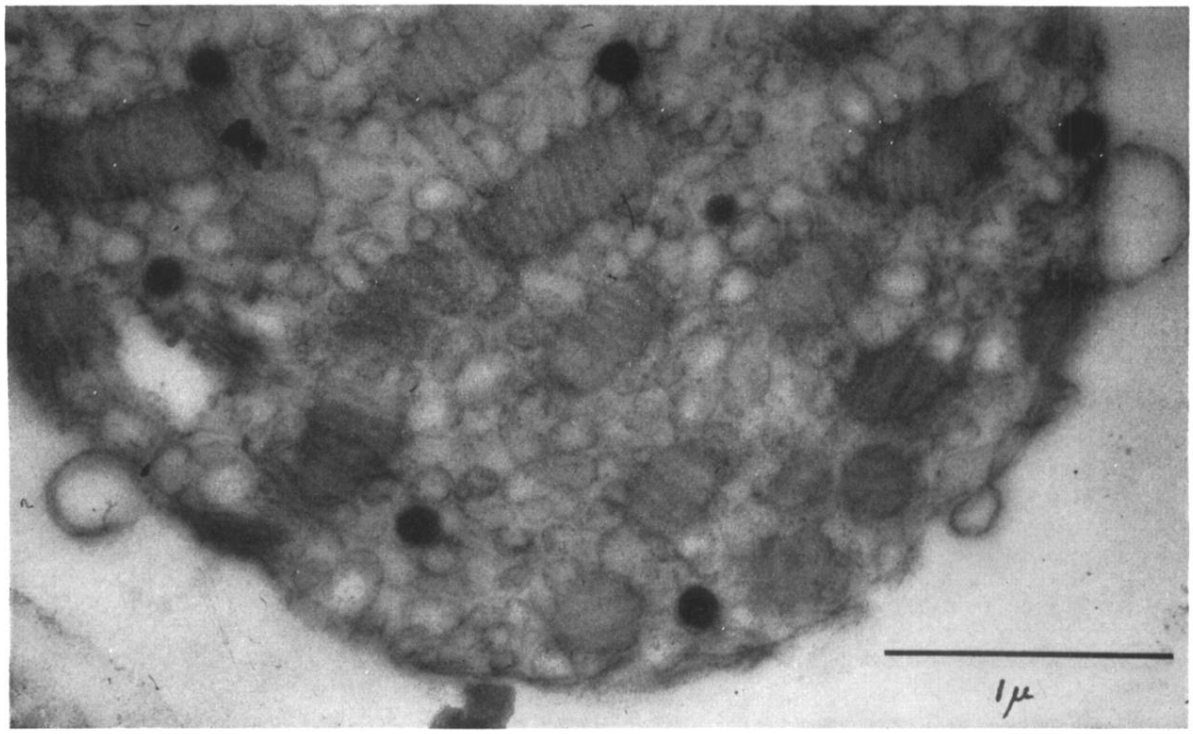

FIG. 13. Mesophyll chloroplast of *Zea* isolated in 0.5 M glucose in neutral phosphate buffer, showing a marked swelling reaction. (Compare with Figs. 4 and 6.) Note that the intergrana lamellae have broken up and formed large numbers of vesicles. × 40 000.

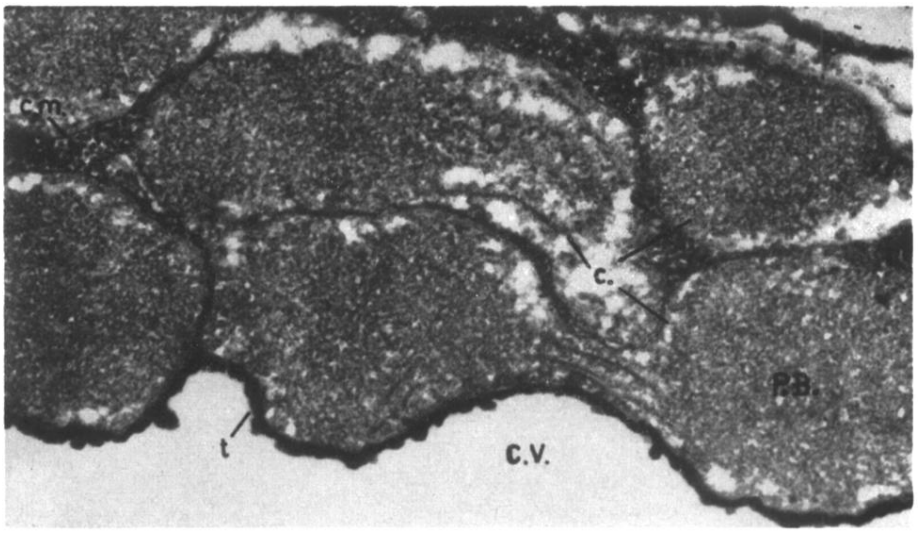

FIG. 14. Plastids in an etiolated leaf of *Zea*. The plastids contain masses of minute vesicles, but are devoid of lamellae, presumably because the absence of chlorophyll prevents their formation.

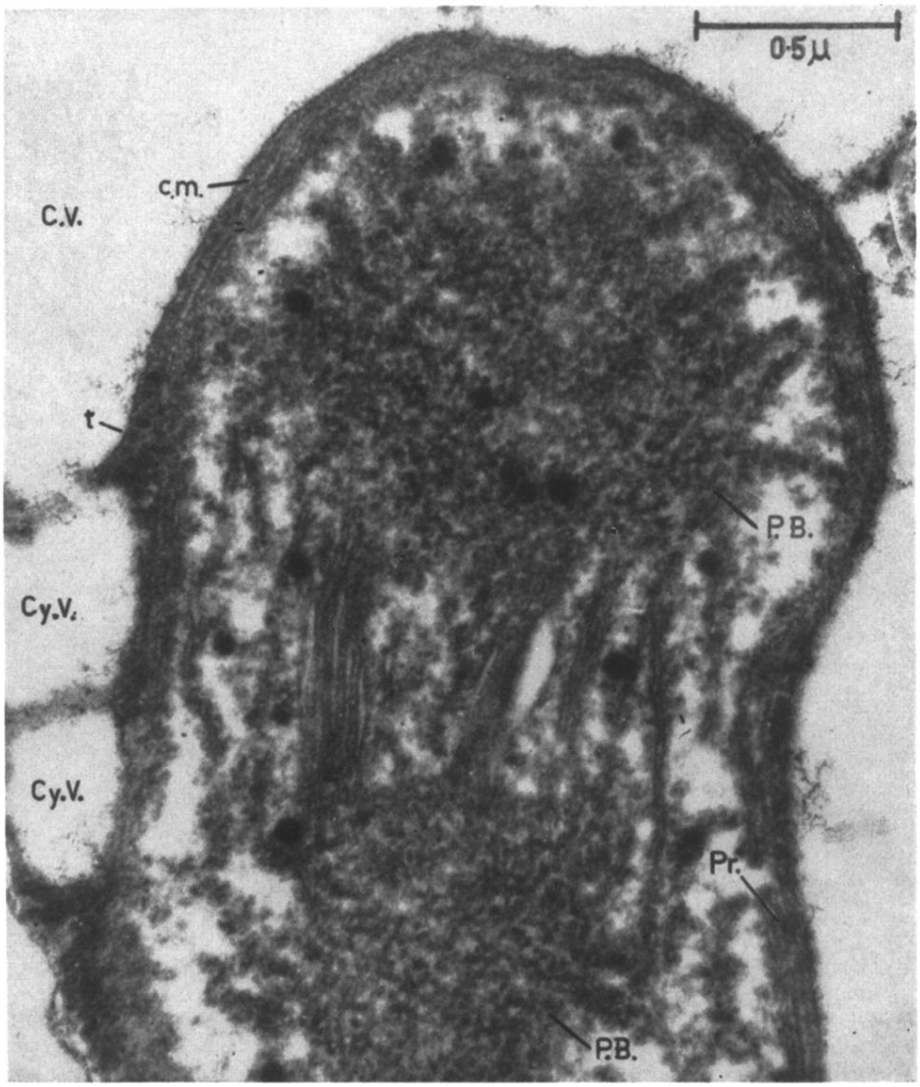

FIG. 15. Plastid from an etiolated leaf of *Zea* after several hours exposure to daylight showing an early stage in the formation of lamellae. Note that the lamellae appear to arise initially as double membranes, apparently by a process involving an orderly fusion of the small vesicles comprising the prolamellar body *P. B.* ×55 000.

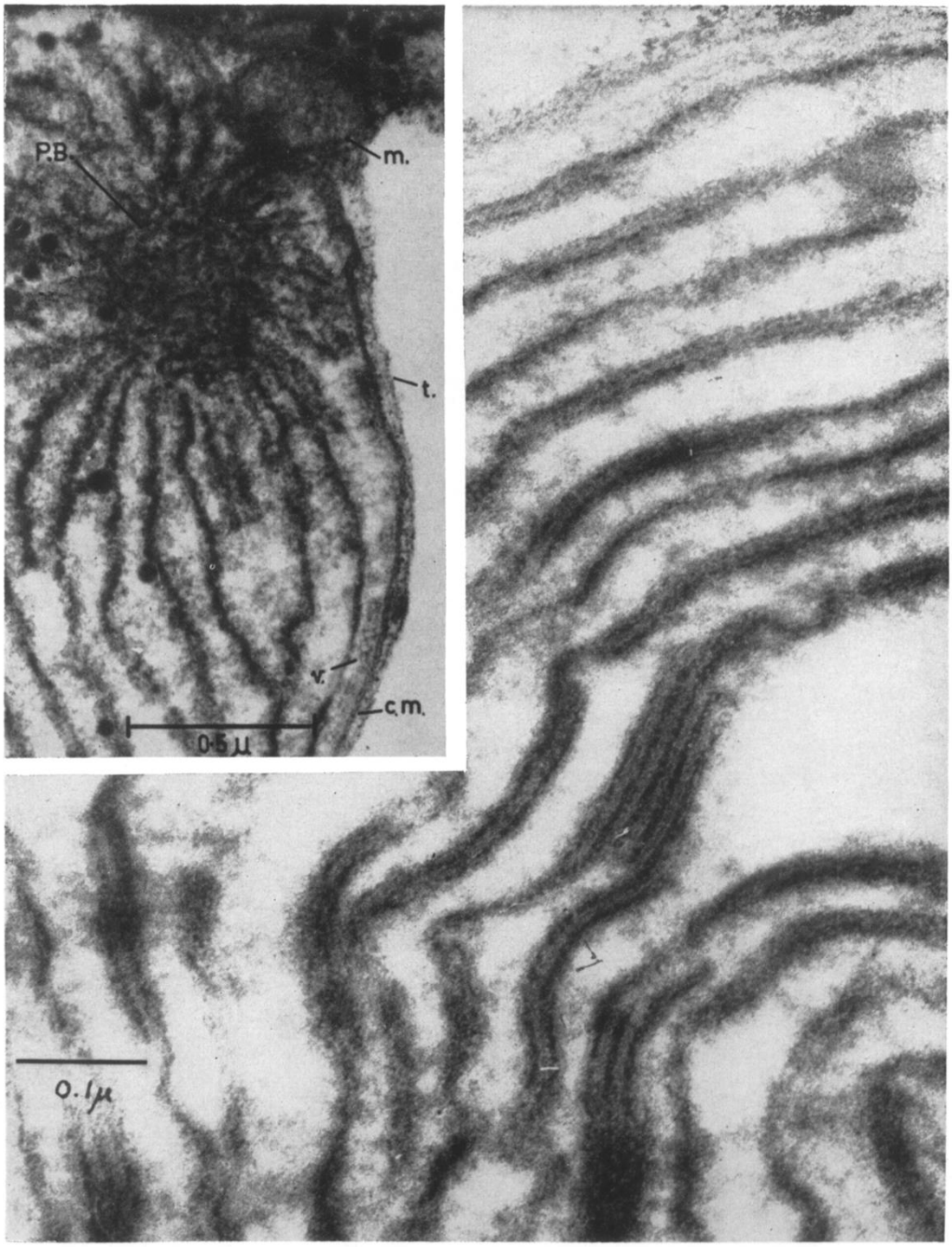

FIG. 16 (upper left). A somewhat later stage in recovery from etiolation than that shown in Fig. 15, illustrating the progressive reduction in size of the prolamellar body *P. B.* and formation of typically dense chloroplast lamellae.

FIG. 17 (upper right and lower area). Mesophyll plastid of etiolated *Zea* after 20-hr exposure to daylight showing the beginning of grana formation. ×210 000.

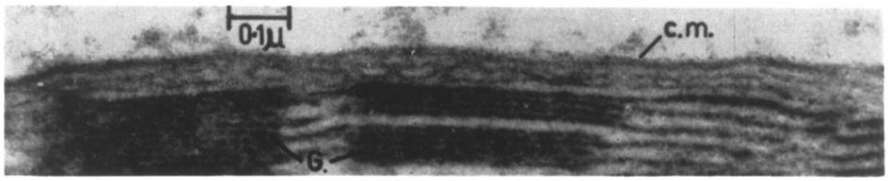

FIG. 18. Mesophyll plastid of etiolated *Zea* after 48-hr exposure to daylight. Typically dense chloroplast lamellae and grana are present. Note the presence of less-dense vesicles and cisternae in the region immediately beneath the chloroplast envelope *c. m.* ×85 000.

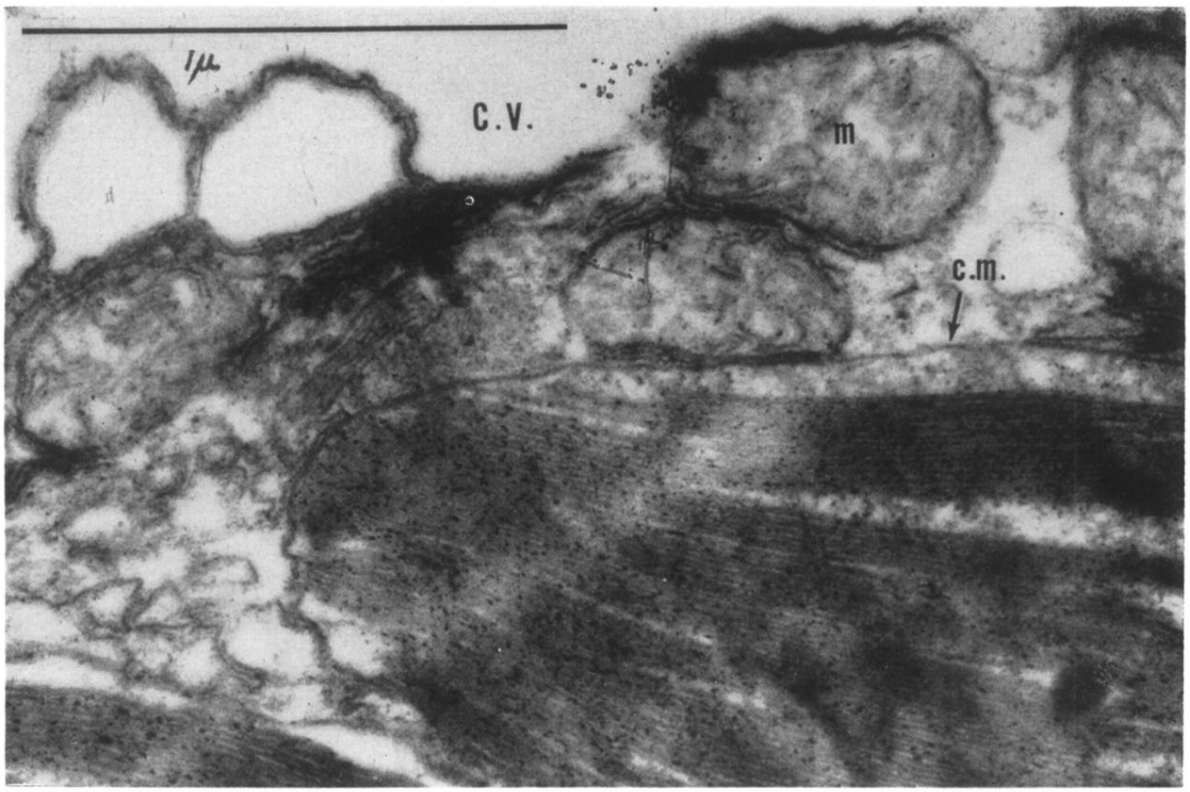

FIG. 2. Transverse section through the cytoplasm of a *Nitella* cell, showing the lamellated structure of a chloroplast surrounded by its limiting membrane *c. m.*, mitochondria *m*, various membranous elements within the cytoplasm, and the tonoplast lining the lumen of the central vacuole *C. V.* ×75 000.

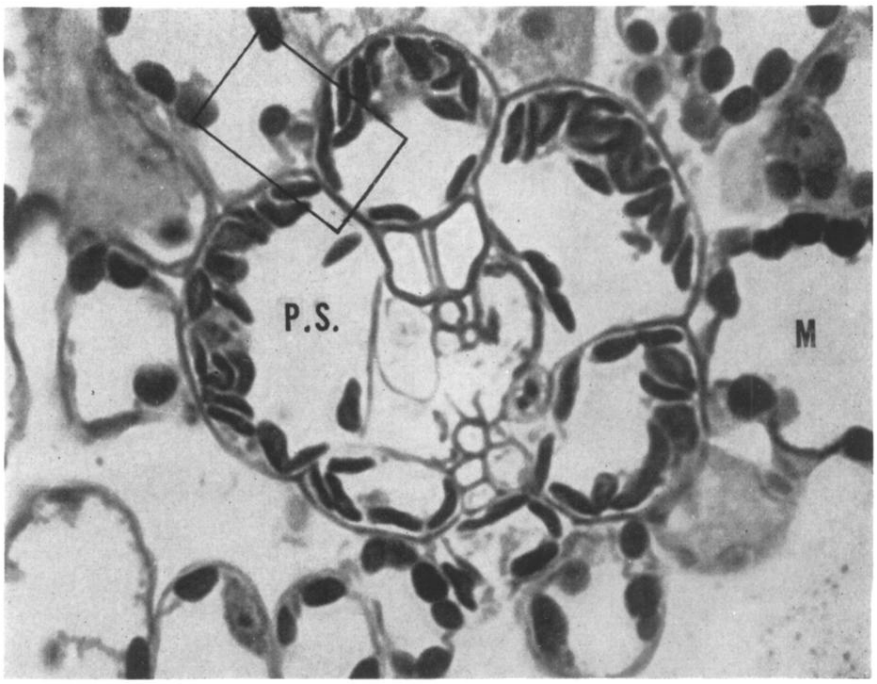

FIG. 3. Phase-contrast micrograph of a relatively thick transverse section through the leaf of a three-week-old plant of *Zea mays L.*, illustrating the difference in appearance between the chloroplasts of the parenchyma-sheath cells *P. S.* and those of the mesophyll cells *M*. A vascular bundle lies in the center of the field.

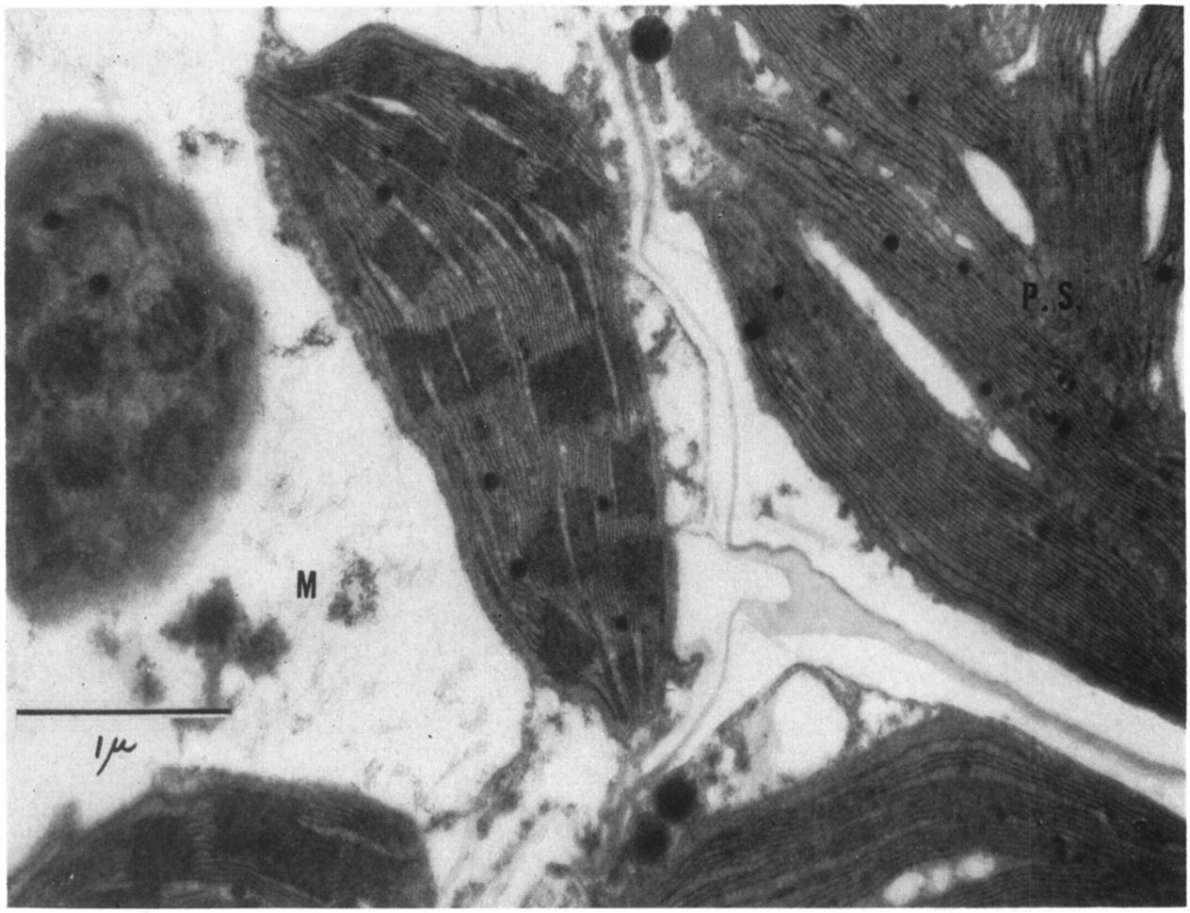

FIG. 4. Electron micrograph of a region similar to that outlined in Fig. 3, illustrating the lamellar structure both of parenchyma-sheath cells *P. S.* and mesophyll *M* chloroplasts of *Zea*. In the mesophyll chloroplasts, the grana appear as dense rectangular regions when the section is normal to the lamellar plane, and as circular regions when the plane of section is parallel to the lamellar plane. ×30 000.

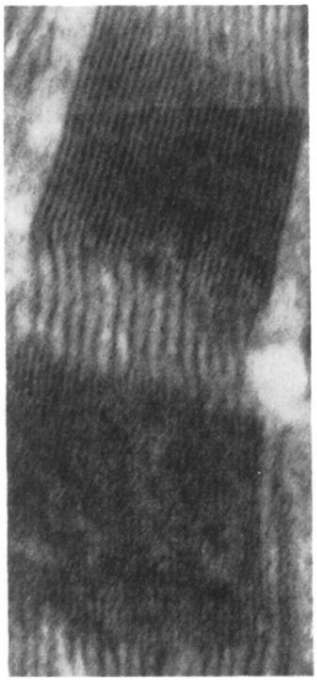

FIG. 5. Two grana in a mesophyll chloroplast of *Zea*, showing the type of connection between the grana lamellae and those in the intervening (intergrana) regions. (Cf. Fig. 11.) ×85 000.

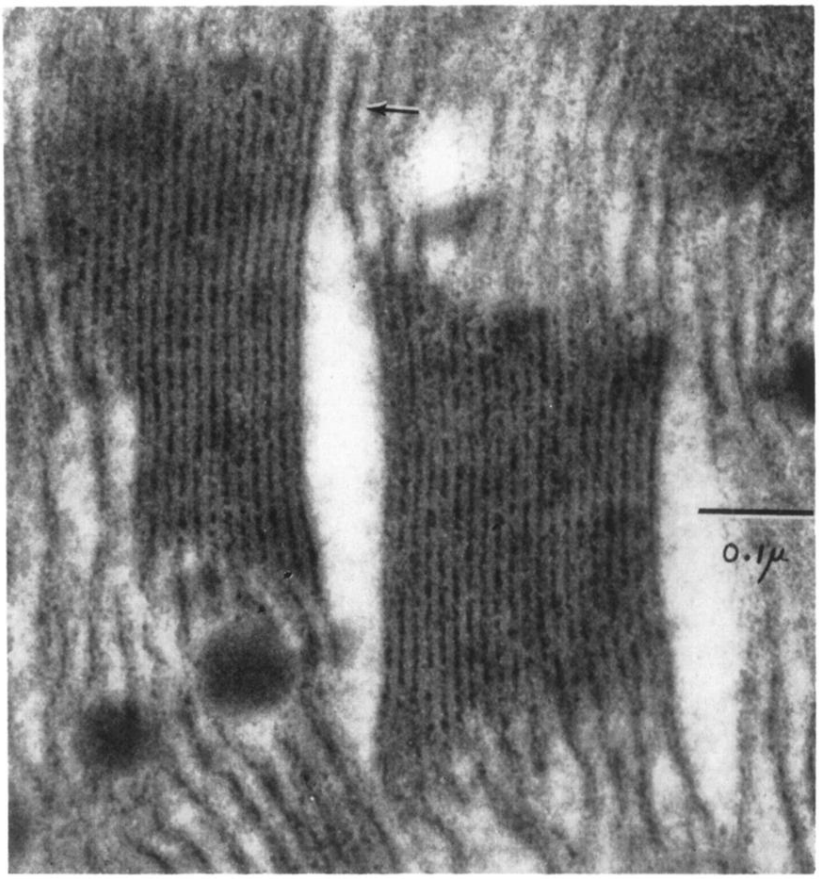

FIG. 6. Mesophyll chloroplast of *Zea*, illustrating the high degree of order within the grana, and the compound-layer structure of the intergrana lamellae (arrow). ×160 000.

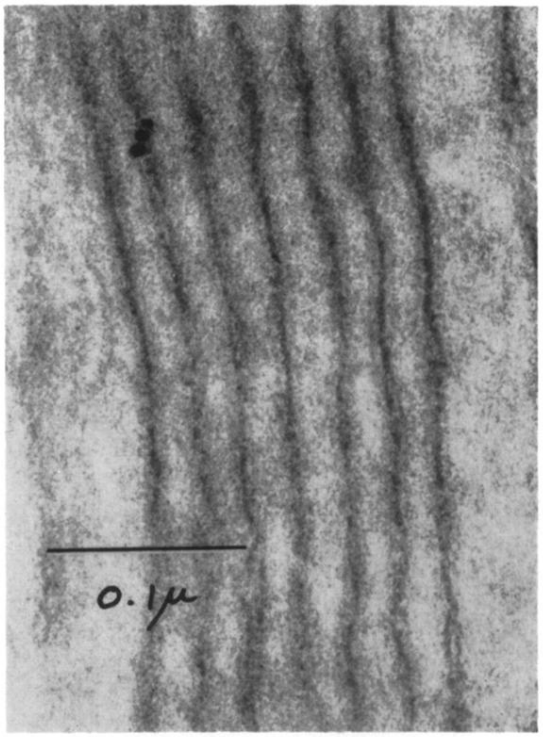

FIG. 7. Compound layer structure of the lamellae in a parenchyma-sheath chloroplast of *Zea*. ×270 000.

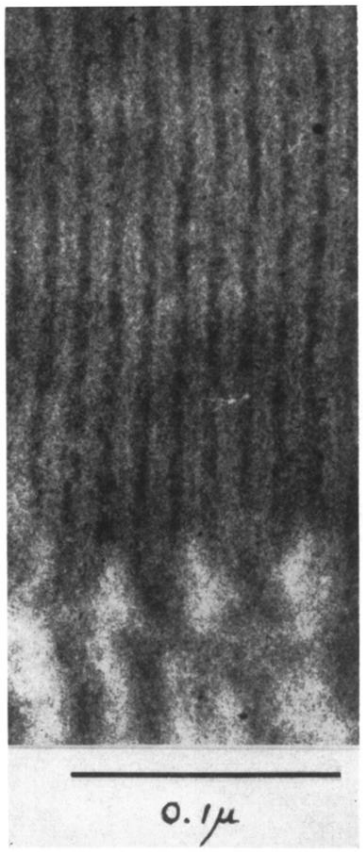

FIG. 8. Mesophyll chloroplast of *Zea* to illustrate the myelin-like structure within a granum. Note the main periodicity of about 130 A and the presence of fainter intermediate lines. ×370 000.

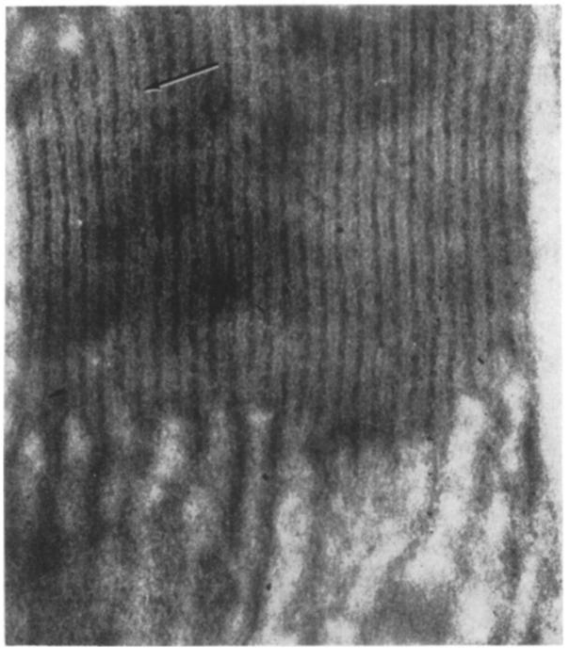

FIG. 9. A granum in a mesophyll chloroplast of *Zea*, illustrating the splitting of the main dense lines (arrow). The central dense lines (*P* zones) of the intergrana lamellae are also occasionally resolvable as doublets, thus showing that the compound lamellae consist of two closely apposed asymmetric unit membranes. ×220 000.