

• • STRUCTURE OF THE PHOTOCHEMICAL APPARATUS IN PHOTOSYNTHESIS*

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INTRODUCTION

The electron microscope has revealed in the 20 or so years of its existence a staggering complexity and diversity in the structure of tissue cells. Since it is potentially useful within a wide range of magnifications (200x to 200,000x approx.), several levels of organization may be visualized: that of the organ, the cell, the intracellular organelles and finally the molecular organization. It is, of course, the latter which has caught the fancy of more than one researcher for many good reasons. Unfortunately it is also the latter which has up to the present proved to be the least rewarding in terms of providing clues for the understanding of cellular mechanisms.

Let me begin by stating that no direct evidence is yet available that would allow us to attribute a particular function to the highly ordered lamellar structures which are observed in photosynthetic cells. Least of all is any direct evidence available on their molecular constitution.

However there are many circumstantial observations that point to the fact that such structures do indeed reflect some sort of molecular organization necessary for photosynthesis.

It may be instructive to note here that other photochemical processes such as phototaxis or photoperiodicity are not to our present knowledge directly connected with the presence of such highly ordered lamellar systems as those present in organelles performing either photosynthesis, vision or the transmission of nerve impulses. When observed by the electron microscope at the molecular level the different cells involved in these intrinsically different processes reveal strikingly similar highly ordered lamellar structures. Apparently the electron microscope, under the conditions employed, does not discriminate between for instance a molecule of rhodopsin and a chlorophyll-protein complex. However as we shall see these processes do have at least one mechanism in

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common and we like to believe that the morphological similarity will turn out to be more than a mere coincidence.

Vision and photosynthesis are admittedly different processes. In photosynthesis the absorbed photon does "work" by moving an electron against the potential gradient. Later on this acquired energy is stored by the reduction of CO_2 to carbohydrates. In vision, light does not do any work. The absorbed photon merely triggers a molecular excitation which results in the isomerization of retinene which in turn (and many steps later) triggers a nerve impulse. Little is known about the nature of nerve impulses but we do know that they are rapidly transmitted and that their transmission is independent of any kind of photochemical reaction. Enough evidence is at hand to claim that a path for conduction of molecular excitation (of whatever form it may be) is present in either case.

Therefore we may assume at least as a preliminary and naive hypothesis that the ordered quasi-crystalline structures seen in the electron microscope do not reflect an organization necessary for the performance of light absorption or the performance of "work" but rather an organization needed for the transmission of molecular excitation in some preferred direction.

We will here be concerned with lamellar structures which occur in cells performing that type of photosynthesis in which the photolysis of water ultimately results in the evolution of oxygen. This occurs in higher plants and green, brown, red and blue-green algae. Ordered lamellar structures are always present in these organisms under photosynthetic conditions.

However, there is another type of photosynthesis which occurs in purple sulfur, non-sulfur and green bacteria. It does not depend on the photolysis of water and does not evolve oxygen. Such organisms photosynthesize in the absence of any highly ordered lamellar structures. Their cytoplasm is usually filled with spherical particles (bacterial chromatophores) about 300 Å in diameter surrounded by a membrane about 90 Å thick (ref. 3). Indeed this type of photosynthesis (as measured by photophosphorylation and photoreduction of DPN has been shown to occur even in the absence of any chromatophores. The active structure was in this case a particle about 250 Å in diameter without a surrounding membrane (ref. 5).

Such facts are at present puzzling but they suggest that the ordered lamellar systems are perhaps also a reflection of the molecular organization needed by the processes of water photolysis and oxygen evolution.

In any attempt to reconstruct from electron micrographs the molecular structure present *in vivo* one has to account for the fact that the specimens have undergone rather drastic chemical treatments prior to observation. They are usually "fixed" in a strong oxidant containing a heavy metal atom, dehydrated by polar solvents, and infiltrated with a plastic monomer which is subsequently converted by heat to a polymer of the desired hardness. This is necessary in order to impart some rigidity to the specimen which has to be cut into slices less than 0.1 μ thick since this is about as much organic matter as the electron beam will penetrate without significant loss of resolution. Aqueous solutions of potassium permanganate or osmium tetroxide are the preferred "general purpose" fixatives (the latter may be used in the vapor state).

Little is known about the in vivo reaction sites of either compounds (refs. 1, 8, 12, 14) and even less about the effect they may have on molecular architecture. The effect of dehydration is no less than obscure at this level, but as will be shown may have a strong influence on the appearance of lamellae in electron micrographs (Table 1).

It will be demonstrated below that the modulation of densities appearing in the direction of the thickness of a lamella depends on the type of fixation. However, the overall thickness of a lamella is constant at approximately 160 Å and remains invariably as a well defined entity under a wide variety of conditions. This is believed to represent the presently meaningful aspect of the information gathered by observing stained thin sections with the electron microscope.

LAMELLAR STRUCTURES

For a general review of the literature dealing with E.M. observations of chloroplast structure, we refer the reader to Wolken (ref. 17). The light microscope literature has been extensively reviewed by Weier (ref. 15) and Weier and Stocking (ref. 16).

The interest in the structure of the photosynthetic apparatus dates back to 1883 when Meyer (ref. 10) first observed the presence of granules which he called "grana" in the matrix of higher plant chloroplasts. These grana were the only parts of the chloroplast that appeared to contain chlorophyll. As such inquiries were extended to lower photosynthetic organisms it was found that chlorophyll was not restricted to grana, but, as in green algae, appeared to be distributed throughout the chloroplast.

Later on the observations of Menke (ref. 9) and Strugger (ref. 13) established that grana were connected to each other by "intergranar" lamellar structures. It was further shown by several investigators that the chlorophyll containing regions of higher plants as well as algae were also lamellated. Although it was then realized that probably all chloroplasts exhibited some lamellated structure, it was found convenient to classify them as "granulated" (those that carried chlorophyll in "grana") and "lamellated" (those that carried their chlorophyll in lamellae).

These conclusions were arrived at mainly on the basis of light microscope observations. And, with the advent of high resolution electron microscopy, it led to some confusion because, as it now turns out, all photosynthetic organisms (with the exception of anaerobic types) are similarly lamellated, the lamellae usually occur in piles, and "grana" are only those piles of lamellae which, because of their size and shape, are easily recognizable by light microscopy (ref. 11).

Figure 1 shows the appearance of a non-photosynthetic hydrogen bacteria while figure 2 depicts a blue-green photosynthetic alga (TX-27). Both are unicellular organisms with a low level of cytoplasmic differentiation typical of bacteria in general: the absence of a nucleus, mitochondria or endoplasmic reticulum is conspicuous.

The striking difference, however, is the presence in the photosynthetic organism of several concentric layers of lamellae that generally follow the contour of

the cell (Fig. 2). Each lamella is approximately* 150-160 Å thick and exhibits a certain modulation of densities in the direction of its thickness (Fig. 3). Such a picture corresponds to scheme IV in Table 1 and is characteristic of potassium permanganate fixation followed by ethanol dehydration. If the same organism were fixed in osmium tetroxide the lamella would again appear approximately 160 Å thick but the modulation of densities would be as in scheme I of Table 1 (for one lamella). If fixed in formalin followed by treatment in potassium dichromate (refs. 2, 4), the thickness of a lamella would still be 160 Å but the modulation of densities in the direction of its thickness would be of a third variety, as in scheme V of Table 1.

Such observations suggest that we are here dealing with a 150-160 Å thick unit, the molecular constituents of which react differently toward various fixatives. The rather unexpected but important observation is that regardless of the fixative, the overall thickness of the unit lamella remains the same. Since the binding sites of the various fixatives are unknown, the significance of the fine density modulations is conjectural at present.

Another significant observation is that such a unit lamella may swell under some conditions (ref. 7). This was interpreted to mean that the lamella consisted of two different phases: an inner space subject to swelling, and a membrane completely surrounding this space. This membrane is about 75 Å thick (Fig. 6) after any of the treatments considered in Table 1, but its apparent density is again determined by the preparative treatment. Therefore, the gross morphology of a lamella may best be described by analogy as similar to a closed flattened sac (see scheme I in Table 1).

As will be shown it is such a structural unit which is used over and over again in the building of more complex systems by higher organisms.

If we now turn to more complex but still unicellular photosynthetic organisms such as the green alga Scenedesmus spec. (Fig. 4) or the green flagellate Euglena (Fig. 7), the greater amount of cytoplasmic differentiation is immediately obvious. We recognize segregated nuclei, mitochondria, golgi bodies, endoplasmic reticulum and other smaller cytoplasmic inclusions. In Scenedesmus one cup-shaped chloroplast is present. Fig. 4 is a section in a plane approximately parallel to the bottom of the chloroplast cup. It therefore appears as a doughnut with the lamellae again generally following the contour of the cell. Some differentiation inside the chloroplast may be recognized: the pyrenoid, a large particle of obscure function, and starch grains (the end products of photosynthesis) scattered in between the bundles of lamellae. Chlorella cells are similar in all these respects except that they are spherical. In Euglena (Fig. 7) in addition to several disc shaped chloroplasts, one recognizes paramylum grains (end-products of photosynthesis) outside the chloroplasts, and in addition to other cytoplasmic organelles, the eyespot consisting of a number of dense granules (the location of carotenoid-like pigments active in phototaxis) at the base of the flagellum (see also Gibbs, S. ref. 6).

At higher magnification (and upon close scrutiny), one recognizes in either organism (Figs. 5, 6 and 8) that each one of the separate bundles, clearly outlined at low magnification, is actually only a multiple of the same 160 Å thick unit

*The qualification of "approximately" should not be underrated. There are very real difficulties in measuring the thickness of lines of this order of magnitude with an accuracy better than 10%.

lamella which was observed in blue-green algae.

Isolated lamellae are seen to extend from one bundle to another in several places on Figs. 5 and 8, and bundles consisting of two to five or six lamellae may be recognized. The 75 Å membrane and the closed ends of each lamella, as well as the manner in which the lamellae unite to form bundles, is clearly seen in Fig. 6. While the thickness of each bundle in these organisms is seldom more than that of 10 juxtaposed lamellae, their length (in some preferred direction in the plane of the lamella) varies from perhaps a tenth of a micron to that of the whole length of the chloroplast. Figures 5, 6 and 8 are representative of scheme II in Table 1. With other preparative treatments the modulation of densities in the direction of the thickness of lamellae (and therefore of each bundle) would be correspondingly modified as in schemes I and III of Table 1. But a periodic unit thickness (a lamella) of approximately 160 Å would remain apparent in either case. These lamellae are also subject to swelling and the width of the inner space of each lamella therefore appears to vary somewhat. The 150 Å thick complex (Figs. 5 and 6) resulting from the opposition of the membranes of two adjacent lamellae is, however, tightly bound together and is not deformed even under conditions of swelling. Its resemblance (only in as much as the modulation of density across its thickness is considered) to the lamella of blue-green algae (see Fig. 3) is striking but should not be misinterpreted since their origin is different (compare scheme II and IV in Table 1).

Therefore, in the quest of a progressively smaller building block functional in the process of photosynthesis, the electron microscope draws our attention at present to the existence of a unit lamella, the overall thickness of which is revealed with great constancy regardless of pre-treatment (as in Table 1).

Figures 9 and 10 illustrate at various intermediate magnifications the general disposition of chloroplasts in spongy mesophyll cells of a Spinach leaf and the distribution of what has been earlier described as intergranar lamellae and grana in a disc-shaped chloroplast of the same spinach leaf cell.

At high magnification (Fig. 11) the spinach chloroplast exhibits essentially the same pattern of organization as the one observed in Scenedesmus and Euglena. The 160 Å thick lamellae are again the units which by coming in close apposition to each other form bundles. The thickness, length and number of lamellar bundles per chloroplast are a function of various physiological factors (ref. 17) and vary from species to species.

However, their thickness (number of lamellae per bundle) is usually greater than in green algae, while their length is always shorter (in the order of half a micron). This holds true for all higher plant species observed up to the present. It explains why only the lamellar bundles of higher plant chloroplasts have been detected by light microscopy and designated as "grana".

The architectural motif used by the lamellar systems of higher plants and lower organisms is, however, identical.

In order to obtain some insight into the effects of dehydration, we have substituted conventional dehydration by simple air-drying (at room temperature and pressure). The results are illustrated in Fig. 12. A lamella is again preserved as an approximately 160 Å thick unit but the modulation of densities across its thickness is now the reversed (see scheme IIIa) of what it may have been under the conditions of scheme I of Table 1. Such images are obtained regardless of

whether the sample is exposed to osmium tetroxide vapors before or after air-drying, implying that the osmium treatment has little to do with the actual preservation of the lamellar structures. It is needed only to increase the contrast by virtue of the fact that it binds to some sites and not to others.

The results of our experiments with a fixation schedule suggested by J. R. Baker and De Robertis (ref. 2, 4) for the preservation of phospholipids are illustrated in Fig. 13 (see also scheme IIIb in Table 1). They result in a general appearance of isolated lamellae and lamellar bundles which is identical to the one obtained after simple air-drying. The thickness of the lamellae is again approximately 160 Å and the manner in which they pile up into bundles is also the same.

It seems obvious that in any present attempt to reconstruct the in vivo molecular architecture of such highly ordered lamellar structures, one should not attach too much significance to any one particular variety of density modulations appearing in the thickness of a lamella. Their interpretation in terms of different classes of molecules (proteins, lipids and pigments) requires more data than presently available on the overall effect of the preservation treatment and on the nature of the binding sites.

However, one little bit of constructive information does emerge from these observations. The photosynthetic matrix indeed appears to contain many multiples of a regularly oriented unit lamella which is approximately 160 Å thick. Such lamellae tend to come into close apposition to each other in higher plants as well as in green and brown algae. Also, some degree of regularity in the position of unknown molecules (or, more precisely: of osmium tetroxide or potassium permanganate binding sites) in the direction of the thickness of a lamella is indicated.

We conclude that the electron microscope is at present revealing only some intermediate level of organization which is on a larger scale than the one at which we would like to reconstruct the in vivo structural relationship between, for instance, chlorophyll and the enzyme chains catalyzing either the reduction of CO₂ or the evolution of O₂.

STRUCTURE AND ACTIVITY

A review of the many observations correlating photosynthesis with lamellar structures is beyond the scope of this report (ref. 17). As further evidence in favor of such a correlation I may quote our studies (ref. 11) where synthesis of chlorophyll and onset of photosynthesis were followed with structural changes in chloroplasts of primary bean leaves grown in the dark. Although isolated lamellae are present in dark grown plastids, the formation of lamellar bundles (grana) follows very closely the time course of chlorophyll synthesis (Figs. 14 and 15). This suggests that active pigment complexes are perhaps located within the plane of apposition of two lamellae (see scheme I of Table 1). If one assumes that this plane is roughly circular and one calculates its average diameter it turns out that approximately 400 chlorophyll molecules may be fitted in a chain-like fashion along the length of any diameter.

With the final "light traps" and "energy converting centers" located at the periphery of such planes (at the ends of the pigment chains), the structural

requirements for the early steps of photosynthesis are conveniently met. A photosynthetic unit would then be represented by any chain of pigment molecules together with its terminal "converting center".

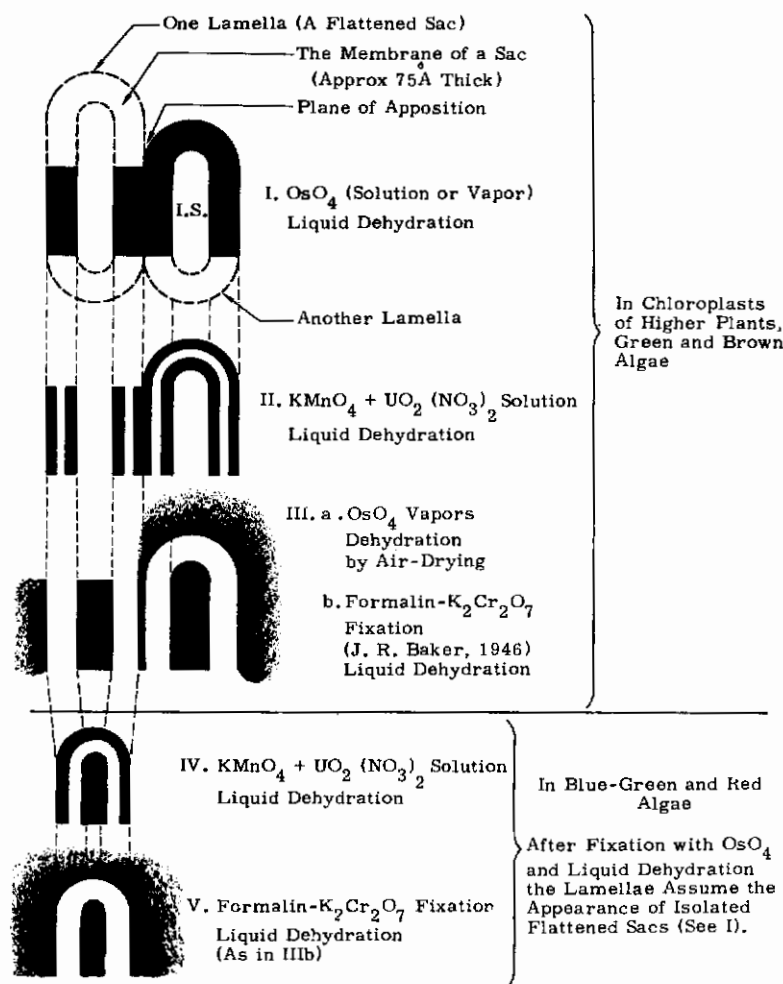
However, apposition planes of this order of dimensions are apparent only in higher plants. Those occurring in lower organisms appear larger and could therefore accommodate pigment chains consisting of more molecules than allowed by average estimates of the size of a photosynthetic unit. Such an arrangement would certainly be hard to visualize in blue-green algae where the lamellae undoubtedly present much too large a surface.

In spite of such difficulties, there remains the fact that the energy absorbed by a large number of pigment molecules is somehow channeled toward a "trap".

Such a focal point must have in its immediate proximity (in order to prevent wasteful dissipation of energy) the enzyme machinery whereby the light is put to work. The present challenge is to reconcile the observed structure with such requirements.

A thought along these lines is that although it takes an anatomist to cut sections, it may require an expert in quantum mechanics to interpret them.

TABLE 1
E.M. IMAGES OF LAMELLAE AFTER VARIOUS FIXATION METHODS



ABBREVIATIONS USED IN FIGURES

S	Starch
M	Mitochondrion
N	Nucleus
L	Lamella
PY	Pyrenoid
GB	Golgi body
E	Chloroplast envelope
C	Chloroplast
PA	Paramylum grains
V	Cell vacuole
G	Grana
PLB	Prolamellar body
I.S.	Inner Space

Figure 1

(right)

Illustrates the appearance of Hydrogenomonas, a non-photosynthetic hydrogen bacteria, in two mutually perpendicular section planes. Note the lack of structure in the cytoplasm. Only the cell wall and the centrally dispersed nucleoplasm containing DNA may be recognized. The equatorial narrowing exhibited by the cell at the right reflects its mode of division. It divides by binary fission. The cell wall is not clearly seen in the organism at the left because it passes at too much of an angle through the thickness of the section. Magnification: 118,000 x.

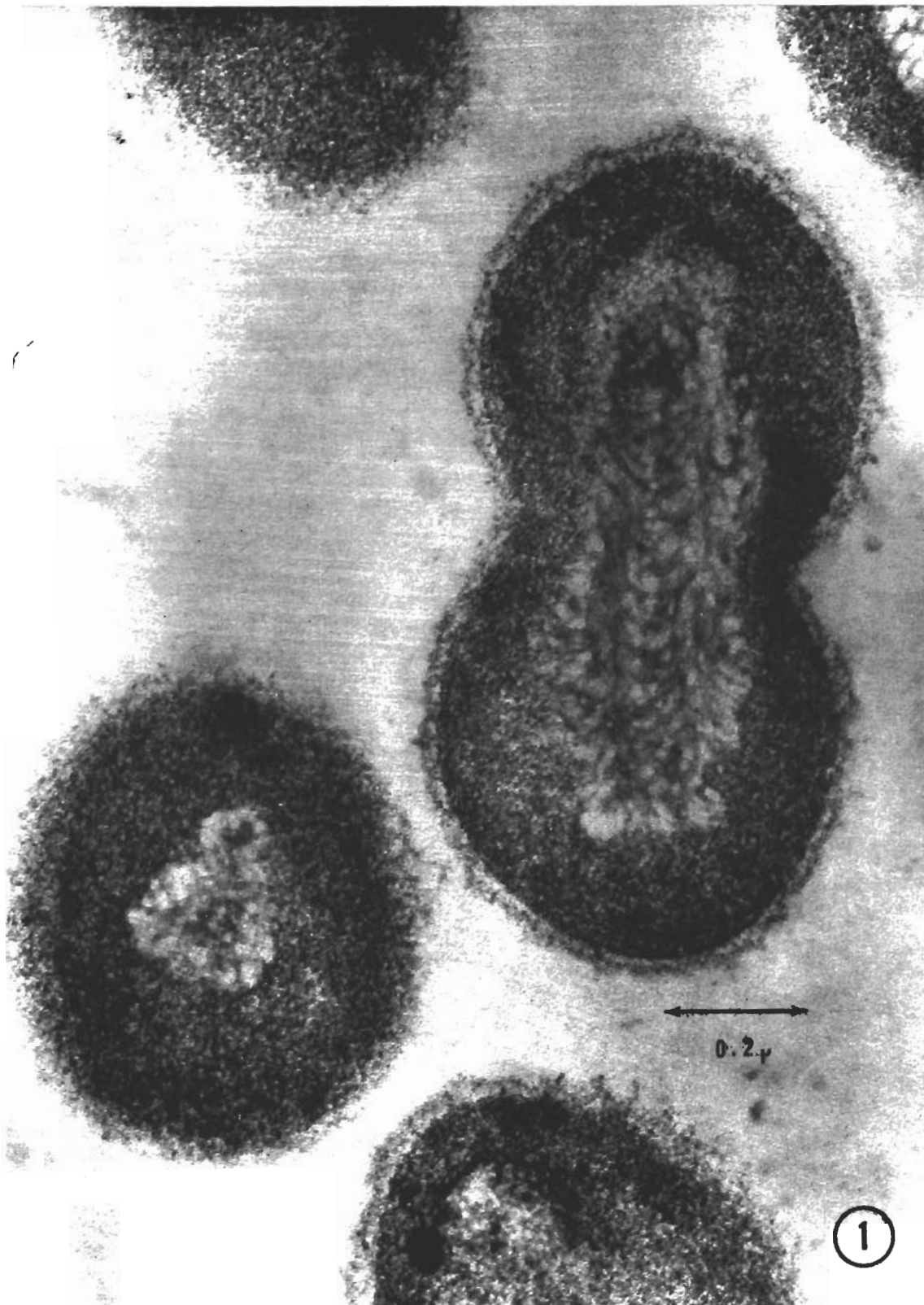


Figure 2

Illustrates several cells of photosynthetic blue-green algae TX-27. This organism also divides by binary fission but the cells do not completely separate from each other and therefore grow in filaments. Note the concentric layers roughly underlying the cell wall. It is not clear whether they are parts of only one spirally wound lamella or many individual lamellae. A space about 500 A thick separates the lamellae from each other.

Cells of Anacystis nidulans would appear very similar under comparable conditions. However, they are smaller, do not remain attached to each other after division, and have fewer lamellae. Magnification: 26,000 x.

Figure 3

Detail of lamellae present in the blue-green alga TX-27. The same applies to Anacystis lamellae. The thickness of each lamella (L) is approximately 150-160 A. The modulation of densities in the thickness of each lamella is characteristic of potassium permanganate fixation (scheme IV in Table 1). After osmium fixation each lamella would appear as a flattened sac and the modulation of densities would be as in scheme I of Table 1. As shown in scheme II (Table 1) the membrane of a lamella may be resolved into two dense lines (each approximately 25 A thick) separated by a light interspace after permanganate fixation.

Therefore, the modulation of densities as seen here reflects an obliteration of the inner space of a lamella and a close apposition of membrane surfaces (within one lamella). Magnification: 300,000 x.

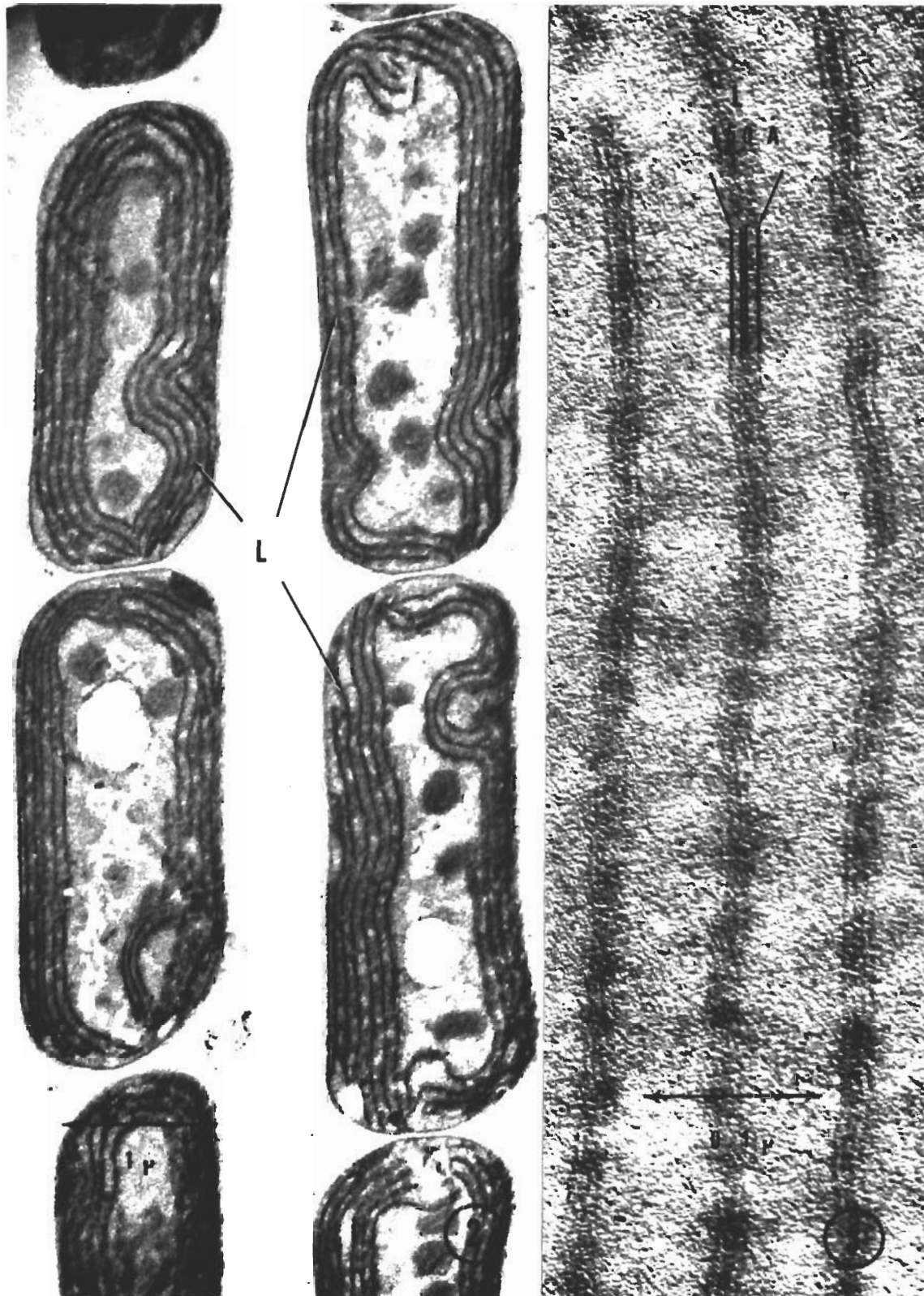


Figure 4

Depicts a cross-section through a Scenedesmus spec. cell. This organism has one cup-shaped chloroplast which in this picture appears doughnut-shaped because of the orientation of the section. The pyrenoid (PY) within the chloroplast, appears structureless but is consistently flanked by "starch plates" (the two clear, sickle-shaped areas). Some starch grains (S) are scattered at random in between bundles of lamellae (L) which appear to follow generally the contour of the cell.

Such a description would apply as well to cells of Chlorella vulgaris, except for the fact that such cells are spherical and that a bundle consisting of two lamellae is invariably found embedded along the diameter of their pyrenoid. Magnification: 13,700 x.



Figure 5

Detail of the lamellar system as seen in *Chlorella vulgaris* chloroplasts. A detail of Scenedesmus would be similar in all respects.

A 160 A unit lamella is emphasized in the upper right corner. The thickness and the modulation of densities within the thickness of any one bundle (G) suggest that they are simply multiples of the 160 A unit lamella (320 A, 640 A, 960 A, etc.). The unlettered arrows point to isolated lamellae extending from one bundle into an adjacent one. Magnification: 243,000 x.

Figure 6

A higher magnification of the lamellar system of the same chloroplast as in Fig. 5. The ends of several lamellae participating in the formation of one bundle are pointed by unlettered arrows. The manner in which each 160 A lamella comes in close apposition with neighboring ones is clearly depicted. The fixation is as in scheme II of Table 1. Magnification: 360,000 x.

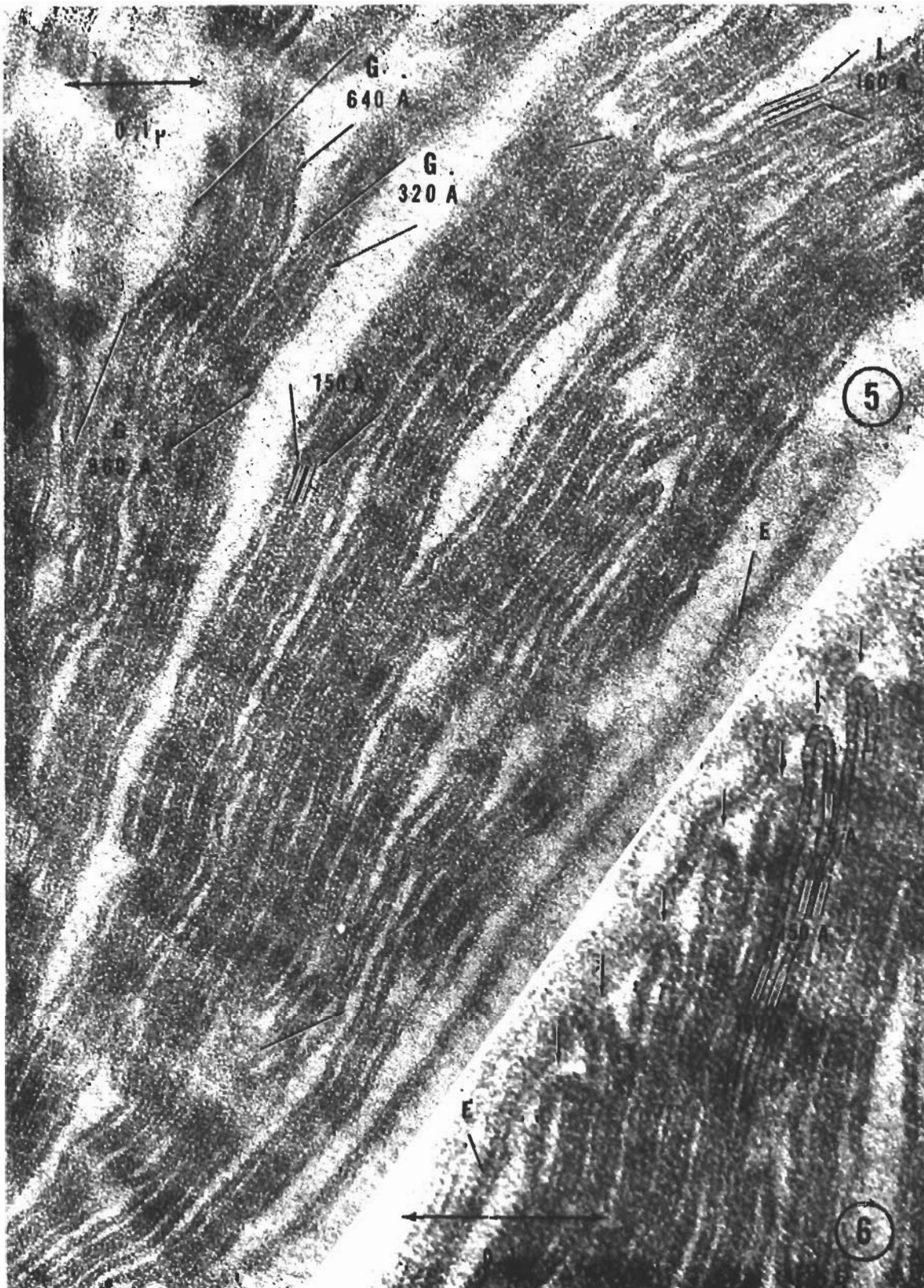


Figure 7

A section through two Euglena gracilis cells oriented along mutually perpendicular planes. The cell at the right is sectioned along its major axis throughout its entire length. The anterior flagellum (F) extends outside of the section plane. The eyespot (E) consisting of a group of dense granules may be recognized at the base of the flagellum. Numerous mitochondria (M) and several golgi bodies (GB) are scattered throughout the cytoplasm.

Ten chloroplasts (C) appear in this section. The lamellar bundles within each chloroplast are generally parallel to the cell surface. Paramylum grains (PA) are in close proximity to chloroplasts but outside of them. No other differentiation within the chloroplasts is apparent. The fixation is as in scheme II of Table I. Magnification: 4,800 x.

Figure 8

Detail of chloroplast lamellae in Euglena gracilis. The bundles (G) are again seen to consist of multiples of a 160 A unit lamella. Although in this picture most of the bundles consist of only two lamellae, thicker bundles are also regularly seen (but no more than 10 lamellae thick). Isolated lamellae, their closed ends, as well as the manner in which they come in apposition is seen in several places. Potassium permanganate fixation (scheme II, Table I). Magnification: 209,000 x.



Figure 9

A cross-section through several spongy, spherical mesophyll cells in a mature Spinach leaf. The cell volume is mostly occupied by a large central vacuole (V) surrounded by cytoplasm. The latter is in turn mostly filled with numerous chloroplasts (C) and only few mitochondria (M). The chloroplast lamellae generally follow the contour of the cell.

Spongy mesophyll cells are attached to each other only along small portions of their surfaces. If the section misses the attached portions, the cells appear (as here) isolated. In the leaf they are, in fact, surrounded by air spaces. Osmium fixation. Magnification: 3,600 x.

Figure 10

A higher magnification of a Spinach chloroplast illustrating the general disposition of lamellae (L) and grana (G). Although the grana (bundles of lamellae) are of a constant length, their thickness varies considerably. Osmium fixation. Magnification: 30,800 x.

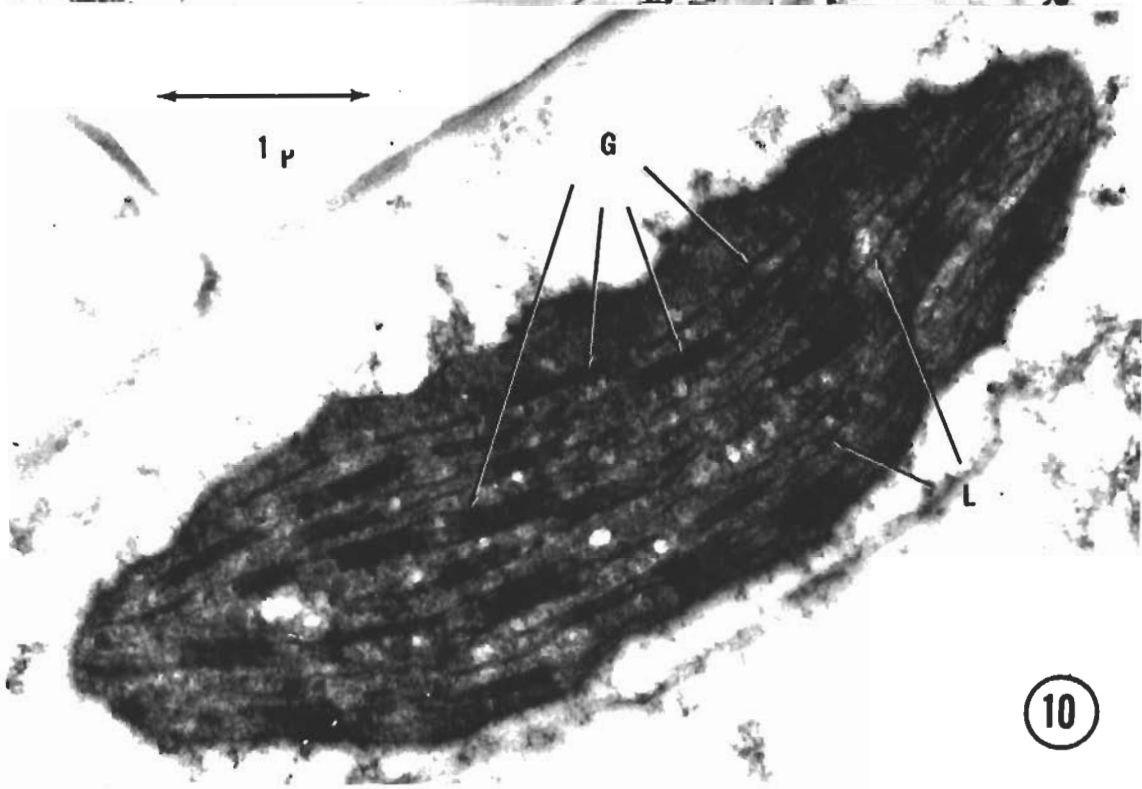
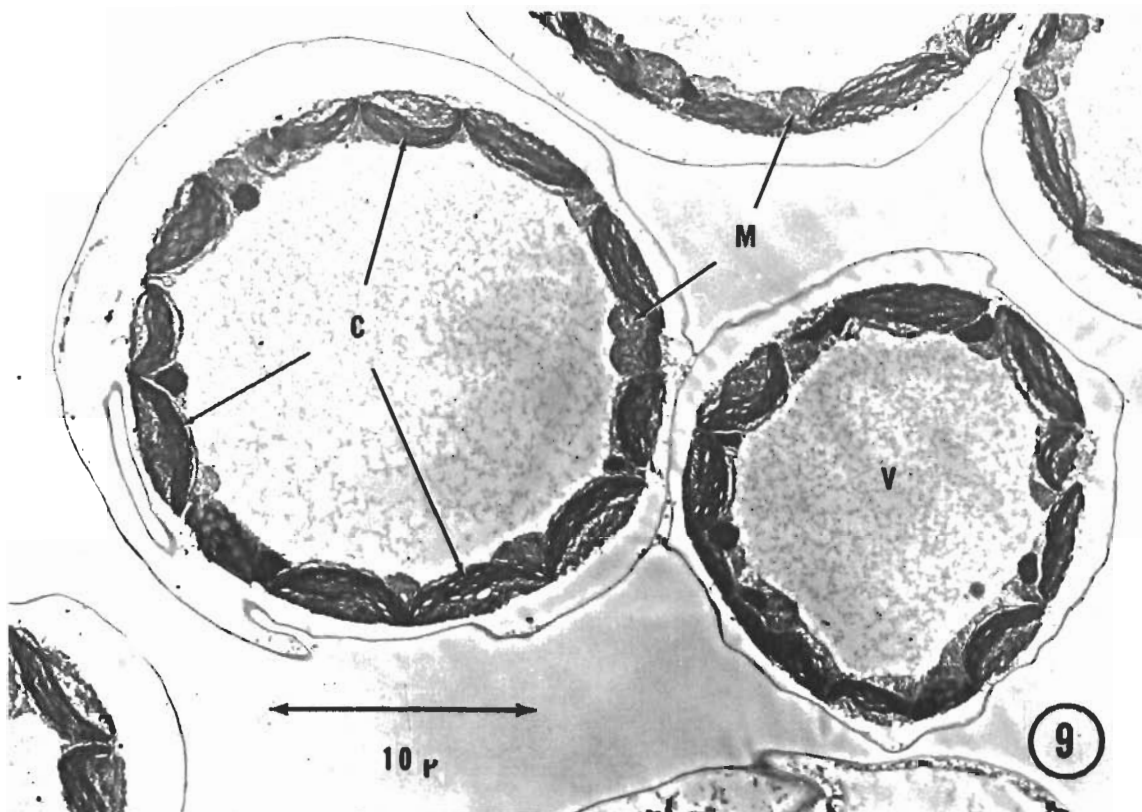
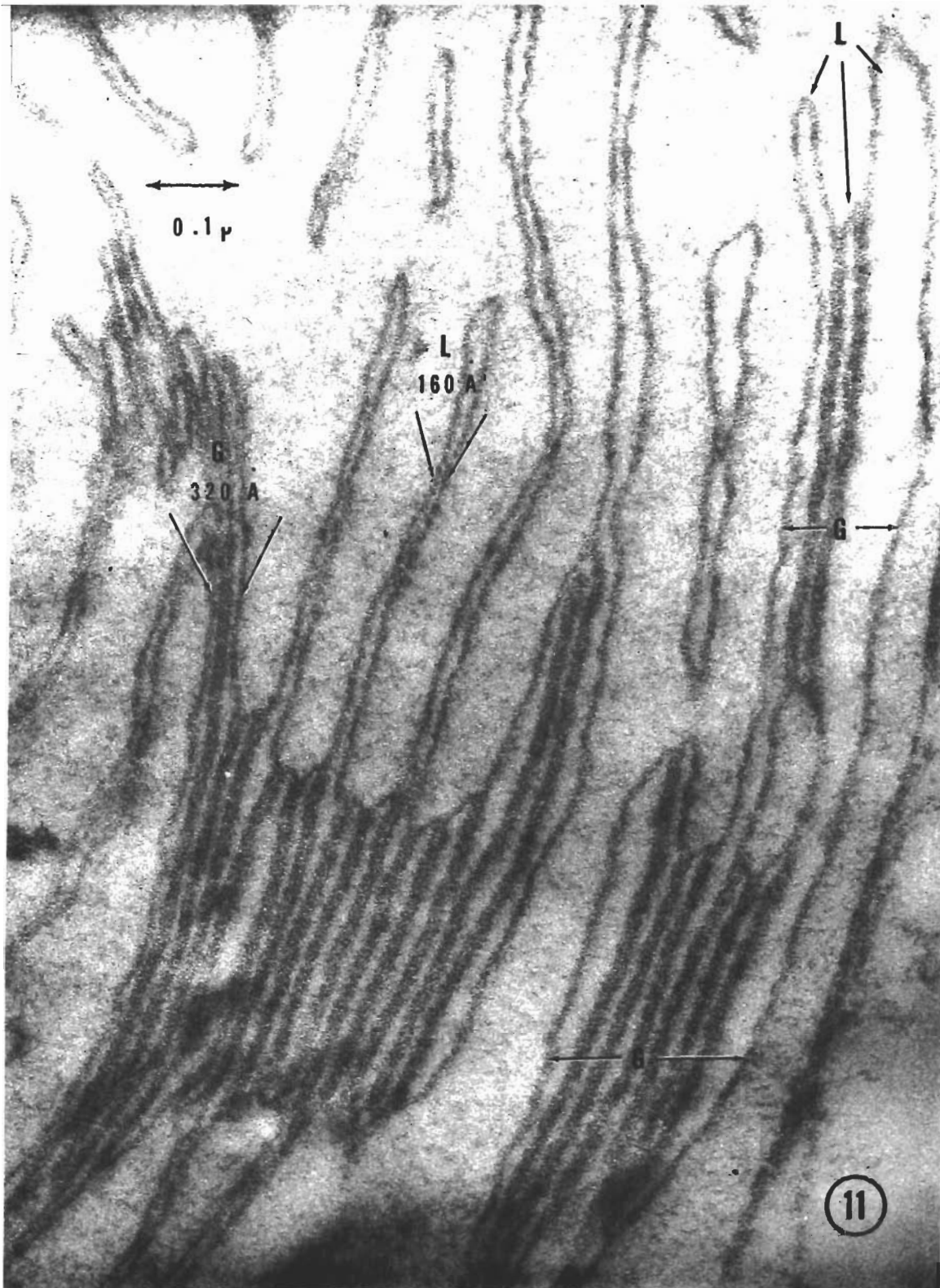


Figure 11

Detail of lamellae (L) and lamellar bundles (G) in Spinach chloroplasts. Potassium permanganate fixation. The lamellar inner space is swollen in some places more than others.

The three lamellae (L) in upper right corner unite to form the granum (G). Only two of these lamellae extend further and associate with other lamellae to form another granum (G) in the lower right corner. The 320 Å thick granum is obviously composed of two 160 Å unit lamellae, although in the course of its length it is seen to enter in the formation of a thicker granum.

The appearance of isolated lamellae and the manner in which they unite to form grana is essentially identical to the one observed in Chlorella or Scenedesmus cells. Magnification: 160,000 x.



Figures 12 and 13

Both illustrate details of the lamellar system of Spinach chloroplasts. The fixation in Fig. 12 is as in scheme IIIa (Table 1) while that in Fig. 13 is as in scheme IIIb (Table 1). Both exhibit identical density modulations in the thickness of a lamella.

The contrast by virtue of which the 160 Å lamella (Fig. 12) is made visible is a reversed one of what it would have been under the conditions of scheme I (Table 1). The modulation of density across the lamellar bundles (G) indicates again that they are simply multiples of unit lamellae. Observe the manner in which two isolated lamellae extend away from a bundle (G) in the upper left corner of Fig. 12. Compare it to the ones seen in the upper right corner of Fig. 11. Magnification: 292,000 x.

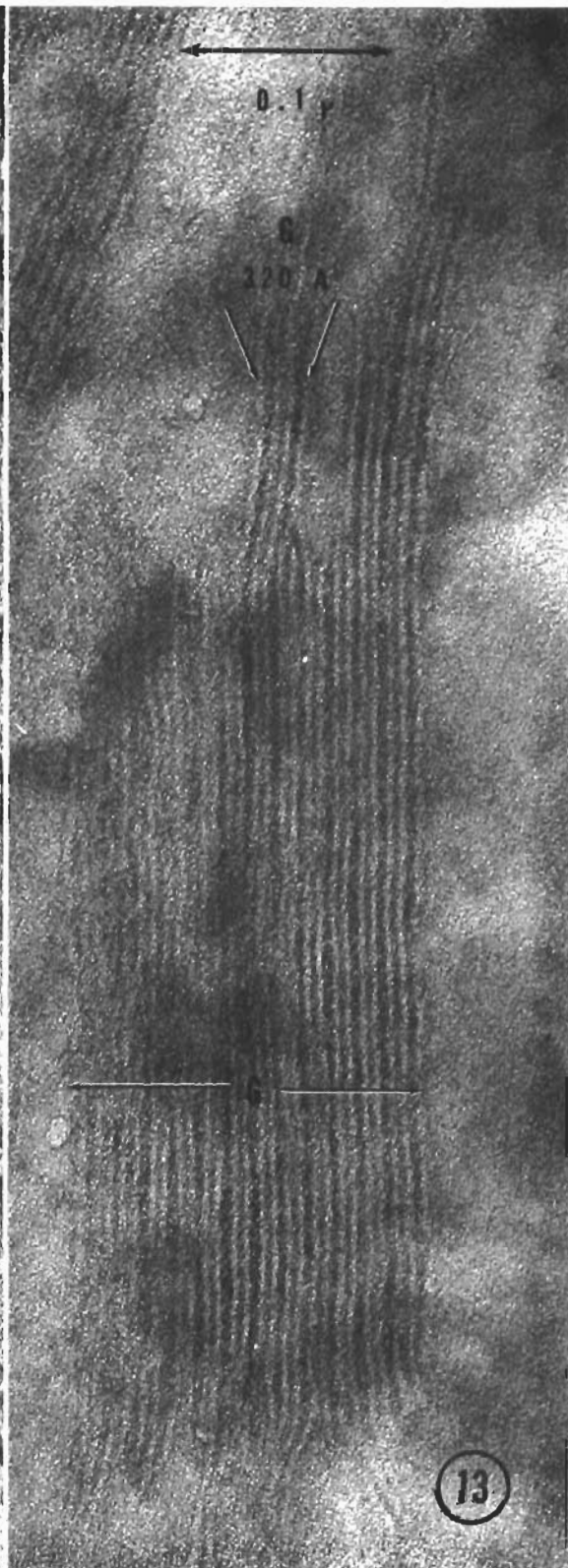
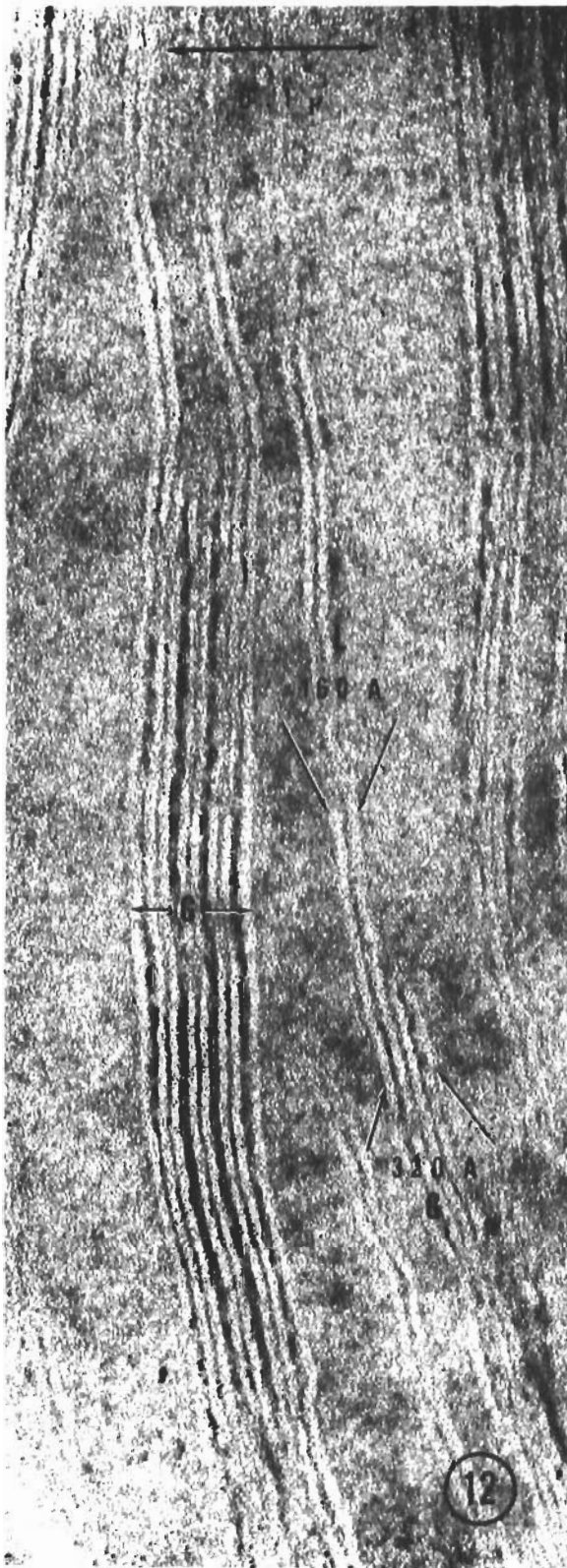


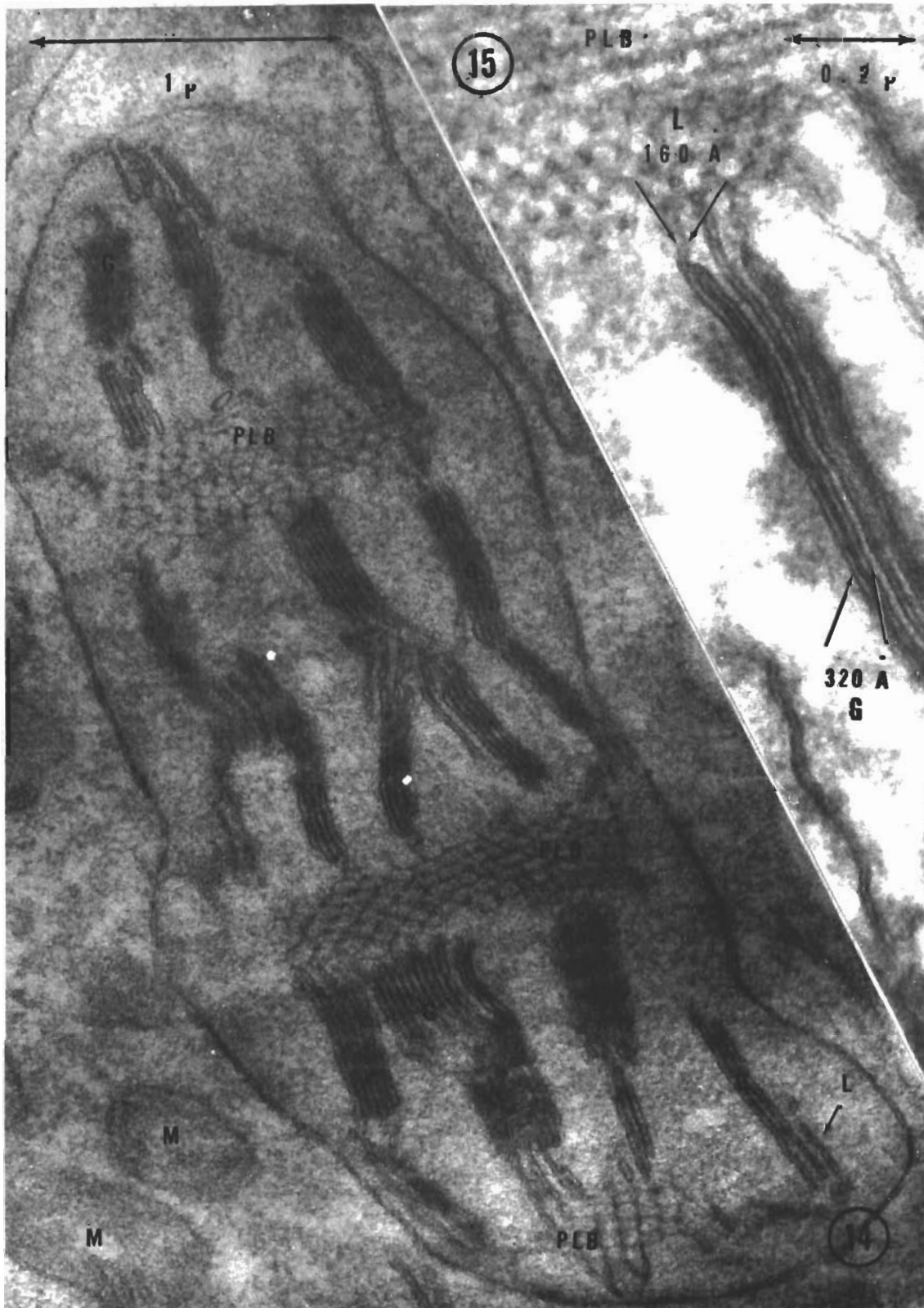
Figure 14

Illustrates the appearance of a chloroplast in primary leaves of a Black Valentine bean plant. The seeds were germinated and grown in total darkness for a period of 6 days. The plant then received alternating 12-hour periods of light and darkness for 3 days and the leaves were fixed (scheme II, Table 1) at the end of a dark period on the third day. In addition to several prolamellar bodies (PLB) numerous grana (G) may be recognized. However, only few isolated lamellae (L) are seen.

If such a sample were fixed at the end of a light period, no PLB would be visible and more lamellae would appear. This indicates that at this age light and darkness have a profound influence on the structure of the chloroplast. Potassium permanganate fixation. Magnification: 55,200 x.

Figure 15

Illustrates the modulation of densities across the thickness of isolated 160 A lamellae as well as a granum (G) consisting of two apposed lamellae. Osmium fixation. The sample is taken from a 10-day old Black Valentine bean plant grown in total darkness and exposed to light for 3 hours. No bundles thicker than two or three lamellae may be seen at this stage. Chlorophyll synthesis is not complete. Note that the prolamellar body has not yet fully disappeared. The lamellae are still in contact with it. Magnification: 110,000 x.



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