Comparative Studies on Chloroplast Development and Photosynthetic Activities in C_3- and C_4-Plants : 1. Studies on Ultrastructure of Developing Chloroplasts within Vascular Bundle Sheaths and Mesophyll Cells of Barley and Maize Leaves

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Comparative Studies on Chloroplast Development and Photosynthetic Activities in C_3- and C_4-Plants

1. Studies on Ultrastructure of Developing Chloroplasts within Vascular Bundle Sheaths and Mesophyll Cells of Barley and Maize Leaves

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Vascular bundles of barley leaves are surrounded by double layers, consisting of an outer parenchyma sheath and inner endodermal layers (mestom sheath). Chloroplasts exist within mestom cells as well as within parenchyma sheath cells, but the rudimentary chloroplasts within young mestom sheath cells disappear during cell differentiation. The primordia of proplastids are suggested to be different from mitochondrial primordia. In early stage of development, they have similar developmental patterns induced by invagination of their inner envelopes. Prolamellar bodies within parenchyma sheath cell etioplasts of maize leaves require light energy to be dispersed into lamellae as do those of etioplasts within mesophyll and parenchyma sheath cells of barley leaves, but those within mesophyll chloroplasts of maize leaves require more light energy. Grana are formed in barley and maize chloroplasts after 6 hours illumination. This suggests no difference in light energy requirements for formation of grana between the chloroplasts of barley and maize leaves. The ultrastructure of chloroplasts within parenchyma sheath cells of maize leaves are similar to those at early stage of chloroplast development within mesophyll and parenchyma sheath cells of barley leaves illuminated by light for 1 or 3 hours. New starch grains assimilated by photosynthesis are observed in the chloroplasts within the barley leaves after 6 hours illumination, but within maize leaves they are observed in the chloroplasts after 18 hours illumination. Etioplasts increase in size -during dark germination, but after illumination and initiation of photosynthetic activities they shrink.

INTRODUCTION

Plants are generally divided into two groups with respect to photosynthetic systems. The C-plants include temperate grasses which belong to the Festucoid group. They have 3-phosphoglyceric acid as the primary product after short-term CO, incorporation. The genera, *Triticum*, *Avena*, *Hordeum*, and *Lolium* have two layers of bundle sheath cells (Bisalputra *et al.*, *1969;* Brown, 1958; Brown, 1960; Esau, 1965). The outer sheath is parenchyma and often develops chloroplasts which are reported to be either similar to those of mesophyll cells (Brown, 1958) or somewhat smaller than the mesophyll chloroplasts (Esau, 1965). The chloroplasts of mesophyll and parenchyma sheath cells have starch grains,

but the inner sheath (mestom sheath) is usually colorless and consists of thick walled endodermal-like cells without chloroplasts (Bisalputra *et al., 1969 ;* Brown, 1960).

The second group, called the C_4-plants which include many tropical grasses have malic and aspartic acids as the primary products after short-term CO, incorporation. Some of the C_4-plants have specialized chloroplasts in the bundle sheath cells (Brown, 1958; Brown, 1960; Esau, 1965; Gutierrez, *et al.*, 1974; Laetsch et *al., 1966;* Osipova *et al., 1965;* Shumway et *al.,* 1967; Weier et *al.,* 1967). Those chloroplasts are sometimes larger than those found in the mesophyll cells and contain many starch grains (Brown *et al.,* 1960; Esau, 1965: Hodge *et al.,* 1955; Laetsch *et al.,* 1966; Shumway *et al.,* 1967). They are generally positioned centrifugally or centripetally within the cells (Brown, 1960; Gutierrez et *al., 1974).* The arrangements of the internal lamellae of the bundle sheath chloroplasts in some C_4-plants are such that grana are lacking (Brown, 1960; Esau, 1965) or not well developed (Shumway *et al.,* 1967; Weier *et al.,* 1967). Kranz cells are thick walled and their plastids store many starch grains, whereas mesophyll cells are thin walled and have fewer starch grains (Johnson et *al., 1973)* and also contain well developed grana (Brown, 1960; Esau, 1965; Hodge *et al.,* 1955; Laetsch, 1971; Laetsch *et al.,* 1966; Shumway *et al.,* 1967).

Furthermore, the single parenchyma sheath cells in maize leaves and the outer cells of the two sheaths in barley leaves originate as part of the ground tissue (Esau, 1965). But anatomical and cytological aspects, and physiological functions of these cell layers are quite different between the two groups of plants.

Prolamellar bodies are formed by vesicles which originate from invaginations of the inner membrane of the etioplast envelope, and upon illumination grana may be formed by an outgrowth of the membranes of prolamellar body, or transformation of prolamellar body into vesicles which are then dispersed, aligned and fused in primary layers from which grana are formed (Gerola *et al.,* 1960; Hodge *et al.,* 1956; Murakami, 1962a; Röbbelen, 1959; Virgin *et al.,* 1963). The formation of prolamellar body occurs, as a rule, in etiolated plants (Ikeda, 1970; Miihtethaler *et al.,* 1959), but they can be formed under dim light condition below 300 lux (Wrischer, 1966) and during dark periods (Ikeda, 1971).

This report emphasizes aspects of chloroplast development and its ultrastructural changes, as comparing with two groups of plants. One is C_3-plant (barley), and the other is C_4-plant (maize).

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) were sterilized in 0.5 % formalin solution for 1 hour, rinsed several times in tap water and germinated in a dark room at 25°C for 6 days. The leaves were fixed in a dark room after 0, 2, 3, 4, 5 and 6 days of germination in the dark. After 6 days of dark growth some of the seedlings were exposed to light of 1,000 ft-c (250 W tungsten lamps, 40 W fluorescent lamps and 500 W mercury

lamps) for 5 minutes and 1, 3, 6, 9, 18, 24 hours, respectively. Leaf segments of about l-mm thickness aparted 1 cm from the leaf apex were prefixed in 2 % glutaraldehyde for 2 hours and postfixed in 2 % osmic acid or $KMnO_4$ solution (Dalton, 1955) for 2 hours. The materials were dehydrated in graded ethanol series followed by propylene oxide and embedded in epon 812 resin (Luft, 1961). Sections were made with a glass knife on a Porter-Blum Ultramicrotome and stained with 2 % uranyl acetate and lead citrate solution (Reynolds, 1963). These sections were examined in an electron microscope, JEM T7.

RESULTS

I) Barley

Vascular bundles of barley leaves are surrounded by double sheaths, an inner endodermal sheath (mestom) and outer parenchyma sheath (Figs. 7, 10, 13). Mestom sheath cells and parenchyma sheath cells contain chloroplasts, but the numbers and size of the chloroplasts in the mestom sheath cells are much smaller than those in the parenchyma sheath cells (Fig. 7). During cell differentiation, rudimentary chloroplasts within mestom cells disappear (Figs. 10, 13), but chloroplasts within parenchyma sheath cells persist in the differentiated cells. The size of chloroplast profiles in parenchyma sheath cells is a little smaller than that within mesophyll cells. Chloroplast development in the parenchyma sheath cells is similar to that of chloroplast in mesophyll cells. Prolamellar bodies are also formed by the invagination of the inner membrane of the chloroplast envelope during dark germination. After illumination, the body is dispersed into lamellae in the stroma (Figs. 10, 11), and grana are formed (Figs. 12, 13, 14). Starch grains can be also observed in the chloroplasts of parenchyma sheath cells as well as of mesophyll cells (Fig. 13).

Parenchyma cells of the embryo's first foliage leaf contain numerous proplastids and mitochondria. Proplastid profiles are approximately 1.3 μm in length and 1.1 μm in width. Mitochondria are about 0.7 μm in length and width. But small proplastids are sometime difficult to be distinguished from mitochondria (Fig. 1).

Ultrastructurally, proplastids without starch grains in the matrix are uniformly stained and some large one contains a single thylakoid-like membrane in the matrix, while proplastids with starch grains in the matrix are not well stained. Mitochondria usually contain electron-opaque inclusions in the matrix. Numerous lipid bodies are arranged uniformly inside of plasmamembrane and scattered in the cytoplasm at this stage (Fig. 1). After 2 days of dark germination, lipid bodies completely disappear from cytoplasm. Proplastids are easily distinguished from mitochondria because of their unique morphology. A few membranes are formed from the inner envelope of the proplastids (Figs. 2, 3). The size of proplastid profiles in these parenchymatous cells of the first foliage leaf germinated during 2 days of dark, 1.4 μm in length and 0.9 μm in width, is almost the same as that in the parenchymatous cells of the embryo's first foliage leaf. Mitochondria also have rudimentary cristae (Fig.

2). Prolamellar bodies can not be observed in these proplastids, but one or two prolamellar bodies are formed in the central region of the etioplasts after 3 days of dark germination. They have less tightly contracted membranes, a somewhat less crystalline appearance than those in etioplasts (Figs. 4, 5). In cross section, the peripheral lamellae appear as rows of vesicles and arise from the inner membrane of the etioplast envelope (Fig. 5). At this stage, the size of etioplast profiles increases to about 2 μm in length and 1.2 μm in width. Cell vacuoles also increase in size (Fig. 4). The starch grains cannot be observed in these etioplast profiles (Fig. 4). After 4 days of dark germination, etioplast profiles, 4 μm in length and 3 μm in width, have increased about 3 times over those in the embryo's first foliage leaf. Cytoplasmic vacuoles become larger and cytoplasm adheres closely to the peripheral region of the cells (Fig. 7). In 5- and G-day dark grown seedlings, one or more crystalline prolamellar bodies are formed. The lamellae are connected with each other (Figs. 6, 8). After illumination, the first morphological response is the disappearance of the quasicrystalline appearance of the prolamallar body. The honeycomb-like quasicrystalline body is loosened and membranes radiate from the prolamellar body (Fig. 9). In chloroplasts of the leaf illuminated for 1 or 3 hours two thylakoids overlap on some of these radiating membranes (arrows in Fig. 11). In some case, the overlaps appear as single short lamellae appressed to longer lamellae (single arrows in Fig. 11) or single lamellae appear to be flattened (double arrows in Fig. 11). It seems to be the first grana. The quasicrystalline prolamellar body is dispersed into thylakoids in the stroma by further illumination. The body cannot be observed in chloroplasts illuminated for 6 hours. In this stage, grana of 2 or 3 thylakoids are formed in the chloroplasts (Fig. 12). Thereafter, grana increase in number and size by further illumination (Figs. 13, 14). Starch grains formed by photosynthesis are first observed in chloroplast illuminated for 6 hours after 6 days of dark growth (Fig. 12). There are few grains at this stage, but many grains can be observed much in chloroplasts illuminated for 18 hours after 6 days of dark germination (Fig. 13). The shape of chloroplast profiles is changed from short ellipsoid to long ellipsoid types by the decrease in width. Chloroplast profiles are about 5.9 μm in length and 1.8 μm in width when a leaf is illuminated for 24 hours (Fig. 14).

II) Maize

The maize leaf does not have a mestom sheath around each vascular bundle. However, the parenchyma bundle sheath cells are very well developed. The sheath cells are very large and are reported to have large pale green chloroplasts containing many starch grains (Brown, 1958; Rhodes *et al.*, 1944).

There are also many small proplastids and mitochondria in the mesophyll and parenchyma sheath cells of the embryo's first foliage leaf. Usually, large proplastids can be easily distinguished from mitochondria, but small one cannot. Lipid bodies are also prominent cell inclusions and are arranged inside of the plasmamembrane and scattered in the cytoplasm (Fig. 15). The shape of proplastid profiles is the same as that of proplastid profiles in the embryo's first

foliage leaf of barley leaf. The size of proplastid profiles in the mesophyll cells is almost the same as that in the parenchyma sheath cells. Proplastid profiles are approximately 1.0 μm in length and 0.8 μm in width in mesophyll cells, and 1.0 μm in length and 0.9 μm in width in parenchyma sheath cells. At the 2 days stage of dark germination, iipid bodies disappear from the cytoplasm, while oil droplets can be observed in the cytoplasm. Starch grains cannot be observed in the matrix of the proplastids. In this stage, proplastids and mitochondria are differentiated by the invagination of the inner membrane of their envelope (Fig. 16). At 3 days stage of dark germination, a few thylakoids are formed in the plastids and large starch grains accumulate in them. In this stage, small vacuoles can be observed in the cytoplasm and proplastid profiles can be distinguished from mitochondria. Plastid profiles are approximately 2.2 μm in length and 1.3 μm in width in mesophyll cells, and 1.8 μm in length and 1.1 μm in width in parenchyma sheath cells. Mesophyll cells and parenchyma sheath cells are connected with plasmodesmata. And also many lamellae of the endoplasmic reticulum can be observed near the plasmodesmata (Figs. 16, 17, 18). At 4 days stage of dark germination, proplastid profiles increase in size about 2 times over those in the embryo's first foliage leaf. Plastid profiles are approximately 2.6 μm in length and 2.1 μm in width in mesophyll cells, and 2.5 μm in length and 1.8 μm in width in parenchyma sheath cells. Large starch grains accumulate in the matrix of the plastids and decrease in size during differentiation, while a few long thylakoids appear and are connected with the inner membrane of the proplastid envelope (Fig. 18). After 5 days of dark germination etioplasts, approximately 4.3 μm in length and 3.2 μm in width in mesophyll cells, 4.1 μm in length and 2.9 μm in width in parenchyma sheath cells, are about 3 or 4 times larger than those of the embryo's first foliage leaf. Prolamellar bodies are observed in both etioplasts within mesophyll cells and parenchyma sheath cells (Fig. 19), but they have less tightly contracted membranes (Fig. 20). In the etioplasts within the cells after 6 days of dark germination, prolamellar body appears as quasicrystaline structure and increases in size. Many thylakoids arise by ordered fusion of the minute vesicles. The thylakoids in peripheral zone usually circle inside of the etioplasts. There are also many small vesicles between the circle lameilar and inner envelope of the etioplasts (arrows in Fig. 21). The quasicrystalline prolamellar body begins to be disorganized after illumination. The disorganization of the prolamellar body in parenchyma sheath cells proceeds very rapidly after illumination of 1,000 ft-c, while in mesophyll cells etioplasts, some of the bodies persist until 18 hours of illumination (Fig. 24). One or 3 hours illumination after 6 days of dark germination completely disperses the prolamellar body in parenchyma sheath cells into lamellae, and single parallel thylakoid extends from one end of the chloroplast to the other. But the thylakoid lamellae become enlargement and are more darkly stained by lead acetate than any other parts of the lamellae (Figs. 22). In mesophyll cells, the prolamellar body is dispersed into lamellae in a circular form (Figs. 22, 23), and grana form in the chloroplasts after the leaf is illuminated for 6 hours after 6 days of dark germination (Fig. 23). After further illumination, grana increase in size (Figs. 24,

25). New starch grains, formed by photosynthesis, are formed in the chloroplasts within mesophyll and parenchyma sheath cells illuminated for 18 hours after 6 days of dark germination. There are few starch grains in the mesophyll cells (Fig. 24).

DISCUSSION

Esau (1965) stated that the bundle sheaths of monocotyledons, the single parenchyma sheath in Panicoideae and outer sheath of Festucoideae were same origin, a part of ground tissue and also had chloroplasts in the cells. We can also observe many well-developed chloroplasts in parenchyma sheath cells as well as in mesophyll cells of barley leaves, but the numbers of chloroplasts in parenchyma sheath cells are less than those of chloroplasts in the mesophyll cells. In maize leaves illuminated for long periods, well developed chloroplasts with grana can be observed in the mesophyll cells, but not in the parenchyma sheath cells (Hinchman, 1972; Hodge *et al.*, 1955; Laetsch, 1971; Osipova *et al.*, 1965; Shumway *et al., 1967).*

Zirkle (1929) stated that large mitochondria developed to be plastids and small one developed to be mitochondria in maize leaves. But Hinchman (1972) stated that proplastids (ca. 2 μm) tended to be larger than the mitochondria (1 μm) and usually contained one or more characteristic inclusions, but small proplastids were sometimes difficult to be distinguished from mitochondria. Our results also show that large proplastids can be easily distinguished from mitochondria in the embryo's first foliage leaves from barley and maize plants, because of their similar patterns of differentiation. Perforated lamellae invaginate from the inner membrane of the envelope in early stage of development, but by further germination they can be easily distinguished from each other, because the perforated lamellae become thylakoids and cristae. Therefore, we think that plastid primordia are different from mitochondria primordia. Only those have similar developmental patterns in early stage of germination.

Growth of barley chloroplasts in the dark has been studied by Smith (1954), Henningsen *et al.* (1969) and Robertson *et al.* (1974). Smith (1954) found that the numbers of chlororoplasts per cell in the apical 1 cm of successively older leaves increased during early growth and then reached a plateau. Henningsen *et al.* (1969) showed that chloroplast's size increased through 7 days of growth in dark and then declined thereafter. Robertson *et al.* (1974) showed that plastids in all regions of the leaf were capable of enlarging when exposed to light, whether in the intact plant or excised sections, but plastid replication occurred predominantly in the younger regions of the leaf (regions 4 and 5) where some cell division and elongation were taking place. Our results show that plastids grow remarkably after 4 days of dark germination in barley leaf and 5 days of dark germination in maize leaf. After illumination, chloroplasts shrink. Therefore, the shape of the chloroplast profiles changes from short ellipsoid to long ellipsoid type. We suggest that chloroplast shrinkage is due to energy transfer which leads to synthesis of adenosine triphosphate and the effusion of ions according to active photosynthesis (Hilliard *et al.*, 1969 ;

Siegenthaler *et al., 1965).*

The prolamellar body developed from membranes apparently produced by the inner membranes of the plastids. According to Virgin *et al.* (1963), the etioplasts of etiolated bean seedlings attained a relatively large size in darkness. Vesicles were formed by a continuous process of invagination and blebbing off of the inner membrane of the chloroplast envelope. Some of the vesicles became associated in the prolamellar body and others became aligned in primary layers throughout the stroma. With continued growth in darkness the vesicles were transformed into relatively long and evenly spaced tubes which emerged to form the quasicrystalline prolamellar body. Upon subjecting the etiolated proplastids to the light the tubes in the prolamellar body were transformed into vesicles, These vesicles were dispersed throughout the stroma where they became arranged in primary layers. The dispersed vesicles fused into discs which aggregated to form grana. A similar type chloroplast development in etiolated seedlings has been described by many authors (Gerola et *al., 1960* ; Hodge et *al., 1956;* Murakami, 1962a ; Murakami, 1962b). But Engelbrecht et *al. (1967)* showed that proplastids contained a few irregular internal membranes. During dark germination, sheets or sac-like membranes were produced by the activity of the inner component of the proplastid. These continuous membranes became reticulate and aggregated to the center of the proplastid to form after 7 days germination a quasicrystalline prolamellar body. Weier et *al. (1970)* found that from 2 to 6 days there was an increase in plastid size and starch content and synthesis of a system of porous lamellae which appeared to arise from the inner component of the plastid envelope. From 6 to 8 days much of the starch disappeared accompanied by rapid membrane synthesis resulting in an extensive prolamellar body. In our experiments there are numerous perforated small lamellae at the peripheral zone of the chloroplasts by invagination of inner components of envelope. The small lamellae aggregate to form prolamellar body usually in the central zone of the etioplasts and by subjecting to light the lamellae again aligne each other in stroma from prolamellar body to be fused. Grana can be observed in the chloroplasts illuminated during 6 hours in barley and maize leaves. These results show that C_3- and C_4-plants require the same light energy for grana formation.

On the other hand, many crystalline prolamellar bodies are formed under dim light below 300 lux condition (Wrischer, 1966) and appear in the case where the rate of vesicles formation exceeds the rate of conversion of vesicles into lamellar systems and light energy is need for the conversion of vesicles into lamellae but not for the synthesis of vesicles (Ikeda, 1971). In our experiments the prolamellar bodies are completely dispersed into lamellae in the chloroplasts of barley leaf illuminated by 1,000 ft-c of light for 3 hours after 6 days of dark germination, while in the chloroplasts within mesophyll cells of maize leaves the body persists during 18 hours under the same light condition and cannot be observed in the chloroplasts of parenchyma sheath cells illuminated for 6 hours. These results suggest that the chloroplasts within mesophyll cells of maize leaf require more light energy than those within chlorenchyma cells of barley, but those within parenchyma sheath cells of maize leaf have the

same requirement of light energy for vesicle dispersion from prolamellar body.

Ultrastructural aspects of chloroplasts within parenchyma sheath cells of maize leaves are similar to those of chloroplasts within chlorenchyma cells of barley leaves at early stage of chloroplast development. Single parallel thylakoids extend from one end of the chloroplasts to the other. Occasionally, one thylakoid branches into two, or dark stained and flattened parts of thylakoids can be observed in the chloroplasts of parenchyma sheath cells of maize leaves and chlorenchyma cells of barley leaves illuminated during 3 hours after 6 days of dark germination. These indicate that chloroplasts within parenchyma sheath cells of maize leaves can not be further differentiated and grana persist as a initial stage of development, but chloroplasts within mesophyll cells of maize leaves and those within chlorenchyma cells of barley leaves can be further differentiated.

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Abbreviations

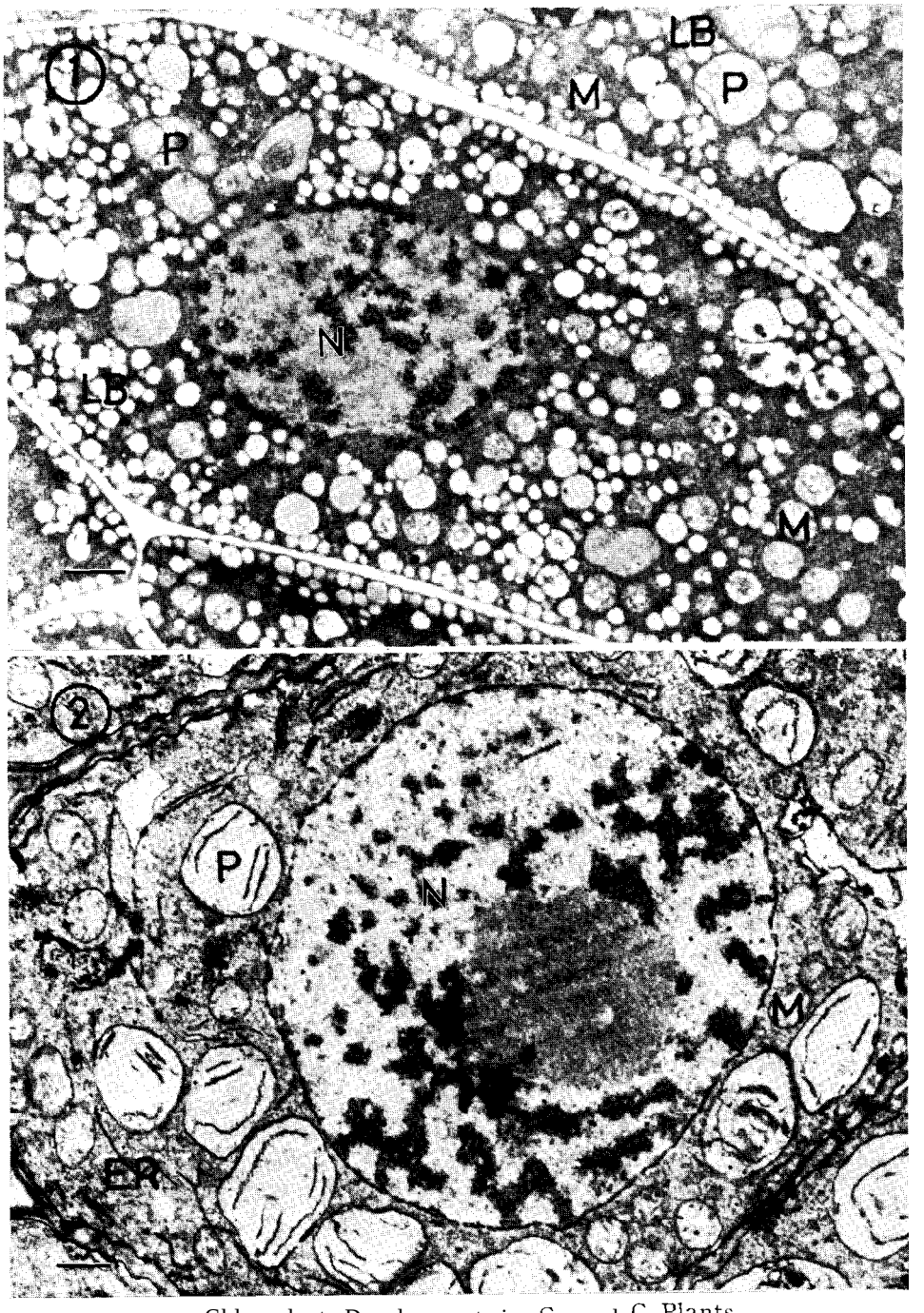
CW=Cell Wall, *D*=Dyctiosome, *E*=Etioplast, *ER*=Endoplasmic Reticulum, G=Grana, Z=Intercellular Space, LB=Lipid Body, M=Mitochondria, *MC*=Mesophyll Cell, *MS*=Mestom, *N*=Nucleus, *O*=Oil Droplet, OG=Osmiophilic Globule, *OL*=Overlaping, *P*=Proplastid, *PB*=Prolamellar Body, *PD*=Plasmodesmata, *PS*=Parenchyma Sheath, S=Stroma, SG=Starch Grain, *V*=Vacuole, *VB*=Vascular Bundle

The scale on each micrograph represents 1 micron.

Explanation of Plate I

Fig. 1. The cells of the first foliage leaf from barley embryo. Many lipid bodies are present inside plasmamembrane and scattered in the cytoplasm. Single thylakoid-like membrane is present in some large proplastids. Mitochondria are usually smaller than proplastids. **~7,100.**

Fig. 2. The cells of the first foliage leaf from barley embryo during 2 days of dark germination. Many thylakoids are present in the proplastids. Each thylakoid is connected with inner envelope of the proplastids and made of row of small lamellae. Rudimentary cristae are developed in the mitochondria. ×7,500.

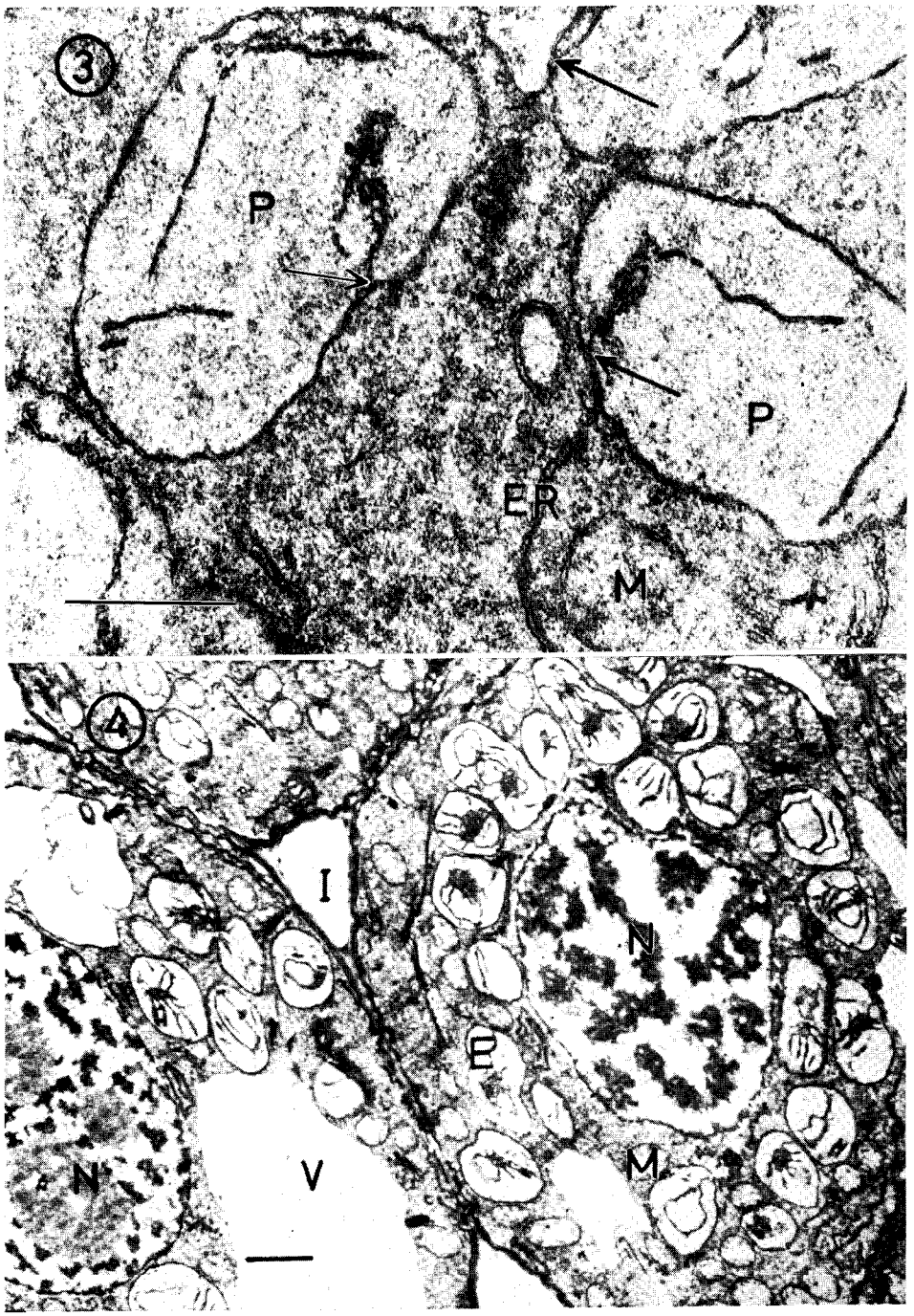


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate II

Fig. 3. The proplastid profiles within the cells of the first foliage leaf from barley embryo after 2 days of dark germination. Each thylakoid is connected also with inner envelope of the proplastid membrane (arrows). Endoplasmic reticula are also connected with outer membrane of the proplastid. x 22,000.

Fig. 4. The cells of the first foliage leaf from barley embryo after 3 days of dark germination. The cells start to be vacuolized. Prolamellar bodies are developed in the etioplasts. but not yet paracrystalline structure. The thylakoids made of minute lamellae perforated from inner components of plastid membrane are connected with inner envelope of the etioplast membrane. ×8,600.

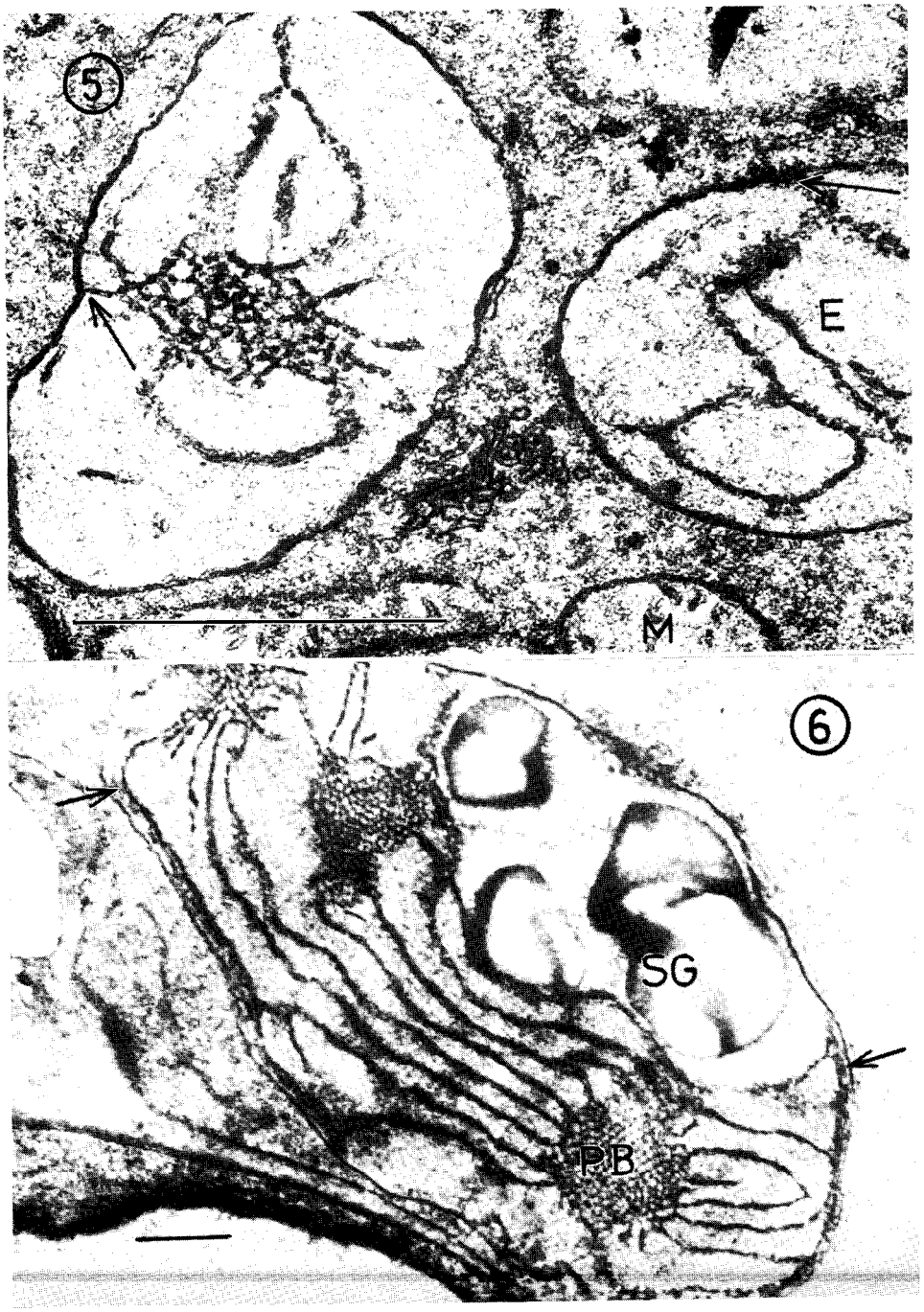


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate III

Fig. 5. Etioplast profiles within the cells of the first foliage leaf from barley embryo after 3 days of dark germination. Prolamellar body was formed usually at central region of the etioplasts. Arrows indicate the thylakoid connected with inner etioplast membrane. ×**50,000.**

Fig. 6. Etioplast profiles within the first foliage leaf from barley embryo after 5 days of dark germination. Prolamellar body occurs as paracrystalline structure. Thylakoids are connected with prolamellar bodies and inner envelope of the etioplast. Arrows indicate the lamellae invaginated from inner components of the etioplast envelope. Starch grains occur in this etioplast and are divided into two or three parts. ×12,000.

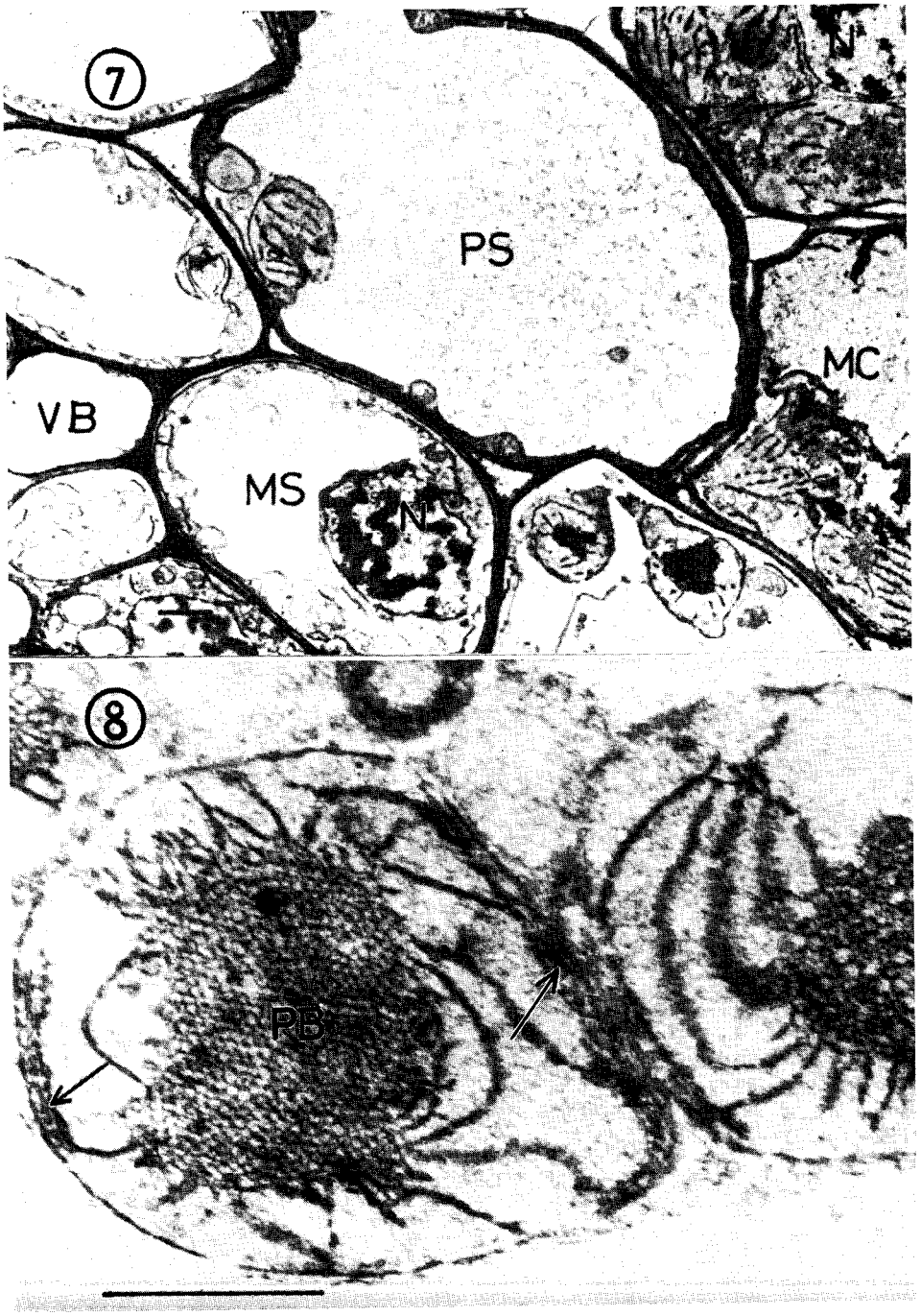


Chloroplast Development in C_3-and C_4-Plants

Explanation of Plate IV

Fig. 7. The cells of the first foliage leaf from barley embryo after 6 days of dark germination. Vascular bundles are surrounded with mestom layer and parenchyma sheath layer. Etioplasts occur within each cell of the layers. ×5,500.

Fig. 8. Etioplast profiles of the first foliage leaf from barley embryo after 6 days of dark germination. Paracrystalline prolamellar bodies become larger according to dark germination and each thyalkoid is connected with inner membrane of the plastid. Arrows indicate perforated lamellae from inner envelope components. x 29,000.

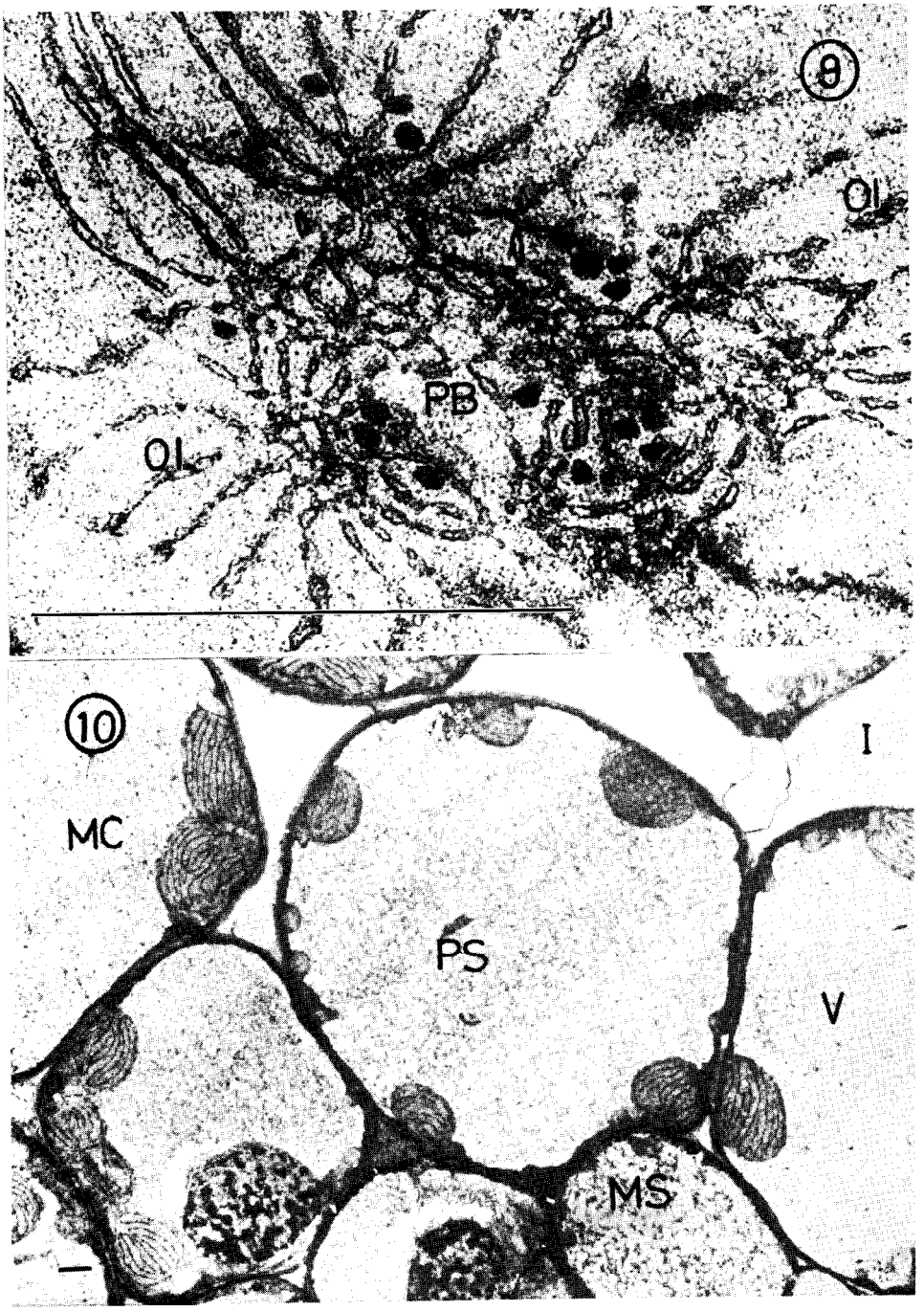


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate V

Fig. 9. Choroplast of the first foliage leaf from barley embryo illuminated for 5 minutes after 6 days of dark germination. Paracrystalline prolamellar body has been loosened by illumination. Small lamellae disperse as a row in the stroma, and then they elongate. Overlappings usually occur at the end between the dispersed lamellae. ×73,000.

Fig. 10. The cells of the first foliage leaf from barley embryo illuminated for 3 hours after 6 days of dark germination. Vascular bundles are surrounded with inner mestom and outer parenchyma sheath. The prolamellar body within the cell of this stage is completely dispersed into thylakoid lamellae. Cells are connected with plasmodesmata. x2,900.

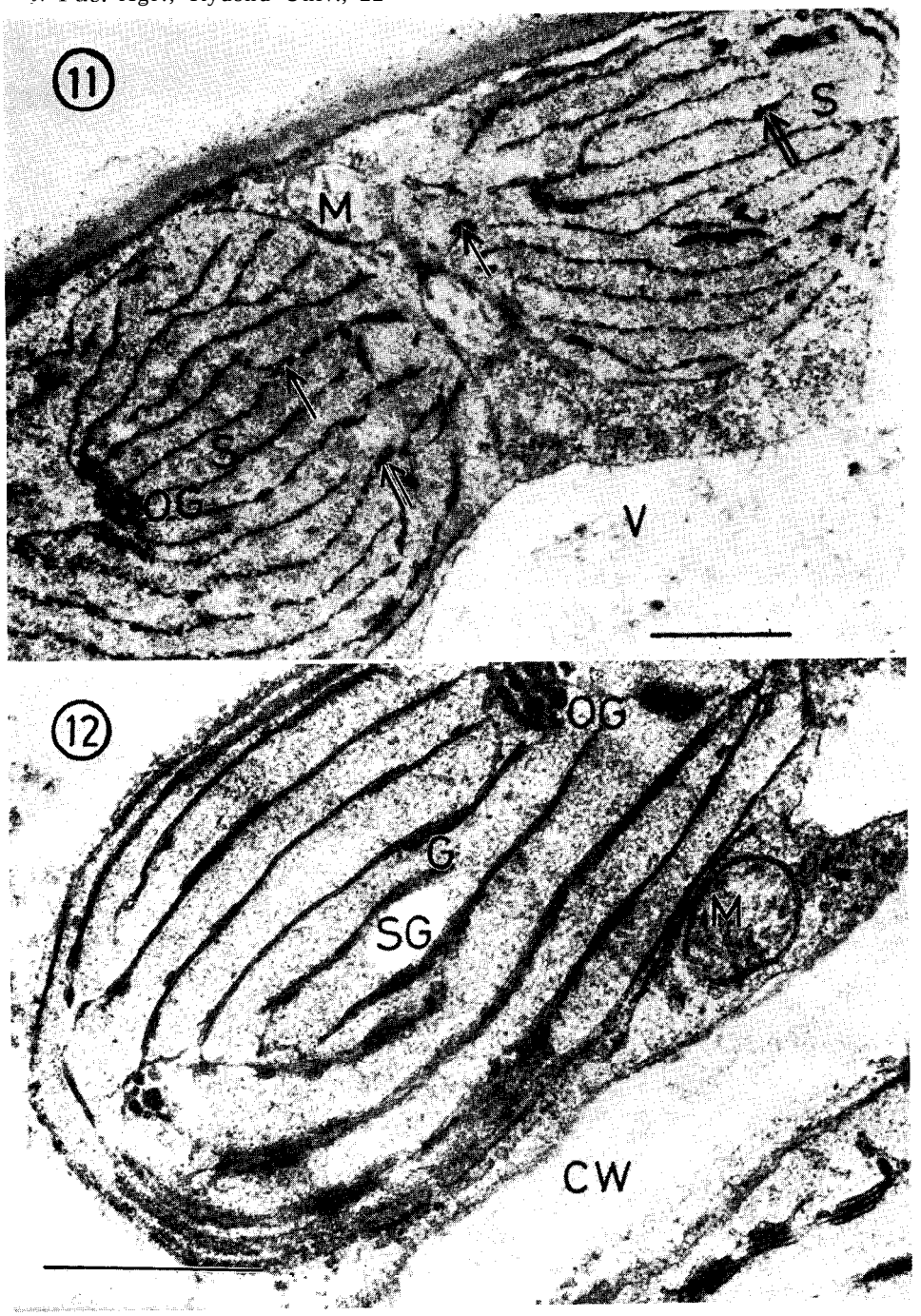


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate VI

Fig. 11. The chloroplasts of the first foliage leaf from barley embryo illuminated for 3 hours after 6 days of dark germination. Note the overlapped thylakoids ; A single short lamella appresses to longer lamella (single arrows), or single lamella appears to be flattened (double arrows). x 19,000.

Fig. 12. The chloroplast of the first foliage leaf from barley embryo illuminated for 6 hours after 6 days of dark germination. Grana are formed from two or three appressed thylakoids. Starch grain formed by photosynthesis is present in the stroma of the chloroplast. ×29,000.

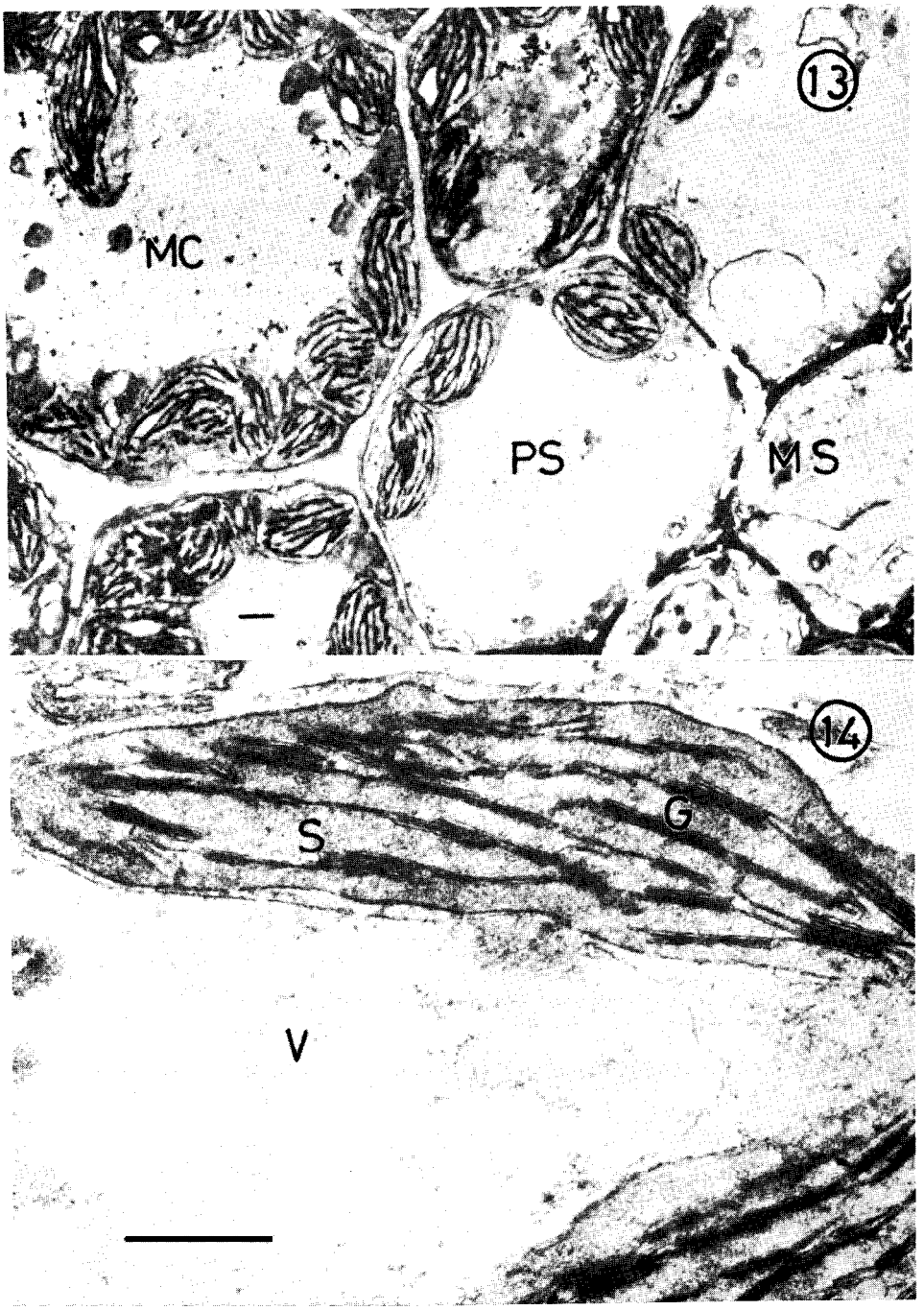


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate VII

Fig. 13. The cells of the first foliage leaf from barley embryo illuminated for 18 hours after 6 days of dark germination. Note the double layers of the bundle sheath and well developed chloroplasts within parenchyma sheath and mesophyll cells. ×3,600.

Fig. 14. Chloroplasts of the first foliage leaf from barley embryo illuminated for 24 hours after 6 days of dark germination. Grana are well developed and chloroplast profiles are a long ellipsoid shape. x 20,000.

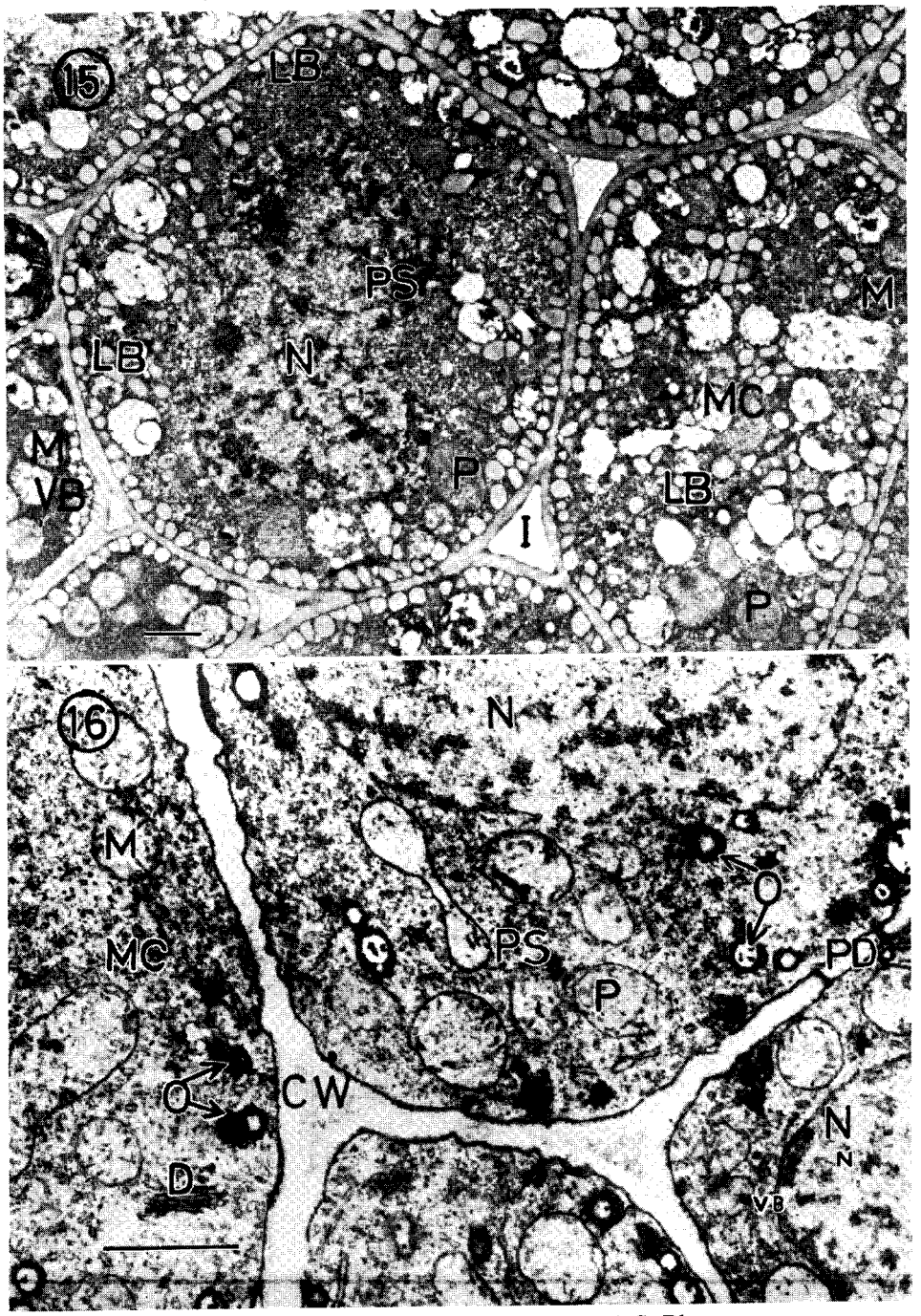


Chloroplast Development in C_3- and G-Plants

Explanation of Plate VIII

Fig. 15. The cells of the first foliage leaf from maize embryo. Lipid bodies are numerous inside plasmamembrane and scattered in the cytoplasm. Proplastids are uniformly stained, but mitochondria are not uniformly stained because of electron-opaque inclusions. x 7,100.

Fig. 16. The cells of the first foliage leaf from maize embryo after 2 days of dark germination. The proplastid profiles cannot be distinguished from mitochondrial profiles. Oil droplets appear in cytoplasm. x 18,000.

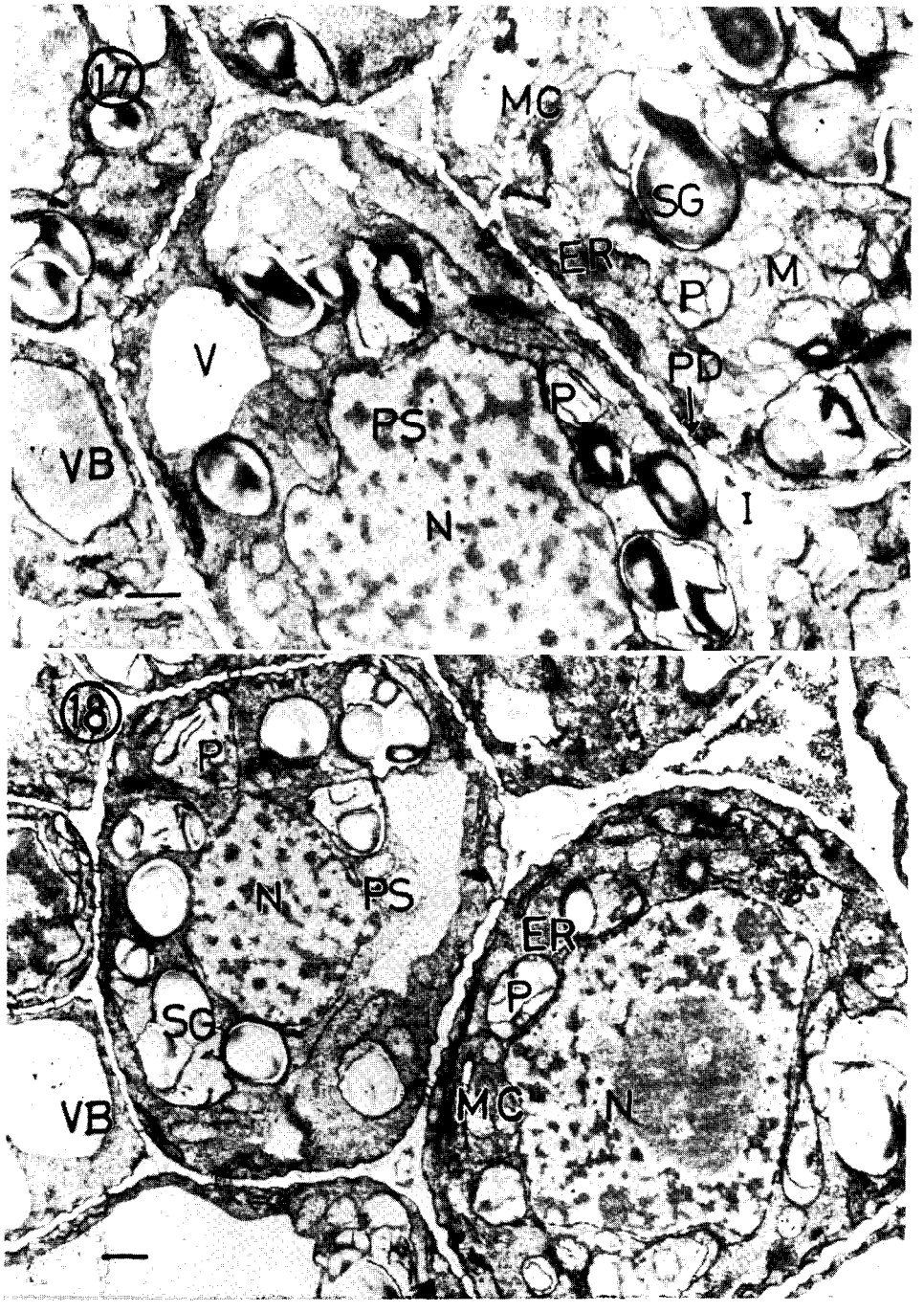


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate IX

Fig. **17.** The cells of the first foliage leaf from maize embryo after 3 days of dark germination. Both mesophyll and parenchyma sheath cells start to be vacuolized. Starch grains appear in the stroma of the proplastid. A few thylakoids are developed in the proplastid. Mitochondria also have cristae. Cells are connected with plasmodesmata. ×7,100.

Fig. 18. The cells of the first foliage leaf from maize embryo after 4 days of dark germination. Note many etioplast profiles with thylakoids. x 5,900.

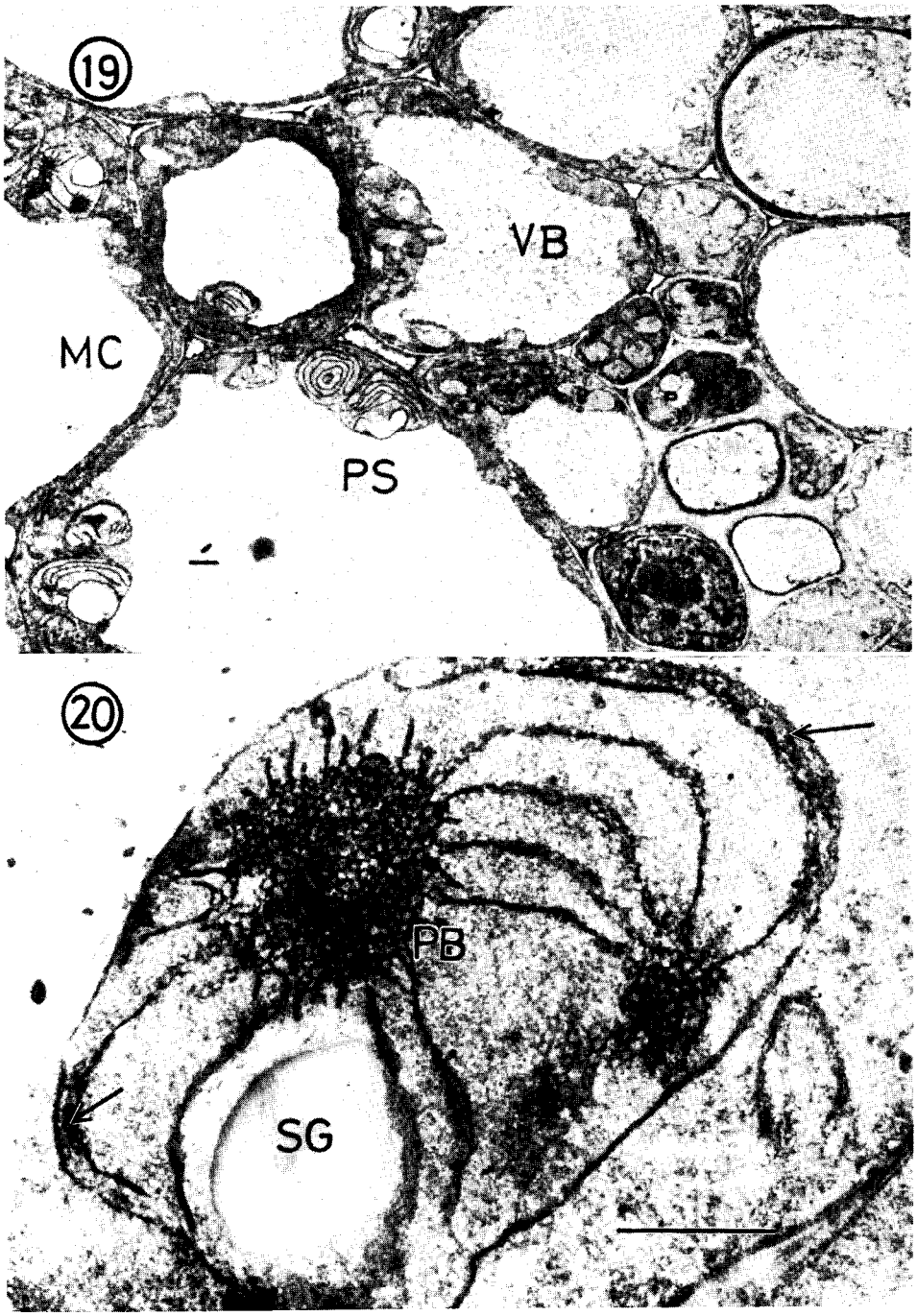


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate X

Fig. 19. The cells of the first foliage leaf from maize embryo after 5 days of dark germination. Prolamellar bodies are formed in etioplasts in mesophyll and parenchyma sheath cells. × 3,600.

Fig. 20. The etioplast of the first foliage leaf from maize embryo after 5 days of dark germination. Perforated lamellae occur between the peripheral thylakoid lamellae and inner envelope of the etioplast membrane (arrows). Each thylakoid is connected with minute lamellae as a row and aggregates at central region to form prolamellar body. x 20,000.

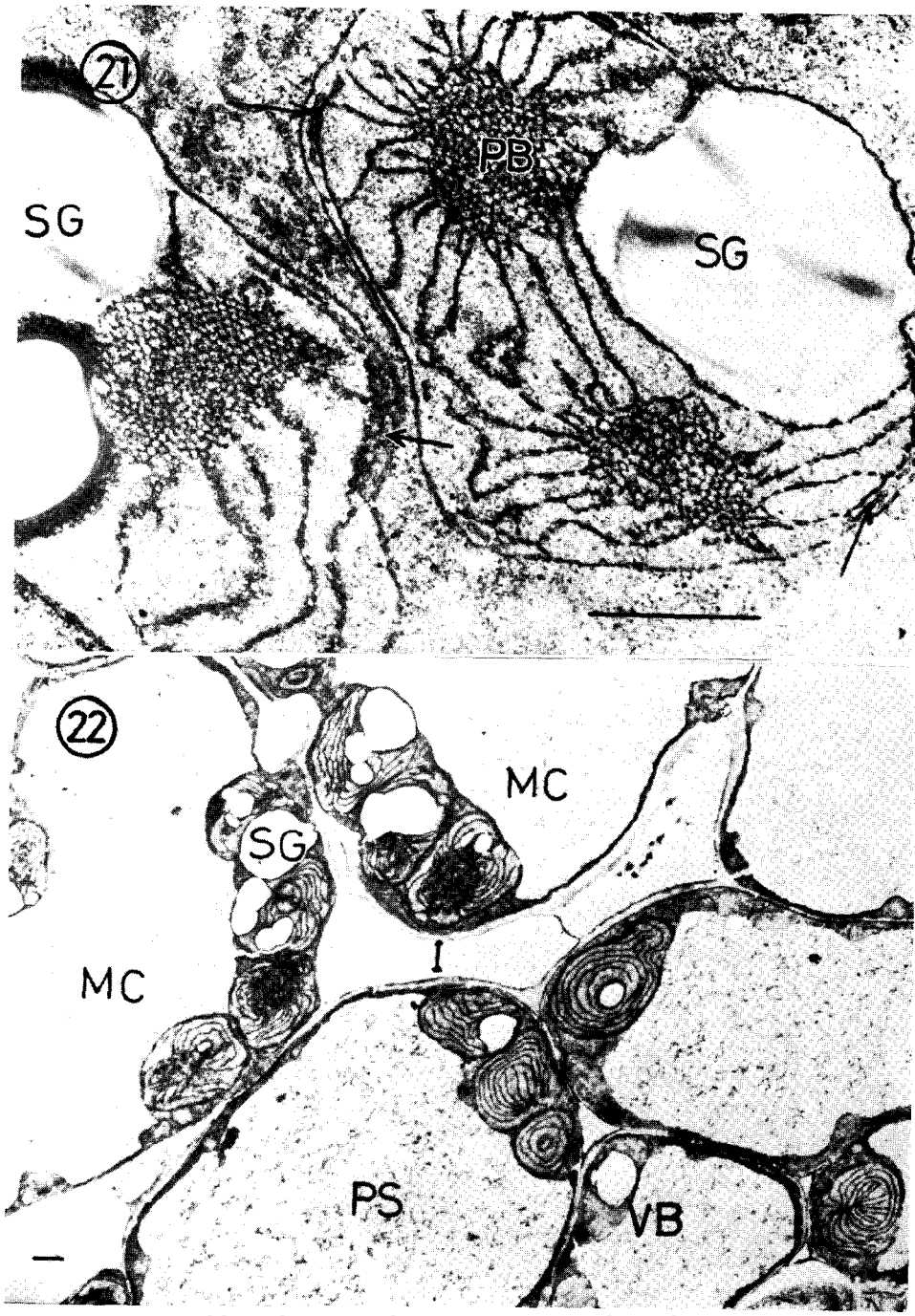


Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate XI

Fig. 21. Etioplasts of the first foliage leaf from maize leaf embryo after 6 days of dark germination. Paracrystalline prolamellar bodies are larger than those of previous stage. Thylakoids in peripheral zone circle inside etioplasts membrane and numerous small vesicles occur between the circle lamellae and inner envelope of etioplast. Arrows indicate small perforated lamellae. x 22,000.

Fig. 22. The cells of the first foliage leaf from maize embryo illuminated for 3 hours after 6 days of dark germination. The prolamellar body has disappeared from etioplast in the parenchyma sheath cells, but is still present in those of mesophyll cells. Prolamellar body in the chloroplast within mesophyll cells loosened by illumination. Parallel thylakoids extend from one end of chloroplast to the other in the parenchyma sheath cells. x 3,700.

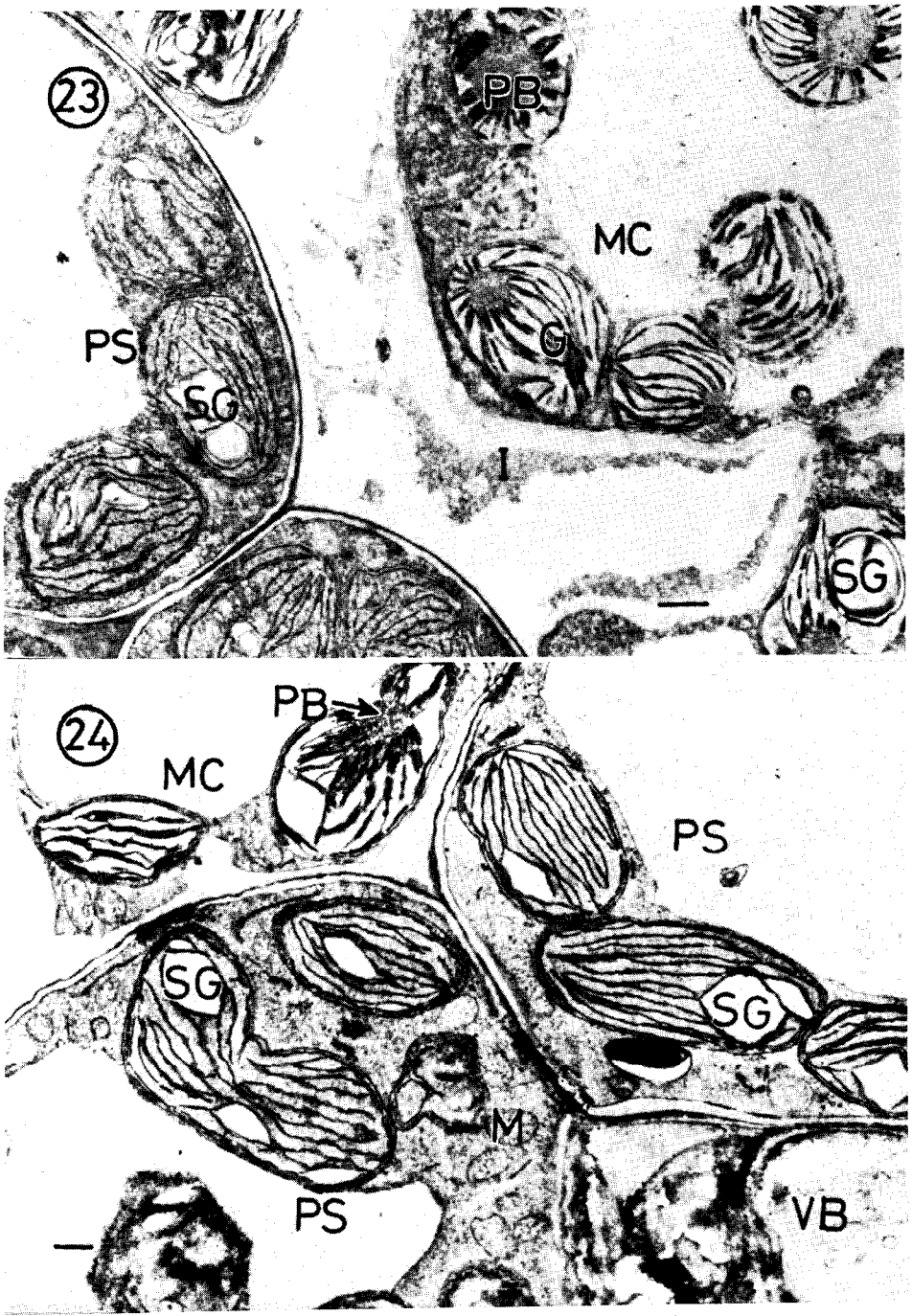


Chloroplast Development in C_3- and G-Plants

Explanation of Plate XII

Fig. 23. The cells of the first foliage leaf from maize embryo illuminated for 6 hours after 6 days of dark germination. Grana are well formed in chloroplasts within mesophyll cells, but paracrystalline prolamellar body is still present in the chloroplast. In parenchyma sheath cells the chloroplasts are similar to those within cells of barley leaves at early stage of development. ×5,700.

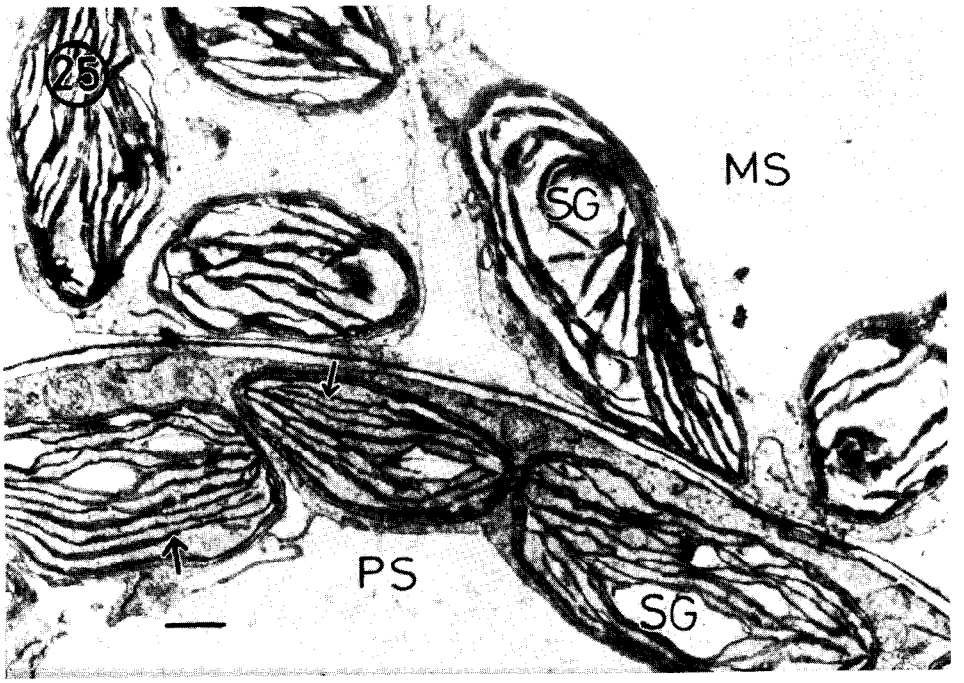
Fig. 24. The chloroplast of the first foliage leaf from maize embryo illuminated for 18 hours after 6 days of dark germination. Prolamellar body is still present in the chloroplast within mesophyll cells. Starch grains assimilated by photosynthesis exist in chloroplast both within mesophyll and parenchyma sheath cells. x 4,800.



Chloroplast Development in C_3- and C_4-Plants

Explanation of Plate XIII

Fig. 25. Chloroplasts of the first foliage leaf from maize embryo illuminated for 24 hours after 6 days of dark germination. The number of grana lamellae is increased by further illumination. Thylakoids in the chloroplasts within parenchyma sheath cells are branched and flattened, or dark stained (arrows). ×7,400.



Chloroplast Development in C_3- and C_4-Plants