ULTRASTRUCTURE OF THE LAMELLAE AND GRANA IN THE CHLOROPLASTS OF ZEA MAYS L.

By A. J. HODGE, Ph.D., J. D. McLEAN, and F. V. MERCER, Ph.D.

(From the Chemical Physics Section, Division of Industrial Chemistry, Commonwealth Scientific and Industrial Research Organization, Melbourne, and the Botany Department, University of Sydney, Sydney, Australia)

Plates 158 to 160

(Received for publication, September 13, 1955)

Electron microscope studies of chloroplasts have confirmed the lamellar structure postulated from polarization optical and other studies (9). In the chloroplasts of lower plants, *e.g.*, *Euglena* (28), *Chlamydomonas* (22), *Chlorella* (1), *Spirogyra* (25), and *Nitella* (18), and in the chromoplasts of brown algae (*Fucus* (17)), the lamellae are fairly well ordered, but grana have not been demonstrated. In the higher plants, grana are present in the chloroplasts, and both the grana and the intervening regions (intergrana regions) are laminated. The grana consist of well ordered stacks of lamellae, *e.g.*, *Beta* (15), *Aspidistra* (7, 15, 16, 26), *Nicotiana* (4), and *Zea* (12).

The present paper is concerned with the finer structural details of the lamellae and grana, and with the interrelationships existing between the lamellae of the grana and those of the intergrana regions. It will be shown that the individual lamellae of both the grana and intergrana regions of *Zea* exhibit a characteristic compound layer structure when ultrathin sections are examined in the electron microscope. A possible interpretation of this compound structure in terms of the lipide, protein, and other components of the chloroplast will be given.

Materials and Methods

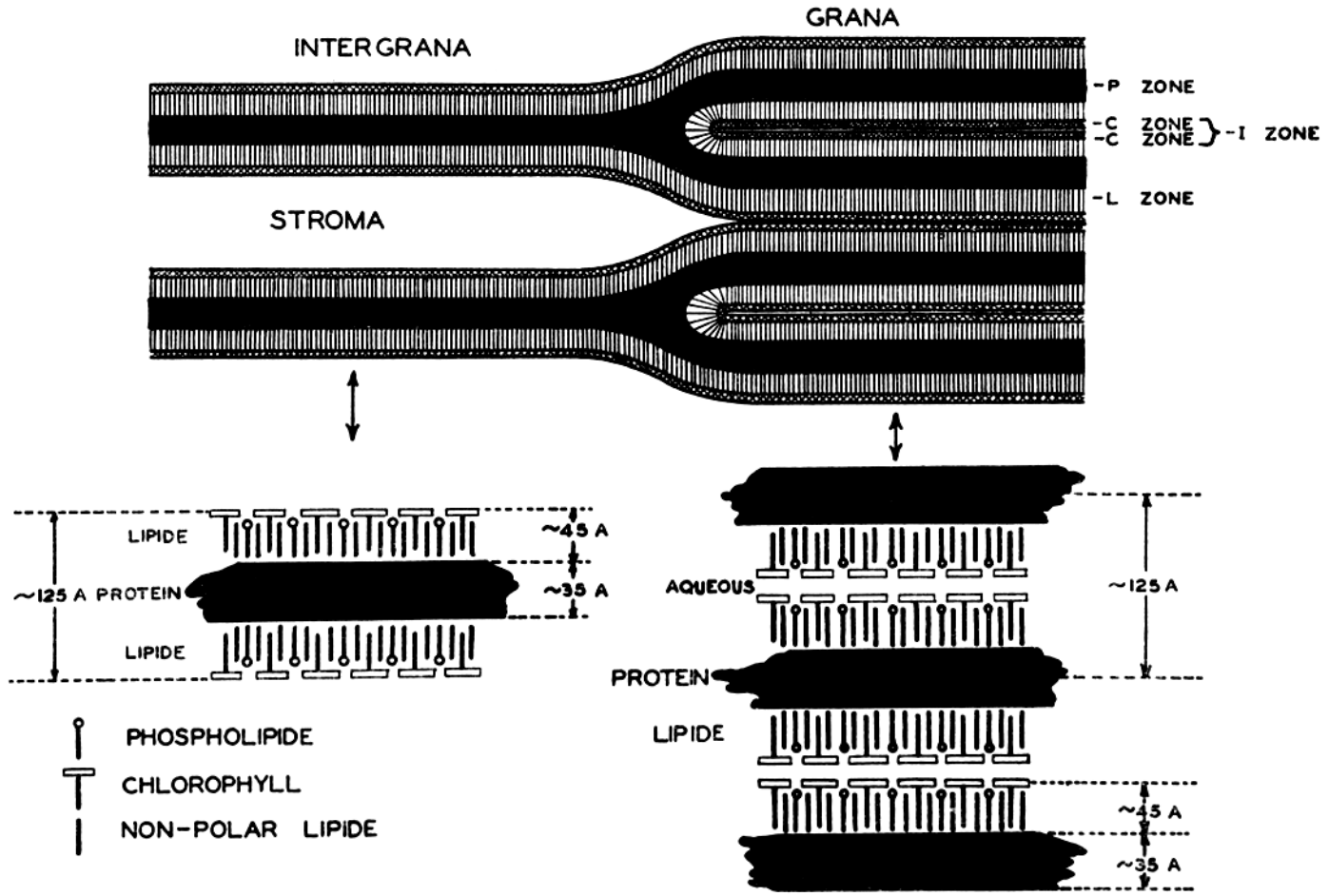
The chloroplasts were examined *in situ*. Small portions of the leaves of 3 to 4 week old maize plants were excised and fixed for 3 to 6 hours in 2 per cent osmium tetroxide solutions buffered to pH 7.2 with acetate-veronal (19) and adjusted to 0.2 M with sodium chloride. After washing and dehydration in an ethanol series, the leaf portions were embedded in *n*-butyl methacrylate, with or without the addition of up to 10 per cent of the methyl monomer. Portions of the leaves of etiolated seedlings were excised at various time intervals after exposure of the plants to daylight, and were prepared for examination in a similar manner. Thin sections were obtained using a microtome and the technique already described (11), and were examined in the electron microscope (RCA model EMU-1, fitted with an externally centerable objective aperture) without removal of the embedding medium.

RESULTS

Two types of chloroplast are found in the leaves of normal *Zea* plants (12, 21). The structural characteristics of these are illustrated in Fig. 1. In the parenchyma sheath cells which surround each vascular bundle, the chloroplasts contain many densely staining lamellae about 125 A thick, most of which extend the full width of the disc-shaped plastids. Grana are absent. The mesophyll chloroplasts, however, contain numerous grana, which are essentially cylindrical in form, rather uniform in diameter (0.3 to 0.4μ), and linked together by a system of lamellae (*ca.* 125 A thick) resembling those found in the chloroplasts of the parenchyma sheath cells. Each granum consists of a pile of circular dense lamellae (2 to 60 or more) which are stacked with high precision, the interlamellar spacing being about 125 A (Figs. 2, 5, and 6).

Both types of chloroplast (parenchyma sheath and mesophyll) are bounded by a thin limiting membrane which often appears as a double structure. This is most clearly defined in young plastids undergoing normal development and in those of plants recovering after etiolation (13). The membrane becomes less distinct as the plastids mature, but has been observed at all stages (*e.g.* Fig. 2). The parenchyma sheath and mesophyll chloroplasts both contain a finely granular matrix material, in which are embedded the dense lamellar system and a number of small dense spherical bodies (Figs. 1 and 2) similar to those reported in the chloroplasts of other plants (7, 16, 18, 26). The extralamellar matrix material will be referred to as the "stroma" of the chloroplast. The peripheral zone of the stroma (*i.e.*, the region of the chloroplast immediately adjacent to the limiting membrane) in both the mesophyll and parenchyma sheath chloroplasts, although devoid of the dense lamellae already described, is often faintly laminated (*e.g.* Fig. 7), particularly in the immature chloroplast. Evidence from electron microscopic studies on developing plastids (13) suggests that this peripheral zone is concerned with the production of lamellar precursors and that the faint laminations represent developing lamellae.

In addition to the components already mentioned, the chloroplasts of *Zea* contain variable numbers of starch grains. These are mainly located within the parenchyma sheath chloroplasts (Fig. 1), but occasional grains are found in the mesophyll plastids. In older (3 to 4 month) plants, the mesophyll chloroplasts usually contain several starch grains while those of the parenchyma sheath may possess starch grains to the extent of 90 per cent or more of their volume (12). Rhoades and Carvalho (21) studied the maize leaf under various conditions with the light microscope and concluded that the parenchyma sheath chloroplasts are specialized for the temporary storage of carbohydrate produced by photosynthetic activity of the mesophyll cells, pending transfer of these products to the vascular system. The functions of these two types of chloroplast will be discussed in terms of their fine structure in a later paper (12).



TEXT-FIG. 1. Diagrammatic representation of lamellar structure in the mesophyll chloroplasts of *Zea mays*. At top is a representation of the density observed in sections of osmium-fixed material, at bottom, an interpretation of this in terms of protein, lipide, and chlorophyll. The structure of the lamellae in the parenchyma sheath chloroplasts is indistinguishable from that of the intergrana lamellae of the mesophyll chloroplasts. Drawing not to scale.

The dense lamellae of the parenchyma sheath chloroplasts exhibit a compound layer structure which is indistinguishable from that of the intergrana and grana lamellae of the mesophyll chloroplasts (Figs. 2 to 5). In each case, the lamella (*ca.* 125 A thick) consists of a dense, rather granular zone (the P zone, *ca.* 35 A thick) interposed between two less dense layers (the L zones, *ca.* 45 A thick), the latter being in turn denser than the stroma. The outermost faces of the L zones are bordered by thin dense lines (Figs. 3 and 4) which will be termed C zones. The P, L, and C zones are only clearly distinguishable where the plane of the section is accurately normal to the lamellar plane. Within the grana, the C zones, by reason of their close apposition, give rise to thin relatively dense intermediate lines (I zones) situated midway between adjacent P zones (Figs. 5, 6, 8, and 9 and Text-fig. 1). It is of interest to note that we have observed a similar compound structure of the intergrana and grana lamellae in *Hydrangea* chloroplasts.

A number of chlorophyll-deficient mutants of *Zea mays* have been examined in the present series of studies on chloroplasts. Severe chlorophyll deficiency, such as for instance occurs in etiolated normal maize and in various white and pale green lethal mutant types, is associated with a failure to form lamellae. These results will be described elsewhere (13). However, it is of interest to note that in the chloroplasts of a non-lethal, yellow-green mutant of *Zea*, the fine structure of the lamellae and grana (Fig. 6) is indistinguishable from that of the normal plant (compare Fig. 6 with Figs. 2, 4, and 5).

In the mesophyll chloroplasts of *Zea*, the number of intergrana lamellae connecting with a particular granum is usually about half the number of P zones (*i.e.*, the number of compound lamellae) within the granum. This disparity appears to be due to a "forking" of the lamellae as they enter the granum (Figs. 2, 4, and 6). In its simplest form, the forking appears as a bifurcation such as is indicated schematically in Text-fig. 1. However, an occasional intergrana lamella is linked to three of the grana lamellae (Fig. 6), and a number of other interrelationships have been observed (12). The situation appears to differ considerably from that reported for *Aspidistra* (26). The granum is thus not simply a highly ordered "micellar" region within the lamellar system of the chloroplast, at least in *Zea*, for if this were the case, the ratio of the number of lamellae in a granum to the number of connecting intergrana lamellae should be nearer 1:1 than the observed value of *ca.* 2:1. Further information on these interrelationships is afforded by observations on chloroplast development under normal conditions and in leaves recovering from etiolation. The formation of the lamellar system in etiolated leaves exposed to daylight closely parallels that in the chloroplasts of plants undergoing normal development (13), but is more easily followed. Rudimentary grana are recognizable in the mesophyll chloroplasts at an early stage during such recovery (Fig. 8). Even at this early point in the developmental sequence, forking is evident at the edges of the immature

grana, and the structure is closely similar to that of mature grana (compare Figs. 8 and 9 with Figs. 5 and 6).

DISCUSSION

It is now generally accepted that the lamellae of chloroplasts represent a lipoprotein system, a concept compatible with the compound layer type of structure described in the present work. For a number of reasons, some of which are given below, we believe that the P zone of each compound lamella represents a layer of protein and the two L zones mixed lipide layers, the molecules of which are oriented with their long axes approximately normal to the plane of the lamellae and are bound in the structure by non-polar forces. Such an alternating arrangement of oriented lipide and protein layers is necessary to account for the form birefringence (see reference 9).

The protein of the chloroplast, much of which is in the lamellae, has a high sulfur content (10), presumably at least partly in the form of sulfhydryl groups and disulfide linkages. These are known to react strongly with osmium tetroxide (3), thus affording a possible explanation of the extraordinarily high density of the P zones in osmium-fixed material. Preliminary experiments involving electron microscopic examination of thin sections of ether-alcohol–extracted chloroplasts also indicate that the lamellar residue (presumably protein) stains rather densely with osmium tetroxide. The lamellar protein is also rather insoluble in aqueous solvents (see reference 20), a finding which is consistent with the protein having a high disulfide content (*cf.* keratin (27)). The protein surface might therefore reasonably be expected to possess relatively few charged groups and an abundance of non-polar groups available for interaction with lipides. The surfaces of the compound lamellae (*i.e.*, the outer surfaces of the L zones) are necessarily hydrophilic since the lamellae are immersed in an aqueous medium (the stroma). This condition can be met in two ways if it is conceded that the P zones represent the protein moiety of the lipoprotein complex. The L zone may comprise either a double layer of polar lipide linked to the protein by ionic forces or a single lipide layer with hydrophilic groups outermost. The dimensions of the various zones within the compound lamellae and the considerations already discussed appear to be most compatible with a model of the type illustrated in Text-fig. 1. The lipide monolayers (L zones) are bound in the structure by non-polar interaction of the hydrocarbon chains of both polar and non-polar lipides with the non-polar groups of the protein. The structure would also be stabilized by side to side interaction of the hydrocarbon chains. This model differs considerably from others proposed (14, 29), and has the advantage that it would allow swelling to take place by a simple increase in thickness of the aqueous layers separating the compound lamellae without disruption of the lipoprotein complex. Our results on chloroplast swelling in *Nitella* (18) and some preliminary electron microscopic observations of swelling

in *Zea* chloroplasts lend support to this concept. The model illustrates only the type of layer structure most consistent with our data, and is not intended to imply any particular stoichiometric ratios between the lamellar components.

The location of chlorophyll within the chloroplast is a question open to contention (see reference 9). One widely held view, based on the results of fluorescence microscopy, is that it is confined to the grana. However, as already pointed out, the chloroplasts of algae contain numerous lamellae but no grana. Moreover, the chloroplasts of the parenchyma sheath in *Zea*, which are green, and similarly laminated but devoid of grana, exhibit fluorescence characteristics indistinguishable from those of the mesophyll chloroplasts (12). It seems likely therefore that chlorophyll is distributed over the entire lamellar system (as indicated in Text-fig. 1). It would seem that fluorescence results should be interpreted with caution since the concentration of lamellar surface within the grana is more than twice that in the intergrana regions, at least in the mesophyll chloroplasts of *Zea mays*. Furthermore, the fluorescence characteristics of chlorophyll could conceivably be affected by its incorporation into highly ordered structures such as the grana.

The manner in which chlorophyll is linked to the lipoprotein of the chloroplast is similarly still open to question. Incorporation of the pigment in a chromoprotein has been suggested (see reference 20). However, the presence on the chlorophyll molecule of a hydrophobic phytol "tail" and the green colour of myelin forms (8) produced from chloroplasts suggests rather that it might be "bound" to the lipide component. Such a scheme does not exclude the possibility of the porphyrin "heads" being associated with stroma proteins, at least in the intergrana regions. Although a number of crystalline lipoprotein-chlorophyll complexes have been isolated, recent work (2) indicates that the pigment is very weakly bound in such complexes. Furthermore, in our experience, chlorophyll is readily extracted with ethanol from leaf material fixed in either formalin or osmium tetroxide solutions. The above considerations appear to be most compatible with an arrangement such as that in Text-fig. 1, in which the chlorophyll is held in the structure by van der Waals interaction of the phytol tail with the hydrocarbon moieties of the lipide layer. Spectroscopic data on chlorophyll indicate that in the plastid the porphyrin heads are situated in an aqueous environment (see reference 9), a condition fulfilled by our model. Moreover, this arrangement has the advantage that it would allow the incorporation of considerable amounts of non-polar lipides in the L zones because of a "screening" action of the hydrophilic porphyrin heads. This is of importance since one of the major difficulties in formulating a satisfactory model of chloroplast structure is the low content of phospholipide (20). The screening action would be maximal for a close packed chlorophyll monolayer. The rather approximate data currently available for the chlorophyll content of chloroplasts (see reference 20) and the lamellar area of *Zea* chloroplasts suggest that the

chlorophyll molecules are quite closely packed. Sterols could conceivably be incorporated within the lipide layers in a manner similar to that suggested for cholesterol in the myelin sheath of nerve (5).

The fine structure of the grana shown in the present work is remarkably similar in detail to that shown by Sjöstrand (24) for nerve myelin sheath, the resemblance extending even to the interlamellar spacing (*ca.* 120 A) and the presence of the thin intermediate lines (I zones). For reasons discussed by Sjöstrand (24), the latter zones must be regarded as real structures. In a model for myelin sheath such as that of Finean (5), in which non-polar interaction between the lipide and protein components is implied, these intermediate lines could arise from a more intense reaction of osmium tetroxide with the hydrophilic end groups of the phospholipides as compared with the hydrocarbon chains. In a model of the chloroplast such as that of Text-fig. 1, a similar differential reaction could account for the densities of the I and C zones. The structural resemblance of the grana of *Zea* to nerve myelin sheath is much closer than was indicated for the chloroplasts of *Aspidistra* in recent papers (7, 26), in which exact agreement was claimed for the values of the spacing (250 A) derived from electron microscopic and x-ray diffraction studies. In nerve myelin sheath, the possibility exists that the x-ray spacing (23, 5, 6) may be twice the value derived from electron microscopic studies. Finean (5) has indicated a possible explanation of this, but has also reviewed various other possible causes of the apparent discrepancy (6). The question of whether such a "doubling" of the measured spacings will be found for the grana of *Zea* must await a low-angle diffraction study of the chloroplasts from this plant.

SUMMARY

The fine structure of the chloroplasts of maize (*Zea mays* L.) has been investigated by electron microscopic examination of ultrathin sections of leaves fixed in buffered osmium tetroxide solutions. Both the parenchyma sheath and mesophyll chloroplasts contain a system of densely staining lamellae about 125 A thick immersed in a finely granular matrix material (the stroma), and are bounded by a thin limiting membrane which often appears as a double structure.

In the parenchyma sheath chloroplasts, the lamellae usually extend the full width of the disc-shaped plastids, and grana are absent. The mesophyll chloroplasts, however, contain numerous grana of a fairly regular cylindrical form. These consist of highly ordered stacks of dense lamellae, the interlamellar spacing being *ca.* 125 A. The grana are interlinked by a system of lamellae (intergrana lamellae) which are on the average about one-half as numerous as the lamellae within the grana. In general, this appears to be due to a bifurcation of the lamellae at the periphery of the granum, but more complex interrelationships have been observed.

The lamellae of the parenchyma sheath chloroplasts and those of both the grana and intergrana regions of the mesophyll chloroplasts exhibit a compound structure when oriented normally to the plane of the section. A central exceptionally dense line (*ca.* 35 A thick) designated the P zone is interposed between two less dense layers (the L zones, *ca.* 45 A thick), the outer borders of which are defined by thin dense lines (the C zones). Within the grana, the C zones, by virtue of their close apposition, give rise to thin dense intermediate lines (I zones) situated midway between adjacent P zones. A model of the lamellar structure is proposed in which mixed lipide layers (L zones) are linked to a protein layer (P zone) by non-polar interaction. Chlorophyll is distributed over the entire lamellar surface and held in the structure by van der Waals interaction of the phytol "tail" with the hydrocarbon moieties of the mixed lipide layers. The evidence in favour of the model is briefly discussed.

BIBLIOGRAPHY

1. Albertsson, P. A., and Leyon, H., *Exp. Cell Research*, 1954, **7**, 288.
2. Anderson, D. R., Spikes, J. D., and Lumry, R., *Biochim. et Biophysica Acta*, 1954, **15**, 298.
3. Bahr, G. F., *Exp. Cell Research*, 1954, **7**, 457.
4. Cohen, M., and Bowler, E., *Protoplasma*, 1953, **42**, 414.
5. Finean, J. B., *Experientia*, 1953, **9**, 17.
6. Finean, J. B., *Exp. Cell Research*, 1954, **6**, 283.
7. Finean, J. B., Sjöstrand, F. S., and Steinmann, E., *Exp. Cell Research*, 1953, **5**, 557.
8. Frey-Wyssling, A., *Discussions Faraday Soc.*, 1949, No. 6, 130.
9. Frey-Wyssling, A., Submicroscopic Morphology of Protoplasm, Amsterdam, Elsevier Publishing Co., 1953.
10. Hanson, E. A., Barrien, H. S., and Wood, J. G., *Australian J. Exp. Biol. and Med. Sc.*, 1941, **19**, 231.
11. Hodge, A. J., Huxley, H. E., and Spiro, D., *J. Histochem. and Cytochem.*, 1954, **2**, 54.
12. Hodge, A. J., McLean, J. D., and Mercer, F. V., in preparation.
13. Hodge, A. J., McLean, J. D., and Mercer, F. V., in preparation.
14. Hubert, B., *Rec. trav. bot. néerl.*, 1935, **32**, 323.
15. Leyon, H., *Exp. Cell Research*, 1953, **4**, 371.
16. Leyon, H., *Exp. Cell Research*, 1954, **7**, 265.
17. Leyon, H. and Wettstein, D. von., *Z. Naturforsch.*, 1954, **96**, 471.
18. Mercer, F. V., Hodge, A. J., Hope, A. B., and McLean, J. D., *Australian J. Biol. Sc.*, 1955, **8**, 1.
19. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
20. Rabinowitch, E. I., Photosynthesis and Related Processes, Interscience Publishers, New York, 1945, 1.
21. Rhoades, M. M., and Carvalho, A., *Bull. Torrey Bot. Club*, 1944, **71**, 335.
22. Sager, R., and Palade, G. E., *Exp. Cell Research*, 1954, **7**, 584.

23. Schmitt, F. O., Bear, R. S., and Palmer, K. J., *J. Cell. and Comp. Physiol.*, 1941, **18**, 31.
24. Sjöstrand, F. S., *Experientia*, 1953, **11**, 63.
25. Steinmann, E., *Exp. Cell Research*, 1952, **3**, 367.
26. Steinmann, E., and Sjöstrand, F. S., *Exp. Cell Research*, 1955, **8**, 15.
27. Ward, W. H., and Lundgren, H. P., *Advances Protein Chem.*, 1954, **9**, 244.
28. Wolken, J. J., and Palade, G. E., *Ann. New York Acad. Sc.*, 1953, **56**, 873.
29. Wolken, J. J., and Schwertz, F. A., *J. Gen. Physiol.*, 1953, **37**, 111.

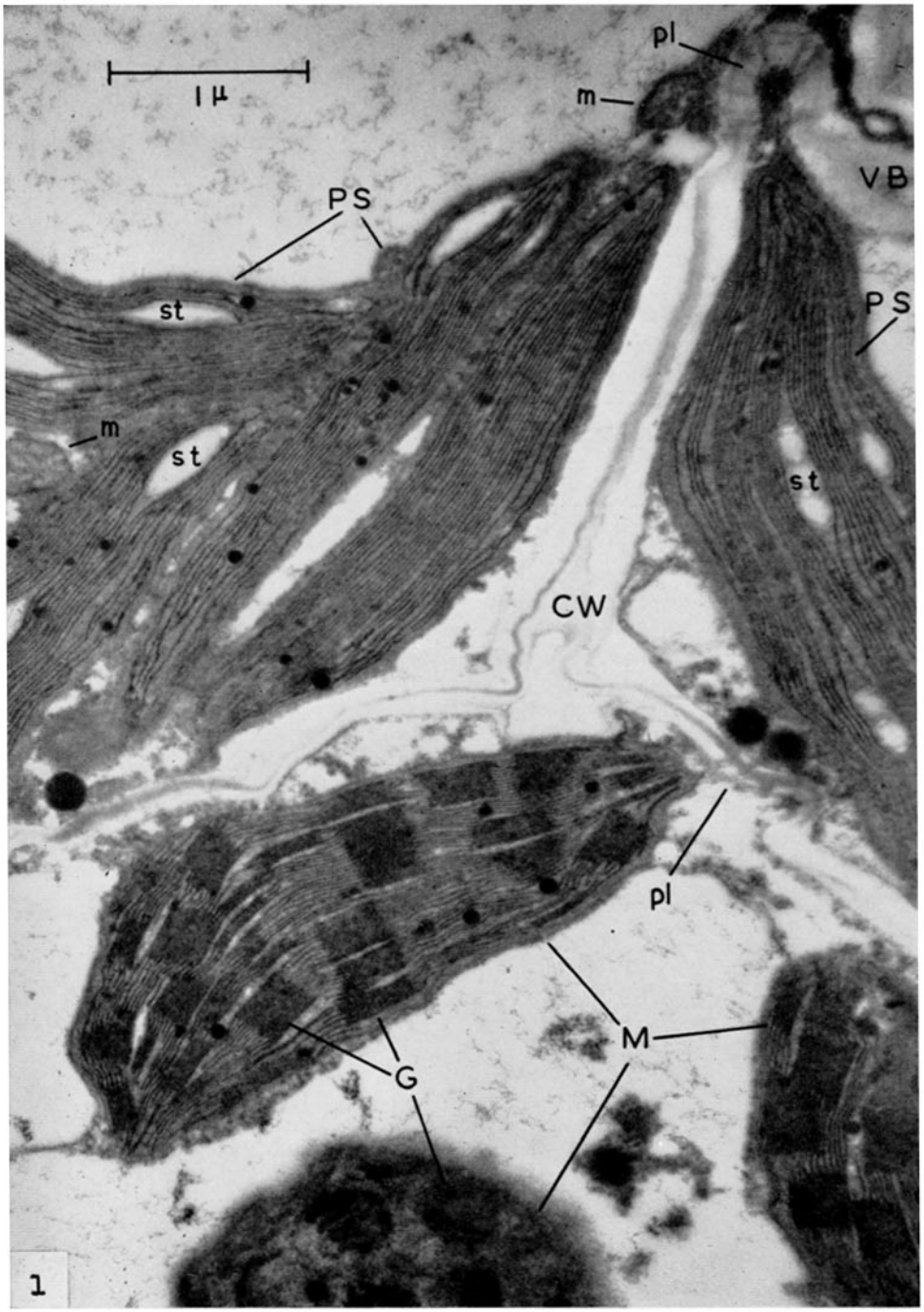
EXPLANATION OF PLATES

(All figures are electron micrographs of thin sections of maize leaves fixed in buffered osmium tetroxide solutions.)

PS, parenchyma sheath chloroplasts.
M, mesophyll chloroplast.
DSB, dense spherical bodies.
CW, cell wall.
st, starch grain.
pl, plasmodesmata.
P, P zone of lamellae.
L, L zone of lamellae.
I, I zone of lamellae.
C, C zone of lamellae.
G, granum.
sr, stroma (finely granular matrix) of chloroplast.
CM, chloroplast limiting membrane.
VB, vascular bundle.
cy, cytoplasm.
m, mitochondrion.

PLATE 158

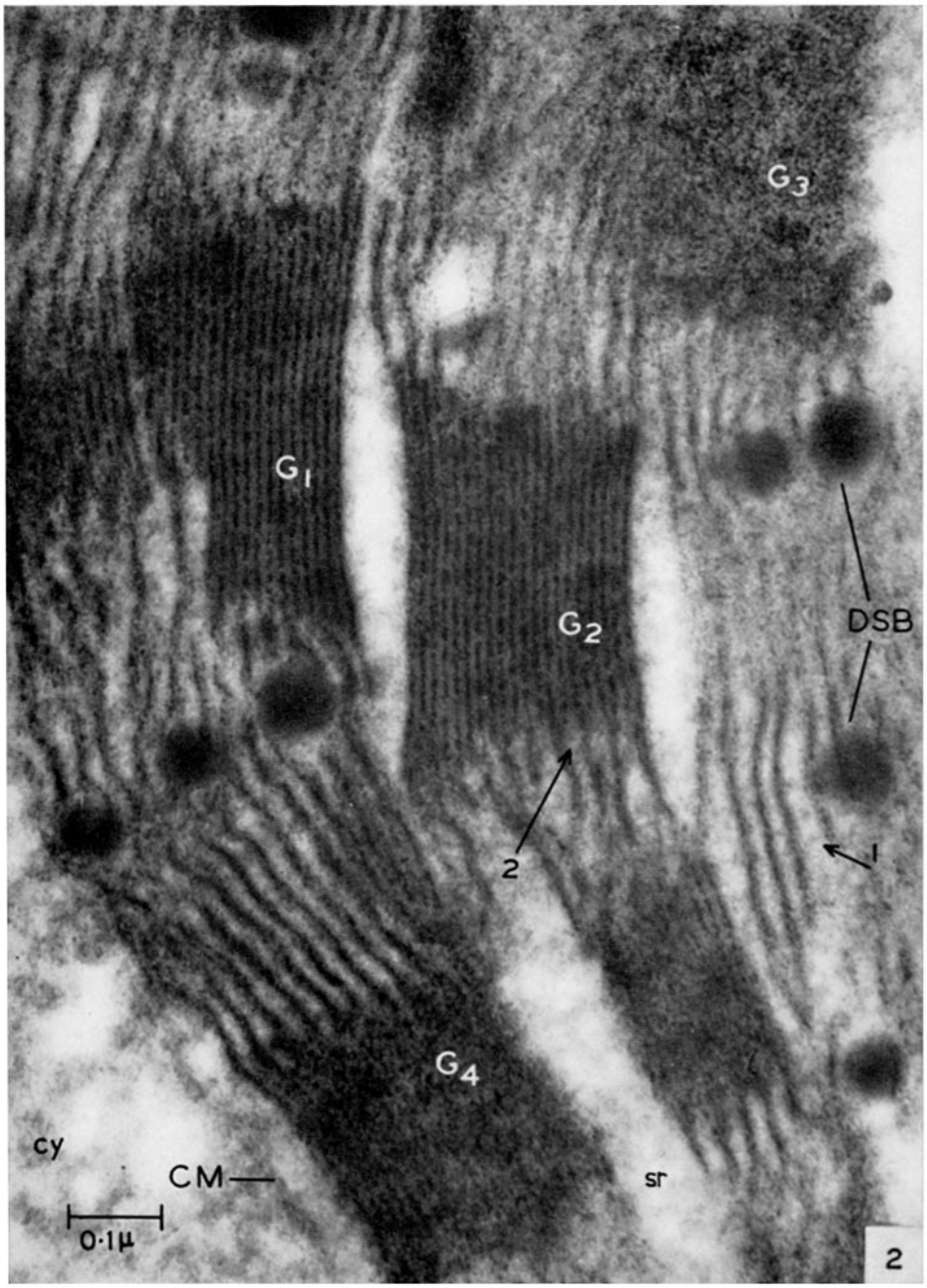
FIG. 1. General view in a transverse section of a 3 to 4 week old maize leaf in a region adjacent to a vascular bundle (*VB*), showing typical parenchyma sheath (*PS*) and mesophyll (*M*) chloroplasts. Note the lamellation of both types, the presence of well defined grana (*G*) in the mesophyll chloroplasts and their absence in those of the parenchyma sheath. The mesophyll chloroplast at the bottom of the figure has been sectioned in a plane essentially parallel to the plane of the lamellae. Under these conditions, the grana appear as circular dense areas. Note the starch grains (*st*) in the parenchyma sheath chloroplasts, and the presence of small dense spherical bodies in both types of plastid. These, together with the lamellar system, are embedded in a finely granular stroma or matrix material, which appears to be rather denser in the parenchyma sheath chloroplasts than in those of the mesophyll cells. Note also the cell walls (*CW*), in which can be seen a number of transverse structures (*pl*), possibly representing plasmodesmata. × 27,800.



(Hodge *et al.*: Lamellae and grana in chloroplasts)

PLATE 159

FIG. 2. Portion of a mesophyll chloroplast from a 3 to 4 week old maize leaf, illustrating in greater detail than Fig. 1 the lamellar structure of the grana and of the intergrana regions. Where the plane of the section is accurately normal to the lamellar plane, the compound nature of the individual lamellae (arrow 1) and the precise stacking of lamellae within the grana is clearly seen (G_1 and G_2). The lamellar structure of the grana is, however, scarcely discernible when they are obliquely oriented to the plane of the section (*e.g.* G_3 and G_4). The disparity between the number of lamellae connected to a particular granum and the number of P zones within that granum is due to forking (arrow 2) at the periphery of the granum (see Text-fig. 1). A number of dense spherical bodies (*DSB*) are present in the stroma and the limiting membrane (*CM*) of the chloroplast is imaged rather indistinctly at lower left. × 130,000.



(Hodge *et al.*: Lamellae and grana in chloroplasts)

PLATE 160

FIG. 3. Portion of a parenchyma sheath chloroplast from a 3 to 4 week old leaf, showing the finely granular stroma (*sr*) and the compound structure of the lamellae (P, L, and C zones, *cf.* Text-fig. 1). The central dense line (P zone) of each compound lamella is interposed between two less dense layers (L zones). The latter are in turn bordered by thin dense lines (C zones). × 260,000.

FIG. 4. Intergrana lamellae and portion of a granum (lower right) of a mesophyll chloroplast from a 3 to 4 week old leaf. Note compound structure and forking of the lamellae as they enter the granum. × 260,000.

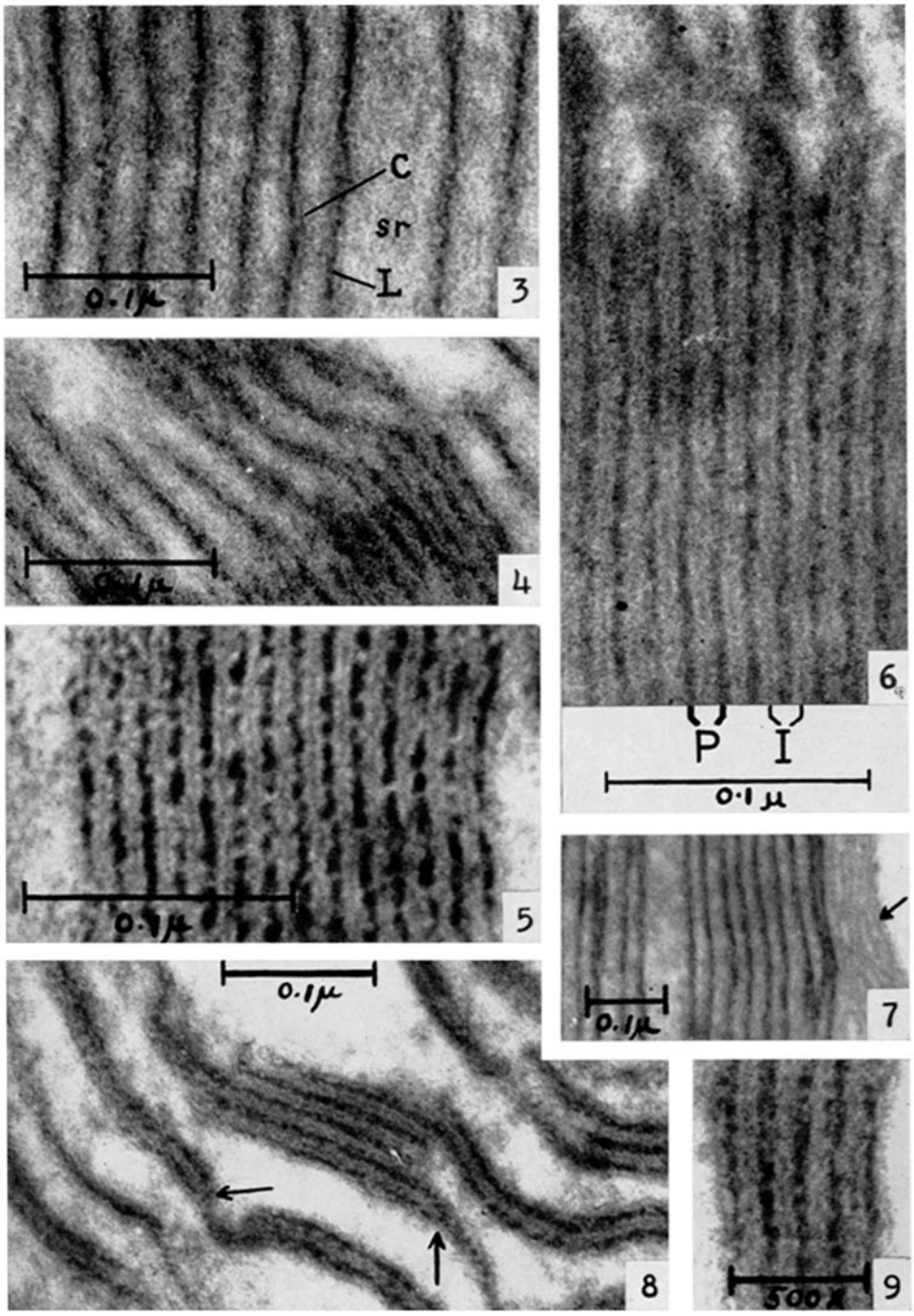
FIG. 5. Slightly underfocused electron micrograph showing a portion of a granum in a mesophyll chloroplast of a 3 to 4 week old leaf. The P, L, and I zones are clearly defined (compare with Text-fig. 1). The absence of correspondence between the course of the I zones and the granular structure of the adjacent P zones indicates that the presence of the I zones in the image is not due to fringe effects arising from the slight degree of underfocus involved. × 370,000.

FIG. 6. Granum from a mesophyll chloroplast in a 3 to 4 week old leaf of a yellow-green non-lethal mutant of maize, showing the compound layer structure and forking at the edge of the granum. The compound structure is indistinguishable from that observed in the grana of normal plants. Close to focus electron micrograph. × 370,000.

FIG. 7. Parenchyma sheath chloroplast in a 3 to 4 week old leaf of normal maize, showing the characteristically dense lamellae in the interior of the chloroplast and the faint lamination (arrow) in the peripheral zone. × 110,000.

FIG. 8. Grana developing in a mesophyll chloroplast of an etiolated maize leaf 20 hours after exposure to daylight. Note the characteristic grana structure wherever two or more compound lamellae are in close apposition, and the forking of the lamellae (arrows). × 210,000.

FIG. 9. Enlargement of part of Fig. 8, showing the compound structure in a developing granum. Compare with Fig. 6. × 370,000.



(Hodge *et al.*: Lamellae and grana in chloroplasts)