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KATY YONG YONG HSU (Name) for the M. S. (Degree)

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Warren E. Kronstad

The seeds of Douglas fir, a representative of the gymnosperm flora, were studied at six stages of germination to discern the structures of food reserves and to study ultrastructural changes occurring during germination in the embryo and resulting seedlings.

The materials were fixed in three fixatives, dehydrated with ethanol series, embedded in Epon, and sectioned to 500-900 Å in thickness, stained with uranyl acetate and lead citrate, and examined with a RCA EMU-2D electron microscope.

Embryo cells contained many fat and protein bodies in addition to the nucleus, a few proplastids and mitochondria. Protein bodies first degraded to form a meshwork and then became completely solublized to form vacuoles in the cytoplasm. Fat bodies decreased in size and number at a rate slower than protein bodies and were utilized completely in root tissue. Amyloplasts increased at the early stages of germination when the fat and protein bodies started

to degrade. At the later stages, the amyloplasts were difficult to observe. Other cellular organelles developed very rapidly with the advancement of germination; their ontagenetic sequences were comparable with plant materials of angiosperm.

Ultrastructural Study of the Embryo During Germination Involving Douglas Fir (Pseudotsuga Menseizii Franco) Seeds

by

Katy Yong-Yong Hsu

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APPROVED:

[redacted]

Assistant Professor of Farm Crops
in charge of major

[redacted]

Head of Department of Farm Crops

[redacted]

Dean of Graduate School

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INTRODUCTION

In the plant kingdom the class Gymnosperm is composed of four major groups, Pteridospermales, Ginkgoales, Cycadales and Coniferales. The coniferales which is the largest of the four are the representative gymnosperms of the present flora. It forms immense forests in the world. Pseudotsuga Menseizii Franco, (Douglas fir) reaches the general size of 100-200 feet in height and six to ten feet in diameter at maturity. It is widely spread throughout North America and is the principal source of lumber in this region. The seed of this species is the only means for reproduction and the first step of reproduction is seed germination.

The physiological and chemical changes during different stages of germination have been studied in detail (6, 7, 8). It was found that the seed of Douglas fir was rich in oils and proteins in the female gametophyte and embryo. The reserve food were utilized gradually for the synthesis of new structural components and soluble enzymes, metabolites, and substrates. The reserve fats were composed of free fatty acids, non-saponifiables, glycerides, and phospholipids. The phopholipids increased gradually in the initial stages and more rapidly in

the later stages of germination, while the glycerides decreased rapidly with the advancement of germination. There was little starch in ungerminated seed; sugars and starch increased with germination at the expense of glycerides. Furthermore starch was only located in embryo and seedling tissues. These findings indicated an active conversion of lipids to carbohydrates in this material during germination. It was further observed that the total nitrogen content of individual seed remained nearly constant throughout the germination process. However, redistribution of nitrogenous compounds was noticed; the reserved protein was localized in protein bodies and was found mainly in female gametophytic tissue. This protein was hydralyzed to soluble forms and transferred to seedling for protein synthesis and further growth (8).

The fresh weight of the seedling increased six-fold and seedling length by thirty-fold during germination. Physiologically, the respiration rate changed from almost zero in the dry seed to 1.5 ml oxygen uptake per hour per germinating seed (5). The types of changes at ultrastructural level in which could be observed in relation to these physiological and chemical changes would be physical evidence for the coordination of structure and function in development process of germination.

The angiosperms with their seeds enclosed in an ovary are structually different from the gymnosperm such as Douglas fir in

which the seeds are borne on open scales in cones. Furthermore, the "endosperm" of gymnosperm seed is developed independent of fertilization from gametophytic tissue, so it only contains half of the somatic chromosome number. On the other hand, the angiosperm seeds contain 3N endosperm which are developed from the fussion of two polar nuclei and one sperm nucleus. Chemically, seeds may vary in their major reserve food with cereal seeds containing mainly starch while legume seeds usually have a high protein content and coniferous seeds always store a high quantity of oils. These anatomical and chemical variations may result in ultrastructural differences.

Some ultrastructural changes in germinating angiosperm seeds have been reported; Bagley, Cherry and Altschule on peanut seeds, (1), Horner and Arnott on Yucca seeds (13, 14), and Klein and Ben-Shaul on Lima Bean (20). There has been no previous work conducted on gymnosperm seeds.

This thesis was initiated to investigate these structural changes which occur in Douglas fir seed and to gain a better understanding of the relationship between morphological developments and physiological changes in gymnosperm. By using the high resolution electron microscope the location of the reserve chemicals and the morphological changes during their utilization, the ontogenetic development of cellular organelles, the organization of cellular components, and

the quantitative aspects of organellar changes during germination were studied.

REVIEW OF LITERATURE

The seed is a product of reproduction from which a new plant will grow when the environment is favorable. Before the root of an embryo grows long enough to absorb nutrients from the soil and the shoot has developed leaves to manufacture food, the seed must provide energy and raw materials for early growth of the seedling from reserved food. Therefore, a large quantity of reserved material is usually stored in the seed during maturation. The degradation sequence of this reserve food at ultrastructural level has been studied in detail in angiosperm seeds. Usually, proteins, fats and carbohydrates are the major storage material for seed; they are deposited in the seed storage organ as distinct structures (4, 10, 30, 32). These structures are different in quantity and in size depending upon the kind of seed or in the type of chemical composition. In starchy seeds, the major stored food is starch grains and protein bodies, while oil and protein bodies are common in oil seeds.

Wheat endosperm and pea cotyledons are filled almost entirely with starch and protein deposits. In embryo tissue, the carbohydrate is stored within the amyloplast which consists of a double membrane involving a diffused matrix with large electron-transparent starch grains embedded in it. The starch grains are bounded by a fairly dense membrane and often show localized internal bands of less

transparent material. Small dense granules of the size of 5-8 mμ in diameter are found in the matrix (30). In wheat endosperm, the starch grains are not enclosed in a plastid. They may represent other carbohydrate materials (4). During the maturation of wheat, one starch granule initiates in each plastid of newly formed cell and grows rapidly in size. Small granules initiate later in the stroma space. The amyloplast membrane often extrudes into the cytoplasm to release or absorb small granules (4). Starch formation and accumulation also takes place in the embryo cells after germination following the above sequence and accompanied by the degradation of lipids and protein bodies (14). The concurrent events of the increase of starch granules and the loss of oil and protein bodies seem to indicate that the degradation of these reserve foods takes place at a rate greater than the immediate needs of developing seed. Thus in order to maintain a satisfactory osmotic balance the excess amount of degraded protein and lipid are converted to starch. These secondary reserves which are intermediate products of lipids and proteins will be used after food reserves have been depleted in later stages of germination.

In addition to starch bodies, there are a large number of small dense granules of 10-20 mμ in diameter scattered in the cytoplasm of pea and wheat embryos (30). Chemical study of these electron-dense granules in wheat have been shown to contain storage

proteins (11) and thus "protein body" is the name given to these granules. Protein bodies vary in size depending on the particular species. They are in general large in size with an average diameter of 3.4 μ in peanut and Yucca seeds. Morphologically protein bodies are classified to two groups: a mesh-work type which is the predominant type containing both electron-dense and electron-transparent regions forming a mesh-work or reticulum structures. Within this mesh-work, structural protein and electron-dense inclusions of various sizes are found. These inclusions are easily shattered during sectioning of the specimen. The dark stained inclusions are non-protein materials as they are not stained in potassium permanganate fixed material. They might be some inorganic crystal such as phytic or other minerals. The second type of protein body is a core-type which is composed of electron transparent core and electron dense matrix. Within the electron dense matrix electron-dense inclusions similar to those found in the mesh-work type protein bodies are also observed. These two types of protein bodies are uniformly stained in potassium permanganate fixed material (1, 13, 14). The protein bodies are enclosed in a single unit membrane (3, 11, 13, 25). The proportion of the two types of protein bodies may be different in various species of seed, e.g. Yucca seed has less core-type than the mesh-work type protein bodies. In the early stage of germination, as a result of imbibition of water, protein bodies swell and develop

cavities within themself. These cavities increase in number and in size forming a network structure which is different to the undegraded mesh-work type protein bodies. Sometimes several small protein bodies fuse together to form a larger one. Finally these network structured protein bodies break down into individual particles which then degraded completely (1). Another way for degradation of protein bodies is through vacuole formation. They do not break down into individual fragments after pit formation, but remain in their original space as a transparent area surrounded by a single membrane (20). Because the feature of vacuole formation Horner et al. suggested that protein body is deposited within vacuole structure during seed formation (13). In developing wheat endosperm, the protein bodies are indeed synthesized within a vacuole structure at the early stage of embryo development. These small protein bodies could be single or several in one vacuole structure. These small protein bodies increase in size and number and then fuse together to fill up the whole vacuole forming a large protein body (18).

There is a large number of small sized osmium dense bodies located near the cell wall which are especially abundant in oil seeds. This is particularly true for Yucca seeds, peanut, and cotton seeds. They are called fat bodies due to their high content of fatty acids. They appeared spherical in shape in glutaraldehyde and osmium tetraoxide fixed material. No internal structure or surrounding membrane was

observed around these fat bodies. On the other hand they show an electron transparent center and irregular shape in potassium permanganate fixed material, since the permanganate is not miscible with lipids. The edge of the empty region of the oil body seems to be bounded by an apparent membrane. It has not been resolved that the membrane-like structure is a true unit membrane or only a phase boundary (10, 14, 20, 32). During germination, lipid bodies are the last ones to be used up completely in Yucca seed. They gradually decrease in number and in size. The first change in the oil bodies is the formation of a fine reticulum. This reticulum increases in size, and the network form begins to break (14). Chemically lipids are hydrolyzed into glycerine and fatty acids, which are converted to carbohydrates (6, 7, 8). This lipid degradation and conversion results in starch formation and the starch grains become morphologically distinguishable.

In ungerminated seeds, these reserve food structures fill up the entire space in the cell, while other cellular organelles are usually obscure. At a later stage of germination other cellular organelles such as dictysomes, mitochondria, and chloroplasts become visible and common in various tissues.

MATERIALS AND METHODS

Preparation of Fixatives, Embedding Resin, Specimen Support Grid and Stains

Four fixatives were used in this study. The phosphate buffer was made by mixing 0. 25 M solutions of Sodium Phosphate Monobasic and Sodium Phosphate Dibasic to a pH of 7 as determined by a pH meter. The glutaraldehyde-buffer fixative was made by mixing one part of 5% glutaraldehyde and one part of 0. 25 M phosphate buffer to prepare 2. 5% glutaraldehyde-0. 125 M phosphate fixative. Glutaraldehyde in 25% solution was obtained from K and K Laboratories, Inc., Plainview, New York. The Dalton's fixative was freshly prepared by mixing one part of 2% potassium dichromate in 1. 7% sodium chloride aqueous solution and one part 2% osmium tetraoxide solution; the mixture was adjusted to pH 7. 2 with 5N NaOH, so the final fixative contained 1. 0% osmium tetraoxide, 1. 0% potassium dichromate and 0. 85% sodium chloride. The osmium-buffer fixative was prepared by mixing one part of 2% osmium tetraoxide solution to one part of 0. 25 M phosphate buffer. The potassium permanganate was made of 1. 5 g potassium permanganate in 100 ml distilled water.

Copper specimen supporting grids of proper mesh size were cleaned in soap solution or 1N HCl, rinsed in distilled water several times, and then coated with a supporting film. The thickness of the

supporting film is important. If it is too thick it will reduce the contrast of the final picture, while if it is too thin it will not be able to support the sections. A solution of 0. 25% Formvar in redistilled dichloroethane was used to make supporting films. Dipping an ordinary microscope slide in this Formvar solution casts a uniform film on the surface of the slide. Upon drying in air, the film was floated off on a water surface and picked up on the grid. To do this the edge of the film was scored first, and then moisture was condensed on its surface by exhaling upon it. The slide was held at about a 30° angle as it was pushed down into the water. The film floated away freely from the glass. A fairly large glass "baking" dish was used for this purpose. It was nearly filled with distilled water, and the water surface was cleaned by passing a piece of tissue paper over the surface. While the film was floating on the water surface, cleaned grids were spaced upon it one by one. They were not spaced too closely together. Each was tapped with the forceps tips to assure good adherence. After the grids were in place, a dry microscope slide was brought down into the water and removed face up with the film and grids on top. Excess water was removed with a filter paper and the grids with film screens were ready to use after drying. Capsules and grids were obtained from LKB Instruments, Inc., 4840 Rugby Avenue, Washington, D. C., 20014.

There are many kinds of embedding resins for electron

microscopy. Epon was selected for its high resolution, better contrast and easy stainability. In addition, the monomer of Epon has an advantage over Araldite in being quite fluid rather than decidedly viscid and thus, it penetrates tissue blocks more easily and rapidly. Made up according to the formula of Luft (21), it is a combination of two mixtures, mixture A is made of 62 ml of Epon 812 and 100 ml of DDSA (dodecenyl succinic anhydride), mixture B is made of 100 ml of Epon 812 and 89 ml of MNA (methyl nadic anhydride). Mixture A is softer than mixture B; usually the ratio of 1:1 is satisfactory for biological materials, but the seed tissue with high content of fats and insoluble proteins is very hard so the ratio of 3:7 (A:B) was chosen to minimize the differential hardness between the tissue and the surrounding resin. These Epon mixtures are stable and can be kept for many months in a refrigerator. A resin polymerizing catalyst or accelerator, DMP-30 (2, 4, 6-tridimethylaminomethylphenol), was added at 1.5-2.0% of the resin volume just before use. The Epon 812 is obtained from the Shell Chemical Company, 3813 Hoke Avenue Culver City, California.

Stains--Uranyl Acetate and Lead Citrate Were Used to Stain the Tissue Sections.

Uranyl acetate reacts with protein and lipid, the solution was made by dissolving uranyl acetate in distilled water to saturation (15).

Lead citrate stains nucleoprotein as well as other organic compounds containing protein. The lead citrate solution was prepared by placing 1. 33 gm of lead nitrate, and 1. 76 gm of sodium citrate in 30 ml of distilled water in a volumetric flask. The resultant suspension was shaken vigorously for a full minute, and allowed to stand with intermittent shaking for 30 minutes in order to insure the complete conversion of the lead nitrate and sodium citrate to lead citrate. Then 8 ml of 1N sodium hydroxide was added (29). The suspension was diluted to 50 ml with distilled water, and mixed by inversion. The stain was ready for use after the lead citrate dissolved in the alkaline solution. The lead citrate solution is unstable and easily produces white precipitate upon standing. The precipitate is lead carbonate which is formed when in contact with carbon dioxide in the air. Therefore the solution was prepared about every month to insure its freshness. Placing sodium hydroxide pellets in a small dish in the staining chamber was a general practice to reduce the formation of the precipitate on specimen during staining.

Preparation of Specimen

Douglas fir seeds were studied at six different stages of germination: Stage A, soaked seeds; stage B, stratified seeds, which were soaked in tap water for 24 hours and then chilled at 3° C for ten days in a plastic box; stage C, radicle emerged, 1 to 10 mm in length;

stage D, radicle 10 to 30 mm in length; stage E, radicle 31-50 mm in length and hypocotyl arch visible; stage F, seedlings with green expanding cotyledons.

The embryo or seedling of these seeds is composed of three parts: root, hypocotyl and cotyledons surrounding a small epicotyl. These three parts were carefully dissected out from the seed of various stages and cut into pieces of about one cubic millimeter in size on an ice cold glass plate. They were divided into three batches and fixed with three different fixatives so that the artifacts produced by one kind of fixation could be determined. One sample was fixed with 1.5% potassium permanganate in small vials for one hour at 0° C, then stayed for three hours at room temperature. The fixative was pipetted out, and the fixed material was washed with water for several times. After that, the materials were dehydrated with acetone solution of 20%, 30%, 50%, 70%, 80%, 90%, 95%, 100%, 100% for ten minutes in each change. The short time used for each change of dehydration step was to avoid the leaching of soluble materials out of the fixed specimens. The small size of tissue specimens made it impractical to transfer them from one vial to another, so pouring fluids out of the containers and replacement with others was conducted during fixation, washing, and dehydration. For better subsequent infiltration of resin, two changes of propylene oxide were used for 30 minutes each then followed with an overnight infiltration of propylene

oxide plus Epon 812 in the ratio of 1:1. Finally the materials were embedded in fresh Epon 812 in polyethylene capsules. This final step was done using a small wooden spatula to avoid the mashing and crushing of the tissue. Filled capsules were labeled with a small piece of paper embedded in resin and polymerized in an oven at 60° C for 30 hours.

The second sample was fixed with 5% glutaraldehyde in 0. 125 M phosphate buffer at pH 7. 0 for one-half hour. Materials were washed with phosphate buffer several times and then transferred to freshly prepared Daltons fixative for four hours in the cold room and washed. The third batch was fixed with 2. 5% glutaraldehyde in 0. 125 M phosphate buffer at pH 7. 0 for three hours and post fixed with 1% osmium tetraoxide in phosphate buffer for 16 hours in the cold room. These fixed materials were dehydrated by a series grade alcohol: 50%, 75%, 85%, 95%, 100% and absolute alcohol for ten minutes each. The dehydrated materials were embedded in Epon 812 as the material fixed with permanganate. Capsules were cured in an oven at 30° C with ten inches degree of vacuum to remove air bubbles and solvent for 30 hours, then polymerized at 30° C for one day and 60° C for two days. After polymerization the capsules were removed from the resin tissue blocks. In order to have uniform serial sections, the block was trimmed with a sharp razor blade before sectioning to make a block face of 0. 3 mm^2. The material was sectioned with a

Porter Blum Ultra-Microtome MT-2, using a diamond knife. Only straight section ribbons with silver or gray interference color indicating the thickness range of 500-900 Å were mounted on the prepared grid. The mounting procedure was carried out by holding the grid with supporting film side down and slightly touching the water surface of the trough where the thin sections were already gathered by an eyelash needle. All the instruments and containers were carefully cleaned before use to eliminate contamination from dirt and oily films or droplets on forceps, needles and fluid surfaces. After mounting, the grids with sections were stained by floating them upside down on a single drop of a saturated uranyl acetate solution for 30 minutes and washed with distilled water. Then they were post-stained with lead citrate for 15 minutes and washed with one drop 0. 2 N sodium hydroxide and followed by thoroughly washing with distilled water to remove precipitate and the excess stain. The purpose of staining is to add a heavy metal to the specimen to increase the electron density and thus enhance the contrast of the picture. The length of staining time in each heavy metal solution determines the reaction time for complex formation. The quantity of metal complex on the tissue section is an important factor affecting contrast and resolution. The staining time used was found to be the best procedure for this material. The stained grids were then examined with an electron microscope RCA EMU-2D and photomicrographs were taken.

RESULTS AND DISCUSSION

The Douglas fir seeds are similar to angiosperm in that they also deposit their reserve foods in characteristic structures of protein bodies, fat bodies and amyloplasts. Their degradative sequence is described below:

Protein Bodies

In dry and soaked seeds, protein bodies were scattered in the cell. The diameter of the protein body ranged 0.5-3.5 μ. The average size varied in different cell types. Generally the average size was 2.5 μ in cotyledonary tissue and 1.5 μ in root tissue. The morphological appearance of protein bodies in this material could be classified into four groups. Type 1: electron-dense body without any inclusions or transparent regions (plate 13, A); Type 2: electron-dense body with one to several electron-transparent spherical regions within it (plate 13, B); Type 3; granular body with many transparent pits (plate 12, B); Type 4: the same as 3 except they contain electron-dense central area (plate 10, A). The protein bodies in potassium permanganate fixed material were one type bounded by a single membrane with uniform internal structure. They are lighter when compared to osmium fixed specimen. The degree of electron transparency of these protein bodies is comparable to cytoplasm.

At the very early stage of germination, there was no type 1 or type 2 protein bodies. All of them were granular type. At later stages those electron-transparent pits increased in their size and finally coalesced together forming vacuole. The degradation sequence may be illustrated by the following diagram (Figure 1). The electron-transparent spherical areas may either represent the original sites of phytin or other mineral deposits or indicate the location of soluble proteins. Type 1 and type 2 both appear to be the undegraded protein bodies but at different angles. When insoluble proteins converted to soluble forms upon imbibition, many small electron transparent pits were formed (plate 12, B and 13, C) as shown by type 3 and type 4 protein bodies. Protein body type 4 might contain different proteineous components which were hydrolyzed at a slower rate. Another possible interpretation of this type 4 protein body is that the edge of the protein bodies absorbed water first so they start degrading first. Very few protein bodies could be seen in stage C and stage E. Most of the bodies coalesced to form big vacuoles with an electron-transparent area in the center and several small dark areas of insoluble proteins around the outer edge (plate 14, A). This part of undegraded proteins did not show granular structure. So it is possible that the coalescence of protein bodies accompanied with coalescence of undegraded proteins. These dark coalesced insoluble protein reduced in size with the advancement of germination. In

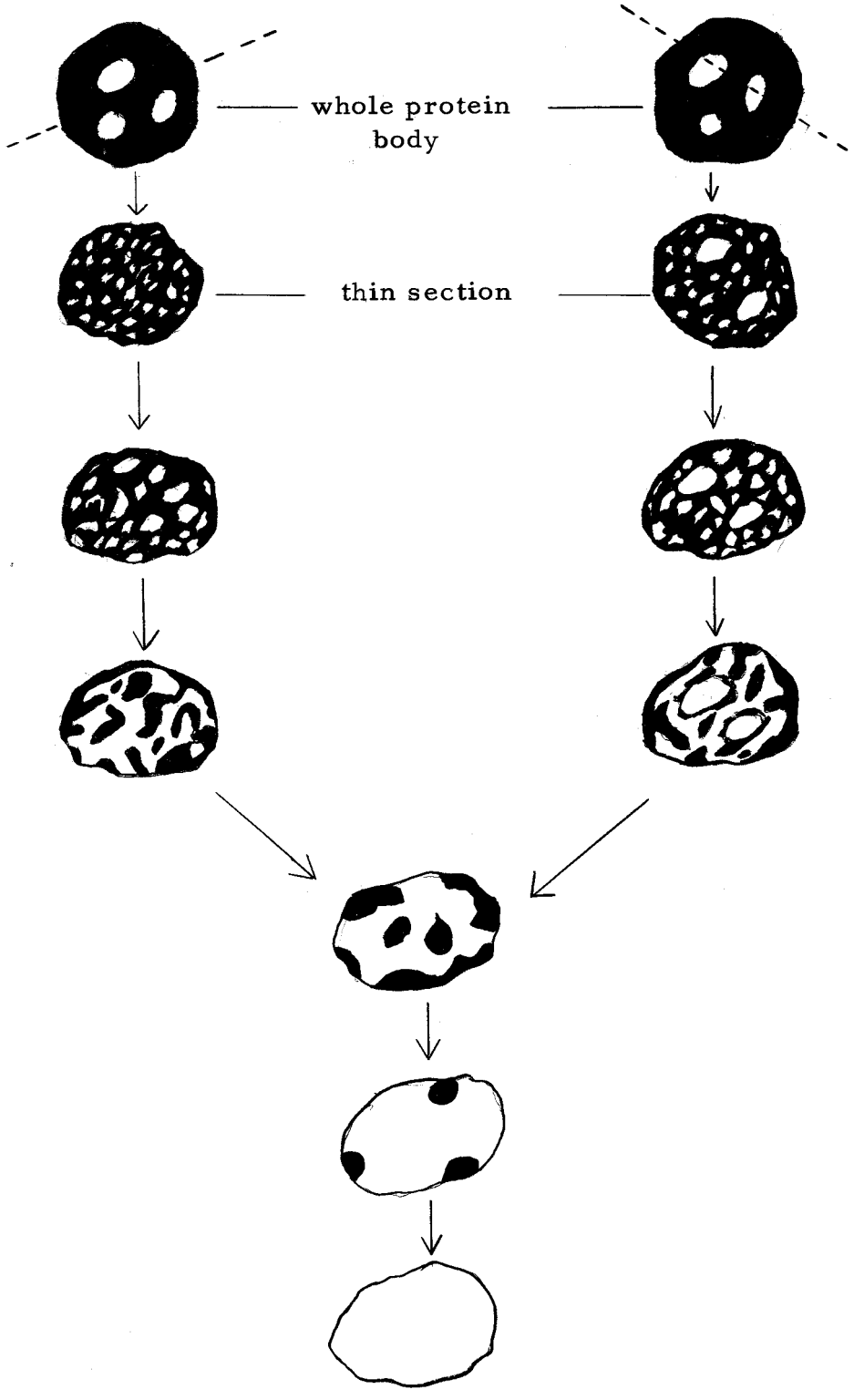


Figure 1. The degradation pattern of protein bodies.

stage E, some of them only contained several dark spots (plate 14, B). So after germination all of those protein bodies become one or several larger vacuoles in a cell with some of these vacuoles still contained very small sized electron-dense areas. Membrane-like and myalin-like structures were occasionly found in these vacuoles (plate 10, C). The gradual solubilization and hydrolysis of reserve protein are reflected in the chemical analysis of this seed (8).

Protein bodies found in root material were not as large as in cotyledenary tissue with a general size of 1.5 - 2 μ. They also showed degradation. Proteins were the first reserve food to be used up in root cells during germination. The rate of degradation was different even in adjacent cells. Vacuoles formed during imbition and the protein bodies disappeared completely in stage B. The degradation pattern of the protein body was similar to those observed in the cotyledonary tissue with the exception of being faster with the resulting vacuole size being smaller.

Fat Bodies

In cotyledonary tissues the spherical lipid bodies showed a homogeneous internal structure and were different in size with a range of 0.2-2.3 μ. The average size was 1.2 μ which was much smaller than the observed protein bodies. In some instances the lipid bodies in dry seeds coalesced resulting in particle sizes larger

than the protein bodies (plate 21, C and D). Relatively smaller fat bodies were lining the cell wall with the larger ones located in the interior space between protein bodies (plate 10, B). In some photomicrographs the fat bodies were bounded by a fine membrane but most of them did not contain a contineous membrane. So the membrane could only be a phase boundary. The boundary of fat bodies, fixed by potassium permanganate showed an irregular shape without any internal contents (plate 21, A). Because the reaction of permanganate, the membrane-like boundary might be some complex of protein.

In the ungerminated seeds fat bodies were closely packed together with very small interspaces. Some of them showed darker center regions indicating different chemical components or a result of poor fixation. Fat bodies also were utilized gradually during germination. They decreased in size, the spaces between fat bodies became larger, and then disappeared gradually. The rate of lipid degradation was slower than protein. At stage C and stage D there were still many fat bodies in the tissue while the protein bodies were nearly used up. Even at stage F some fat bodies were observed in cotyledonary cells (plate 4 and 14, A). In potassium permanganate fixed material, a very thick and dark stained wall was formed around the fat bodies at later stages (plate 21, B). The degradation pattern of fat bodies was simply in situ decrease of size and number. The

reduction of fat bodies coincide with the fat utilization of this material (7, 8).

The number of fat bodies found in root material were less than cotyledonary tissue. The size was smaller with an average of 1 μ. Most of those bodies linned the cell wall (plate 16), while some were scattered in the cytoplasm. They did not surround the protein bodies as closely as in cotyledonary cells. At stage B when protein bodies were degraded completely, the fat bodies were still present but of smaller size. They disappeared very rapidly after imbibition of water. Fat bodies also diminished faster in roots than in cotyledons.

Plastids

Starch granules were embedded in amyloplast in dry seeds. The amyloplasts were concentrated mostly around the nucleus (plate 10, A) and the number per cell was small. Amyloplasts were abundant in root cells particularly at the early stages of germination. Several starch granules were located in one amyloplast which was two to four times larger than those found in cotyledonary tissue of ungerminated seeds (plate 15). The matrix of amyloplast showed more electron-transparent in ungerminated seeds than that at later stage of germination (plate 15 and 7). There were cavities around the starch granules embedded in the amyloplasts. Electron-opaque round granules were also found within the amyloplast. The number

and size of amyloplast decreased after radicle emerged, and finally disappeared. Prior to its complete disappearance, the amyloplasts lost their starch granules and became rod-shaped with denser matrix. The increase of amyloplasts in the root material prior to radicle emergence was due to more rapid degradation of reserve food than the seedling needs so the carbon compounds were converted to starch for temporary storage.

Proplastids were common in root cells. They were surrounded by a double unit membrane and were about the same size as mitochondria but with denser matrix than the mitochondria. Chloroplasts were developed at later stages from proplastids. Round shaped prochloroplast without starch grains and well developed chloroplast were commonly found in stage E and stage F (plate 20). Starch granules with a transparent edge found in chloroplast often had black folds of the section. They were bounded by a double membrane and with an internal structure of lamellar areas. Electron-opaque oil droplets were common in the chloroplast. The greater the number of well developed chloroplasts, the less the number and smaller the size of fat bodies found in the cell.

Another kind of plastids which looked much like proplastids were also observed in germinated seeds. They exhibited the same electron-density of proplastids but with single membrane and granular interior (plate 6). This kind of plastids were found in Yucca seed (14).

Its function is unknown at present.

Other Organelles

Other cellular organelles were very few in number and could be found occasionally in the ungerminated seeds. Owing to the compactness of the storage organelles in the cell, the fine structure of other organelles could not be fixed well for study. Accompanying the degradation of food materials, other cellular organelles gradually become visible during germination. Mitochondria seemed to increase rapidly in number upon inbibition in radicle and cotyledon cells. The morphological feature of gymnosperm mitochondria was similar to that of the angiosperm. They were enclosed by a double unit membrane with many internal cristae and a dense matrix (plate 11).

Neither rough or smooth-surfaced endoplasmic reticulum could be found in cotyledonary cells of ungerminated seeds (plate 1), but they are readily observable in roots (plate 6). They increased with germination in seedling cells. The rough-surfaced endoplasmic reticulum was usually associated with the nucleus (plate 6) and around the outer cytoplasmic membrane. The diamter of the rough-surfaced endoplasmic was about 250 Å.

Golgi bodies deveoped profusely in growing regions of the seedling and consist of three to eight cisternae and many vesicles. Each disc was 0.4 - 0.8 μ in length and 150 Å in width. Usually golgi

bodies were located along the outer cytoplasmic membrane and around the nucleus indicating their secretory function and synthetic activities (23).

Ribosomes and polysomes were observed in meristemic cells of the seedling (plate 6, 11). Such an increase of ribosome and polysomes could also result in changes of RNA contents in the seeds. The ribosome had an average diameter of 150 Å and the polysome generally consists of three to eight ribosomes.

Fibrous primary cell wall and amorphous middle lamella were observable in roots (plate 16). Pigmented intercellular space and cell wall were seen in root cells of completely germinated seedlings (plate 9). Pits could be found in seedling cells at the later stages of germination: plasmadasmata were a rare occurrence in this material except during the formation of the new cell plate at the end of mitosis.

In potassium permanganate fixed material only the structural membrane of various organelles were well fixed, however, the internal structure were usually not well defined (plate 17, 18). The nucleus was surrounded by a double unit membrane which was more widely separated than the double membrane of amyloplastids. Nuclear pores were seen very clearly. Endoplasmic reticulum was well defined. Ribosomes, polysomes, golgi bodies could not be found in this material.

SUMMARY

In Douglas fir seeds, protein and fat bodies were the major structure for reserve food. These structures were smaller and less in quantity in root material than in cotyledonary tissue. They were utilized earlier in root than in the cotyledon during germination. Amyloplasts were present, however, in small numbers in dry seeds, but increased at the early stage of germination. Those starch gains embedded within the amyloplast were the intermediate temporary storage organelles from the excess degradation of proteins and lipids.

Insoluble protein bodies were converted to soluble proteins for the synthesis of cellular components of the growing seedling. Solubilization started at many places in the protein body giving a granular interior structure. Then the soluble pits enlarged and fused together, and the whole protein bodies become fragments enclosed by a single membrane. Finally protein bodies were completely degraded and formed vacuole in the cytoplasm.

Fat bodies were utilized gradually resulting in a decrease in size and number. This was much slower than the proteins as far as the degradation rate was concerned. In the root material prior to radicle emerging most of the protein bodies were used up while fat bodies showed only a very slight degree of degradation.

Amyloplasts were abundant in root material specially at the early stage of germination then decreased rapidly after the radicle emergence.

Accompanying the utilization of reserve food, other cellular organelles were developed for the growth of the seedling. The number of mitochondria increased quickly during germination. Golgi bodies were common in later stages. Endoplasmic reticulum, ribosome and polysomes were visible at the early stage and became very frequent at the later stage. All the organelles were developed much earlier in the root tissue than in cotyledons. Chloroplasts with starch granules also developed at later stages.

ABBREVIATIONS USED IN PHOTOGRAPHS

PB	protein body	CH	chromosome
F	fat body	SO	soluble protein
M	mitochondria	MY	myalin-like structure
CW	cell wall	ML	middle lamella
N	nucleus	NP	nuclear pore
NO	nucleolus	NM	nuclear membrane
PP	proplastid	A	amyloplast
P	polysome	C	chloroplast
PX	proplastid-like structure	G	golgi body
ER	endoplasmic reticulum	S	starch grain
RER	rough surfaced endoplasmic reticulum	V	vacuole
U	undegraded protein		

Plate 1. A portion of a cotyledon cell in a stratified seed fixed with glutaraldehyde and Dalton's fixative. The cell is filled with protein and fat bodies. Different types of protein bodies are shown.

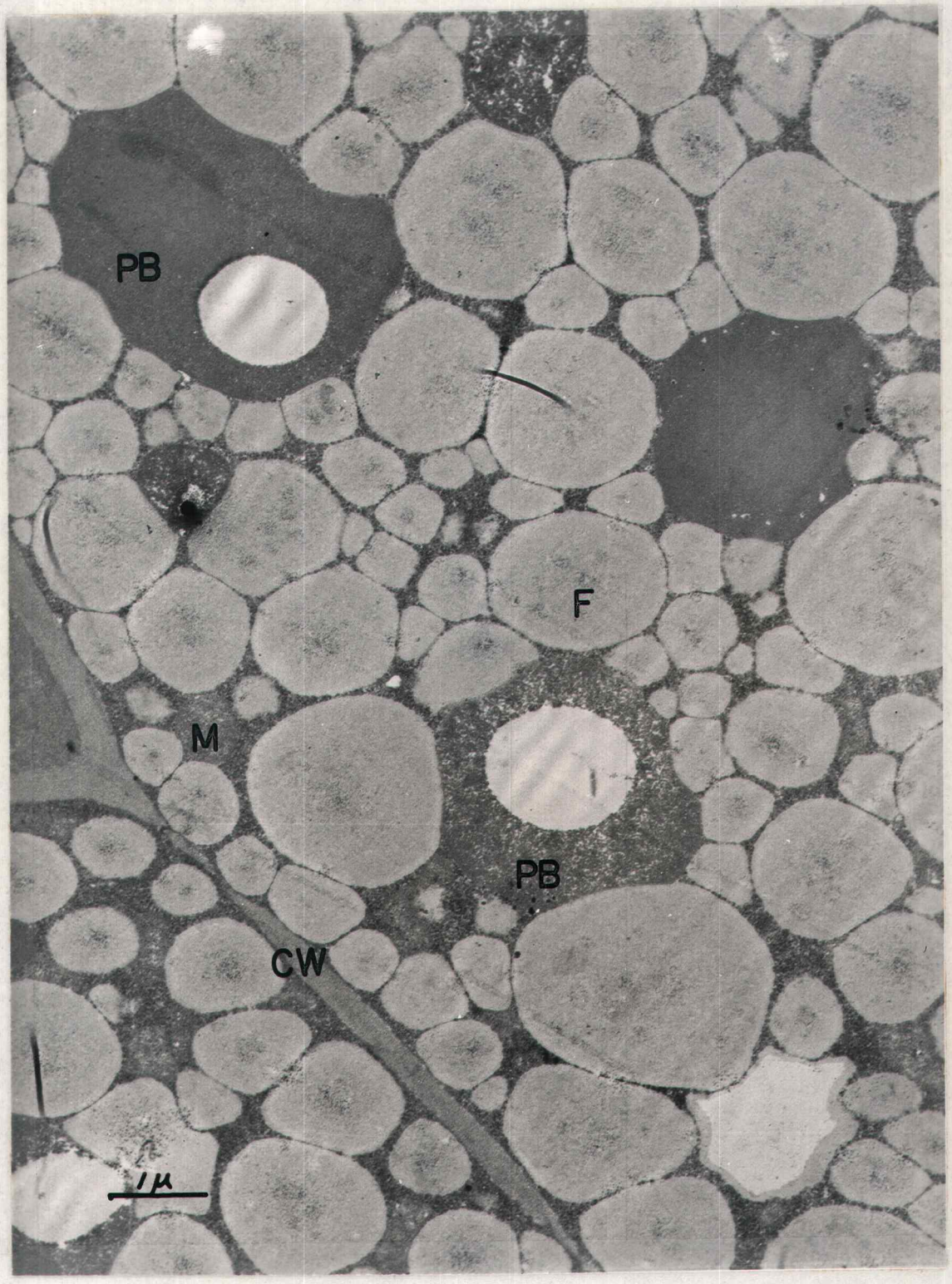
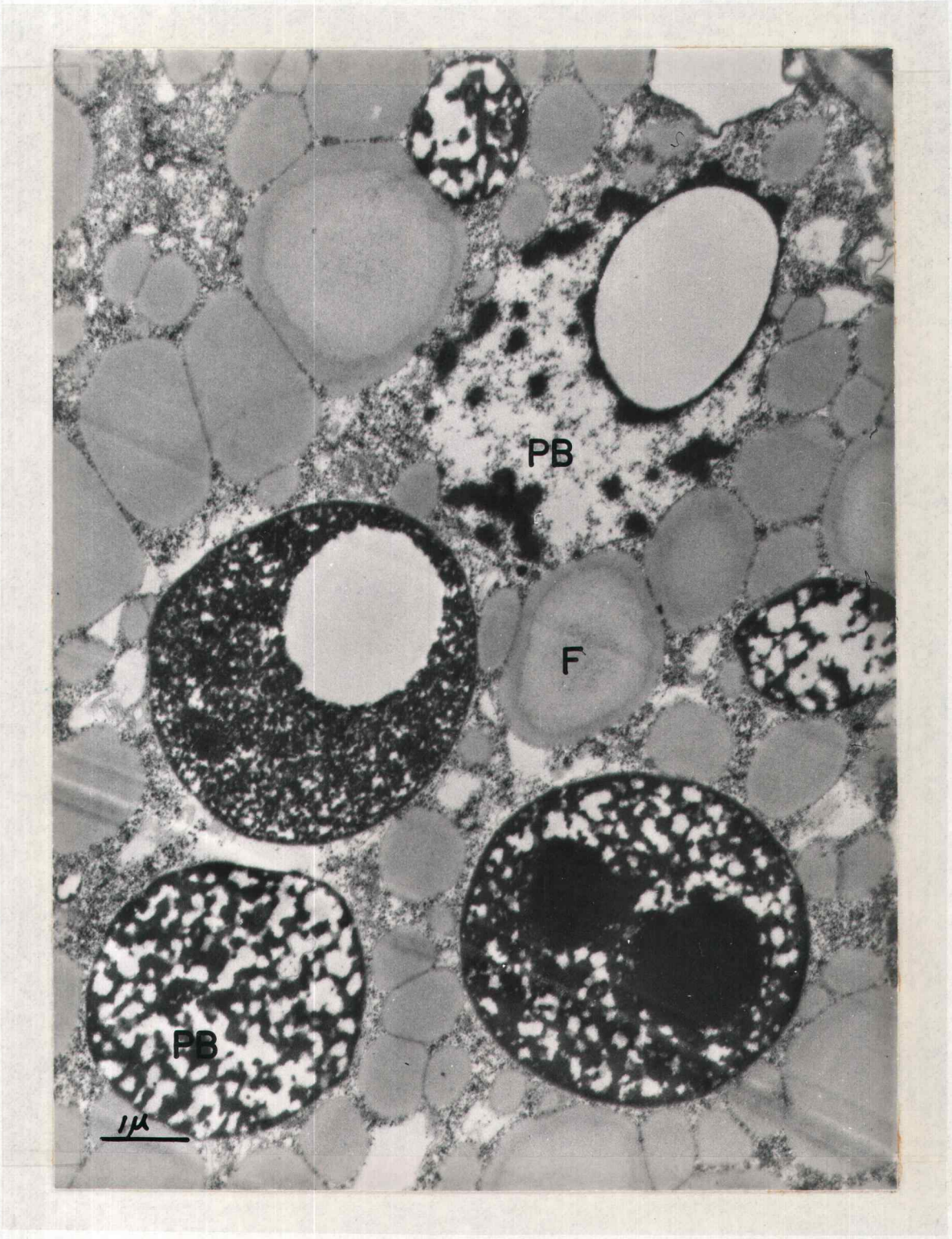

PB
F
M
PB
CW
1μ

Plate 2. A portion of a cotyledon cell in a soaked seed fixed with glutaraldehyde and osmium tetraoxide. Different stages of protein body degradation are shown.


PB
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1μ

Plate 3. Parts of 3 cotyledon cells in a germinated seed fixed with glutaraldehyde and osmium tetraoxide. Protein bodies are degrading leaving vacuoles in the cell. Other cellular organelles are seen.

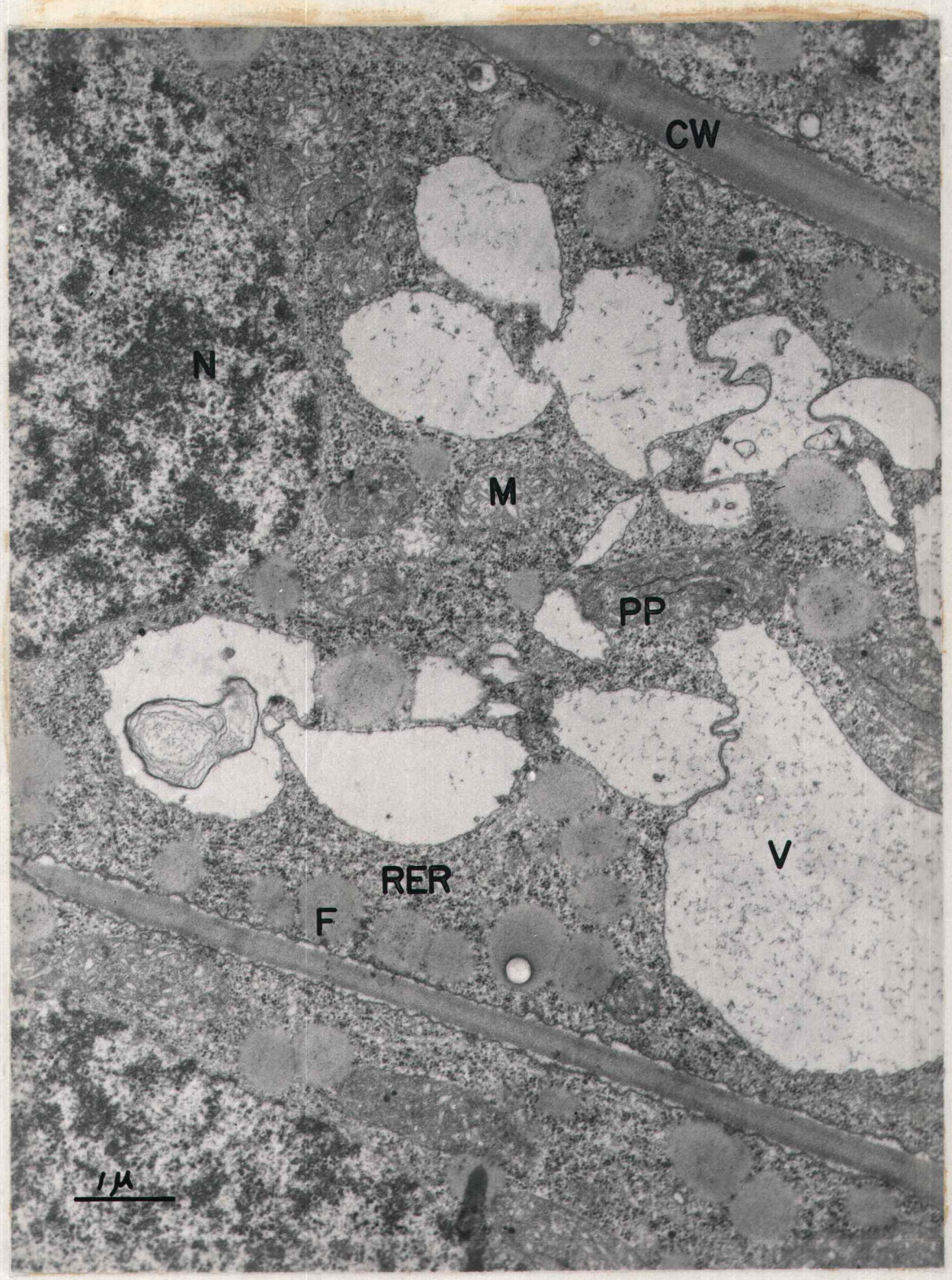

CW
N
M
PP
V
RER
F
1 μ

Plate 4. A portion of a cotyledon tissue of germinating seedling fixed with glutaraldehyde and osmium tetraoxide. A dividing cell in metaphase shows chromosomes without nuclear membrane. Protein and fat bodies are degrading. Starch granules and electron-opaque granules are common in the chloroplasts.

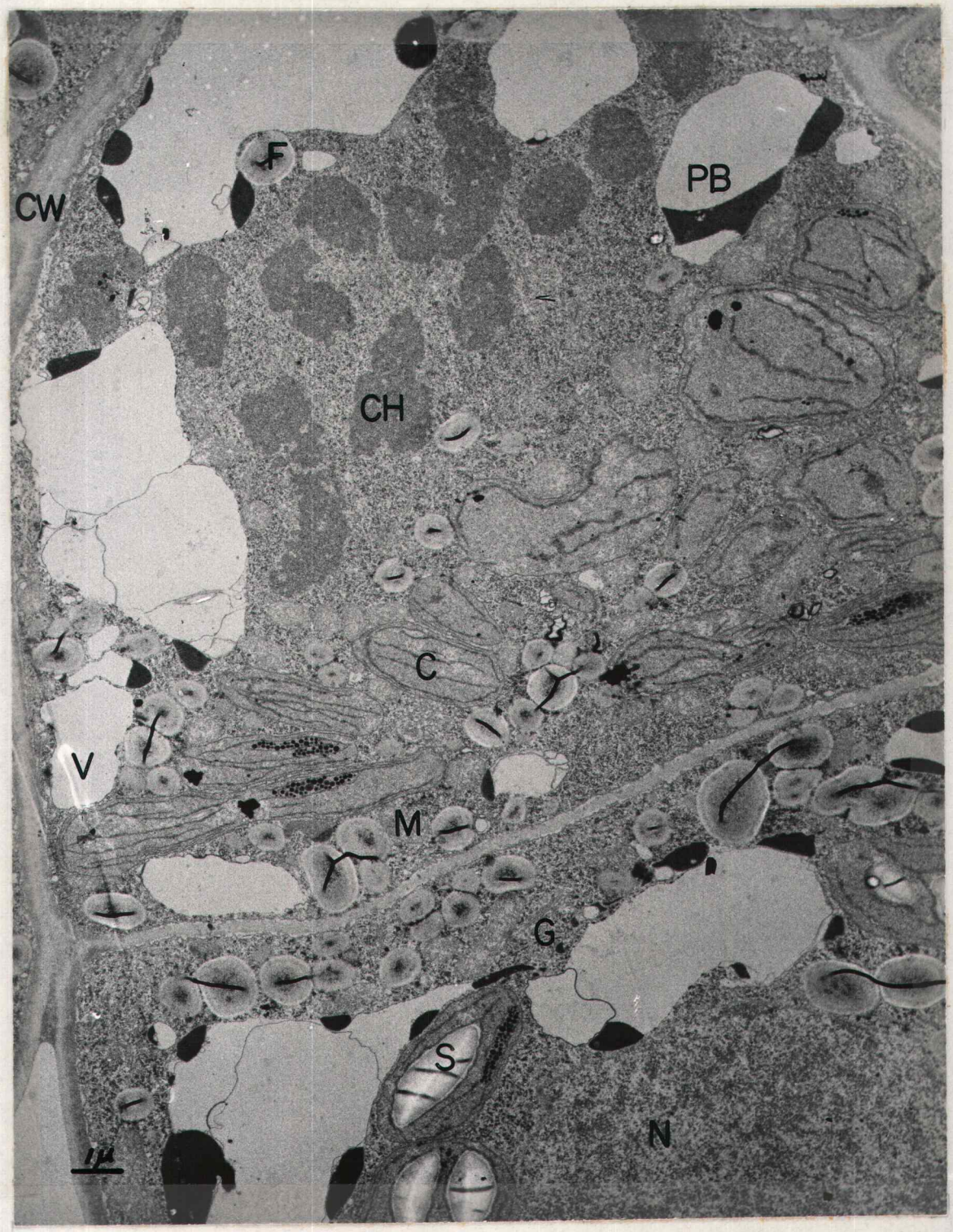
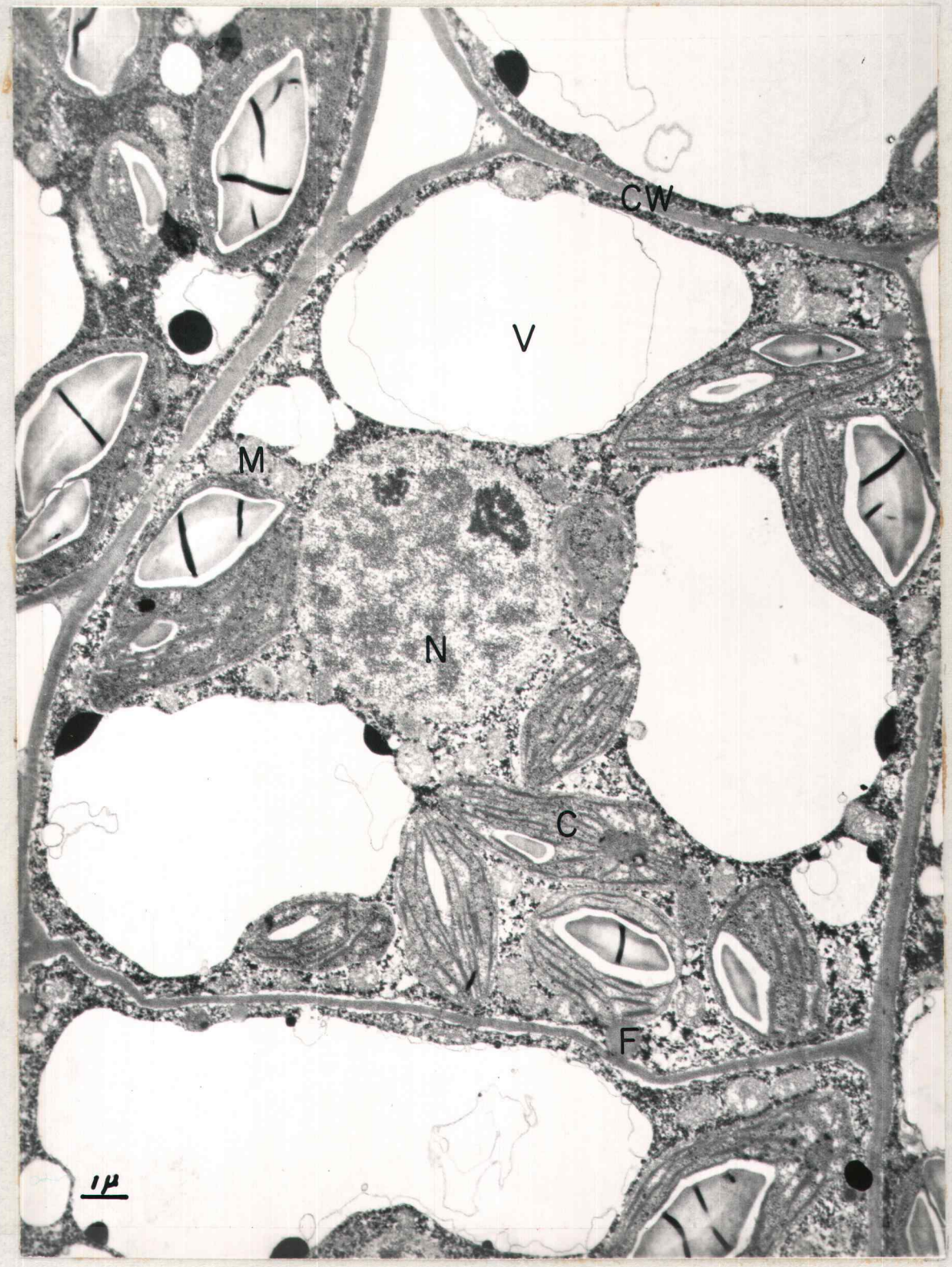

CW
F
PB
CH
C
V
M
G
S
N
1μ

Plate 5. A mature cell in cotyledon tissue of the germinated seedling at a stage later than plate 4. The cell was fixed with glutaraldehyde and osmium tetraoxide. Several big vacuoles are shown. Chloroplasts are well developed.


CW
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N
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1μ

Plate 6. A portion of a root cell in a soaked seed fixed with Dalton's fixative. Protein bodies and fat bodies both are smaller in size and lesser quantity than the cotyledonary cells. Other cellular organelles are visible.

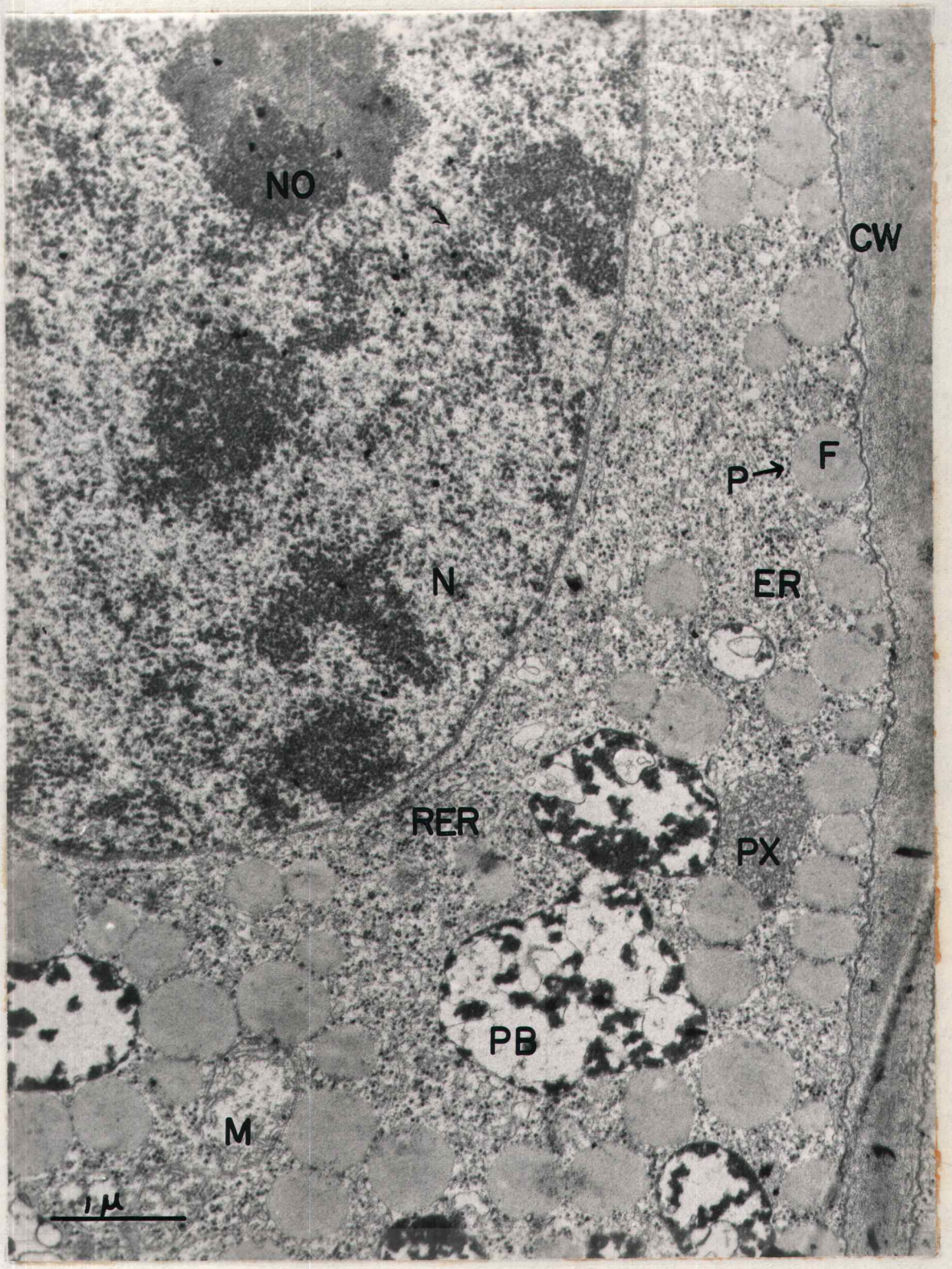
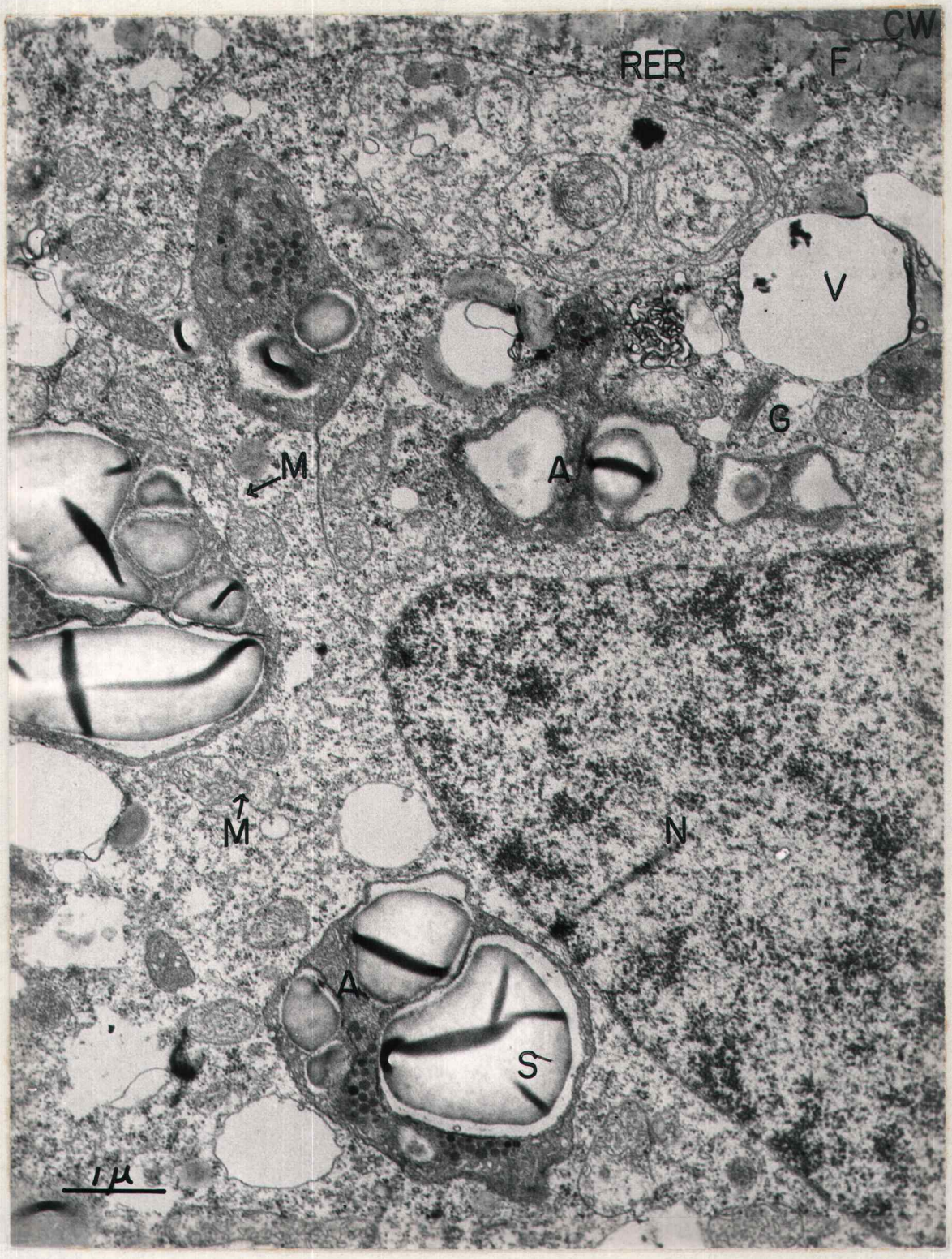

NO
CW
P
F
N
ER
RER
PX
PB
M
1 μ

Plate 7. A portion of a root cell of a stratified seed fixed with glutaraldehyde and Dalton's fixative. Protein bodies are degraded completely leaving vacuoles in the cell. Other cellular organelles become greater in number. Electron-opaque granules are visible in the amyloplasts. Rough surfaced endoplasmic reticulum, free ribosome and polysomes are seen. Lipid bodies appear to be granular.


CW
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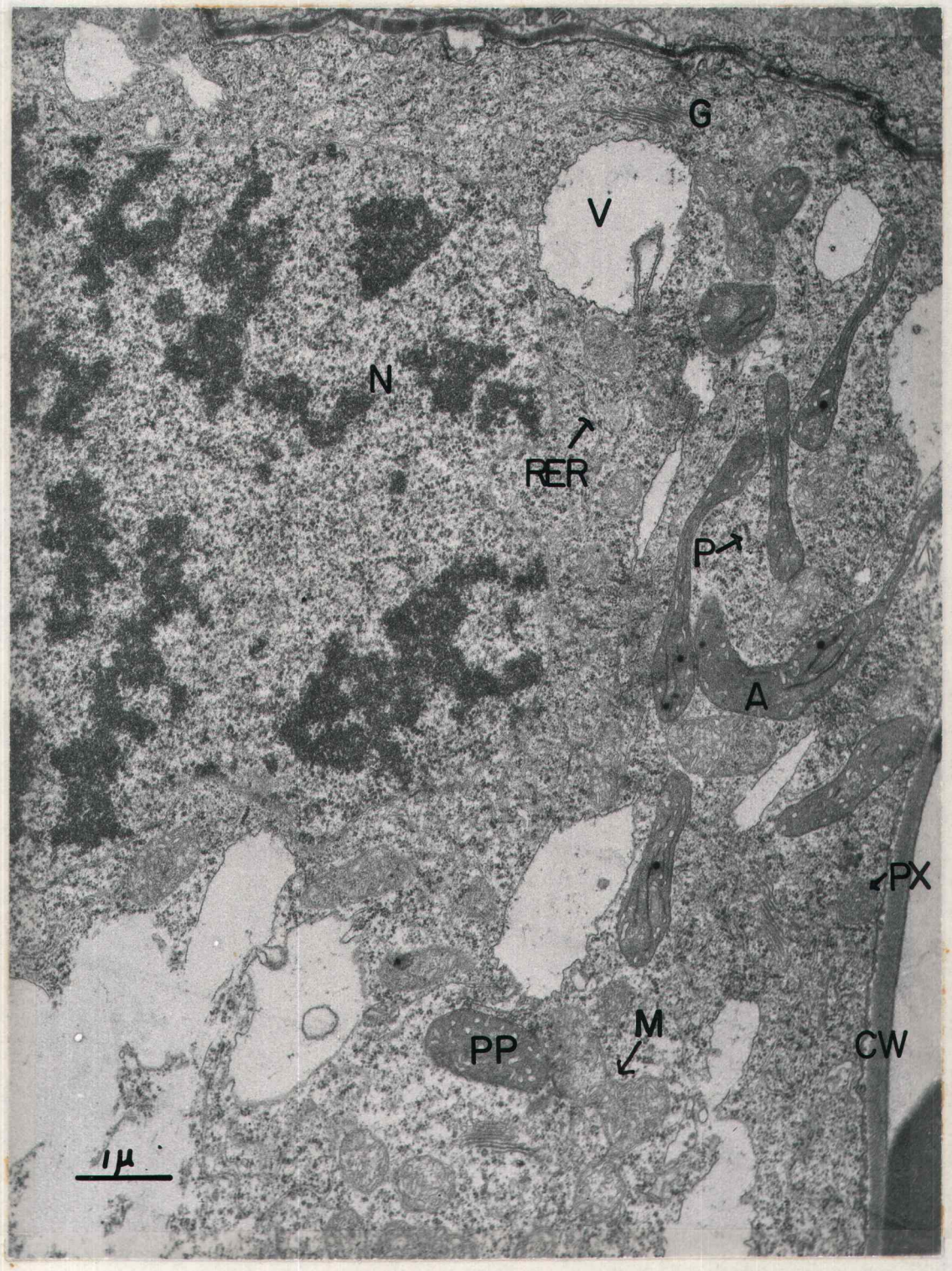
1μ

Plate 8. A portion of a root cell in a germinated seedling fixed with glutaraldehyde and Dalton's fixative. Mitochondria and dictysome are common. Rod shaped amyloplasts are found. Resting nucleus with nucleolus and chromatin material are shown.


G
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1μ

Plate 9. A portion of a lignified root cell in the late germinated stage fixed with glutaraldehyde and Dalton's fixative.

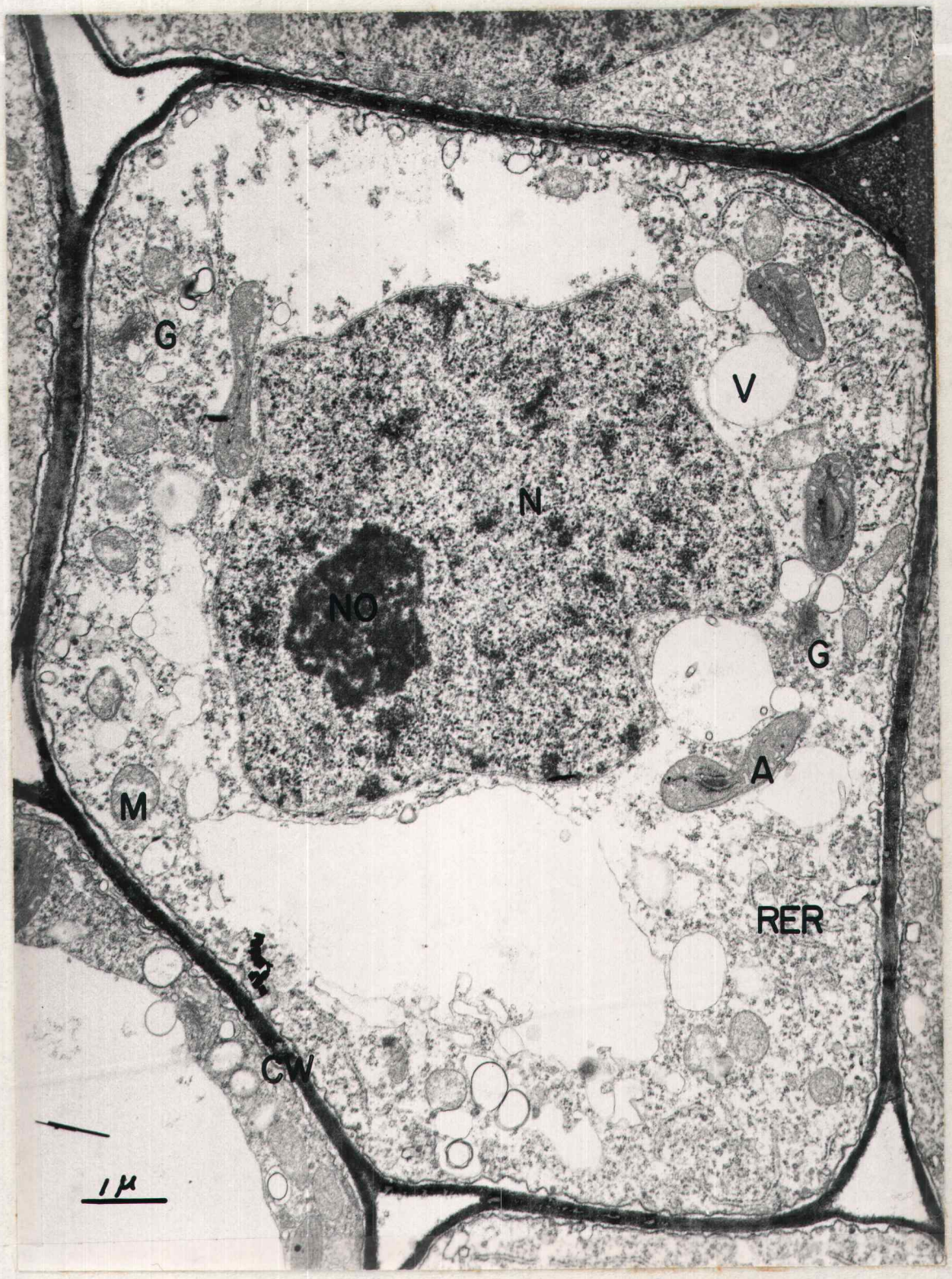
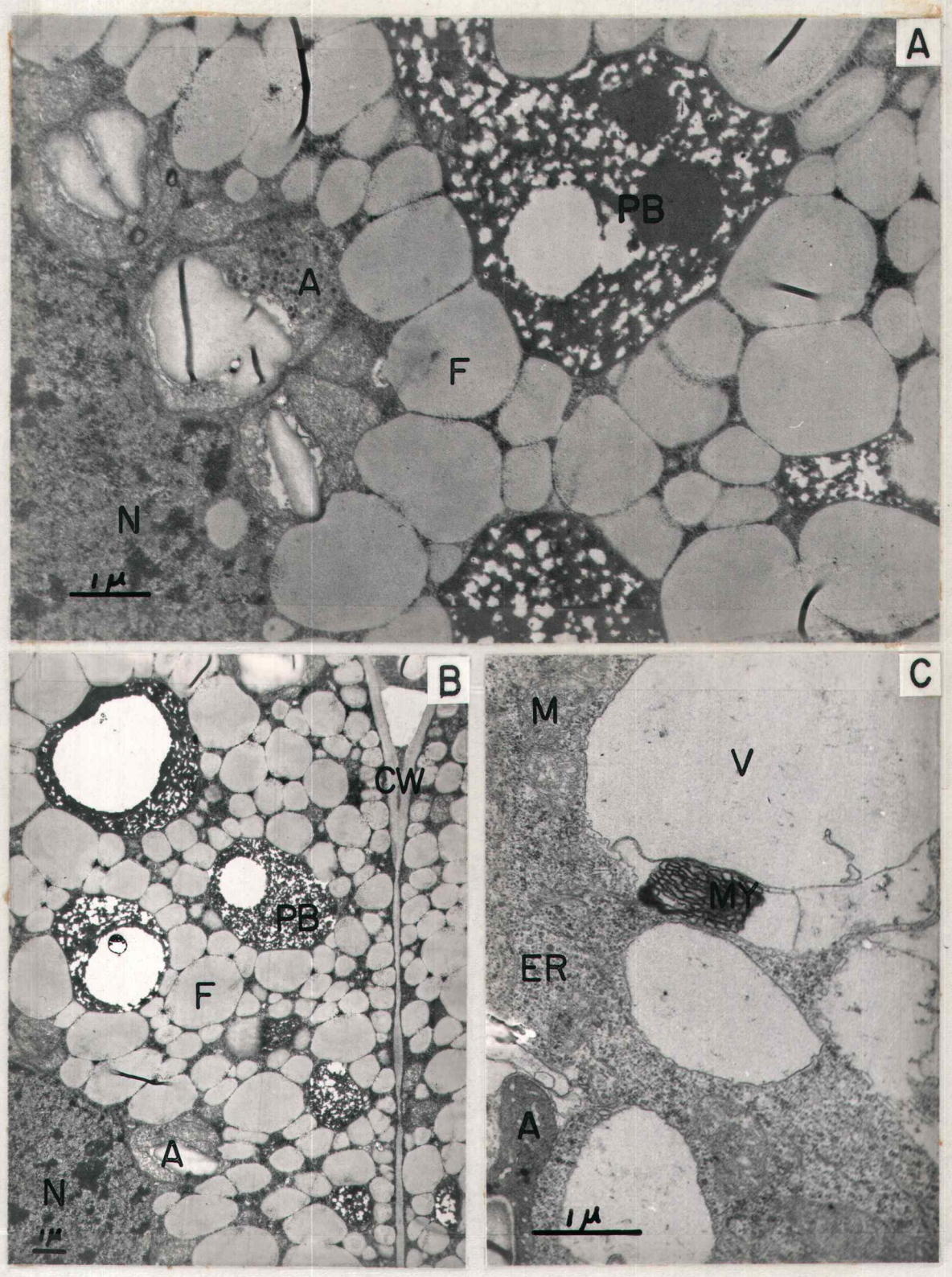

G
V
N
NO
G
A
M
RER
CW
1μ

Plate 10. (A)(B) A portion of a cotyledonary cell fixed with glutaraldehyde and osmium tetraoxide. Amyloplasts with starch granules concentrate closely to nucleus.

(C) A portion of root cell in a later stage of germination fixed with glutaraldehyde and Dalton's fixative. Myeline-like structure is found in vacuoles.


A
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N
1μ
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PB
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1μ
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Plate 11. A portion of a root cell in stratified seed fixed with glutaraldehyde and osmium tetraoside. Even as early as stage B all protein bodies are used up, fat bodies also are few and small. Other cellular organelles are defined.

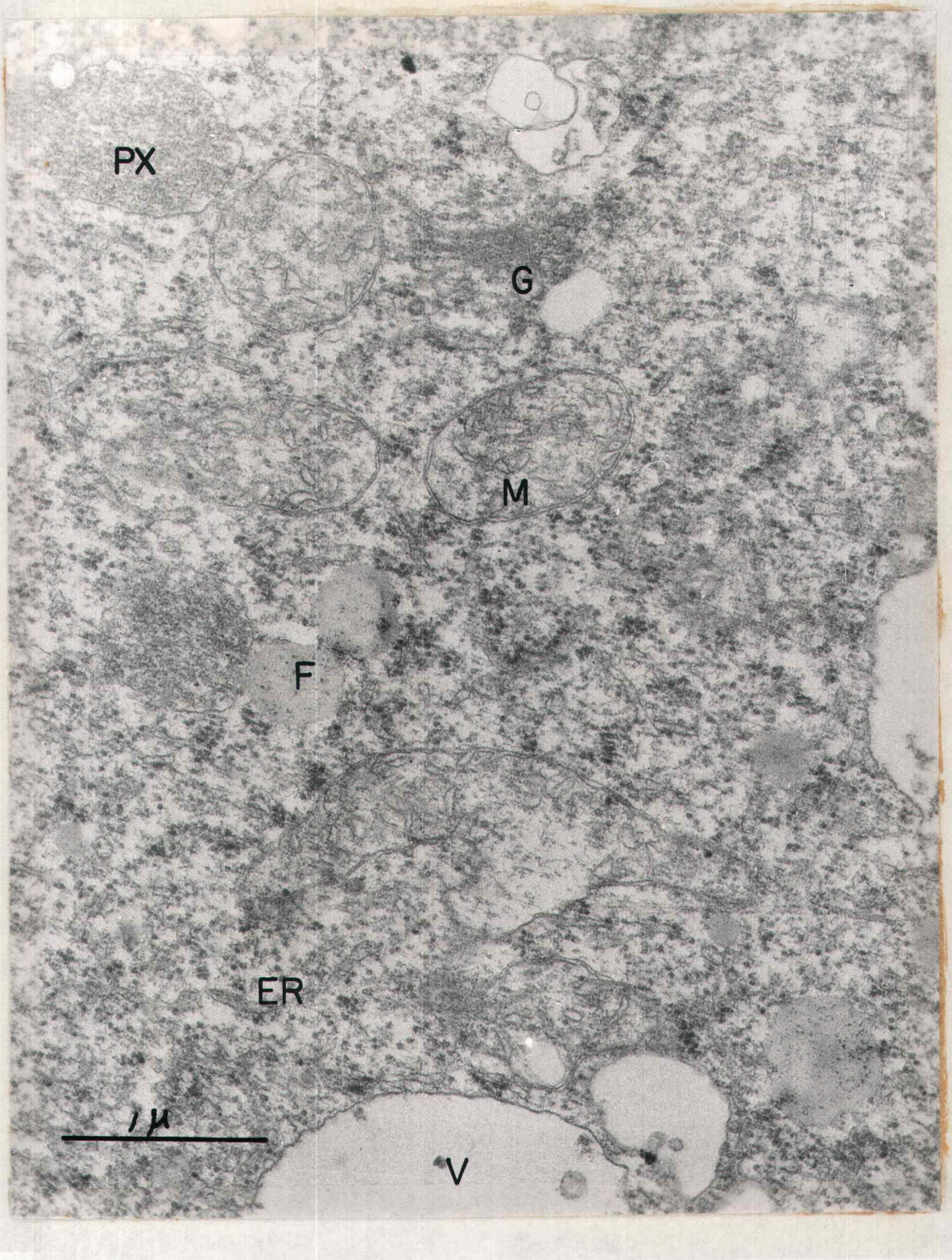
PX
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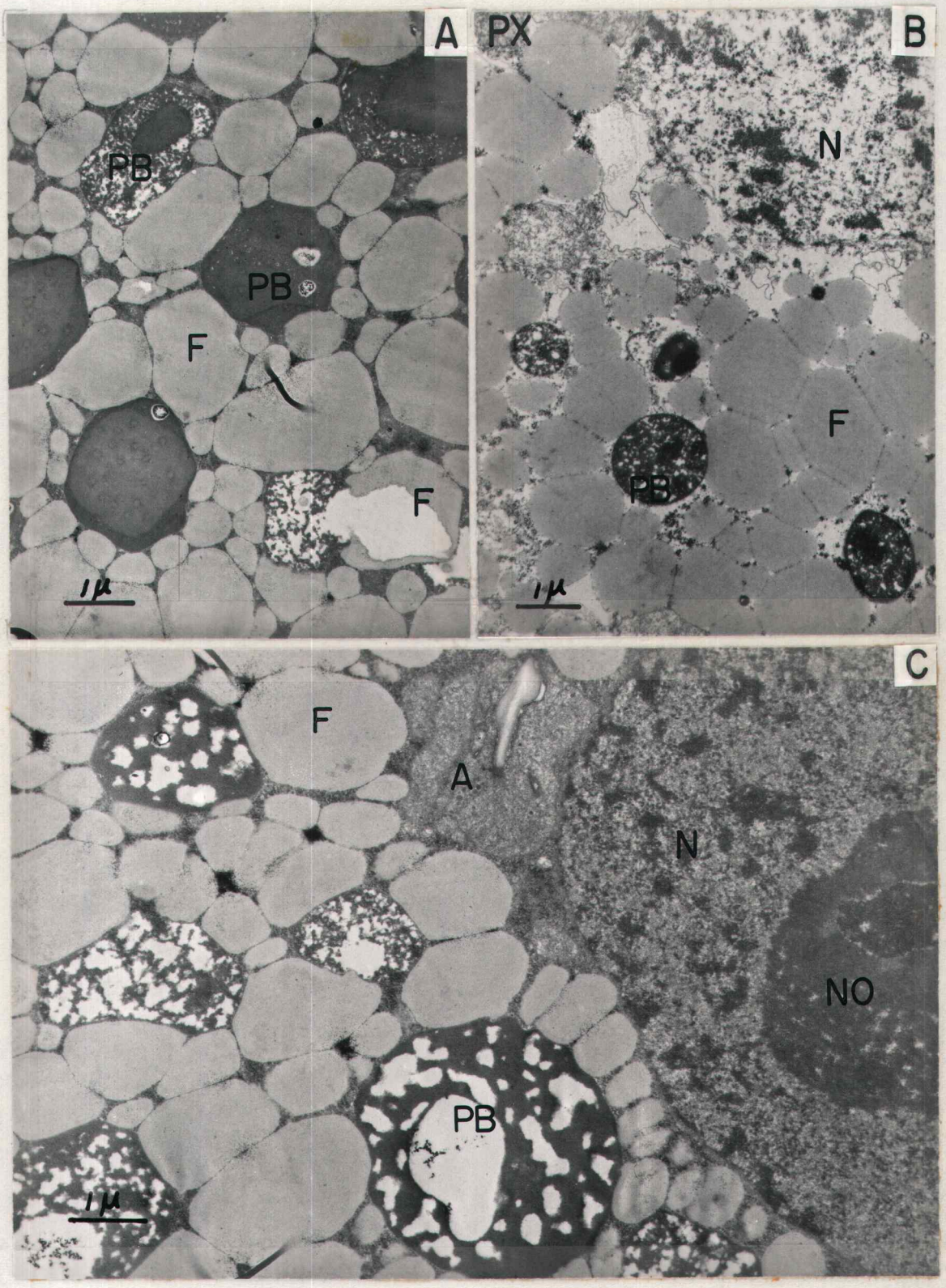
Plate 12. (A)(B)(C) Different degree of protein degradation in a cotyledonary tissue of a stratified seed. Solubilization of protein starts at many places.


A
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F
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F
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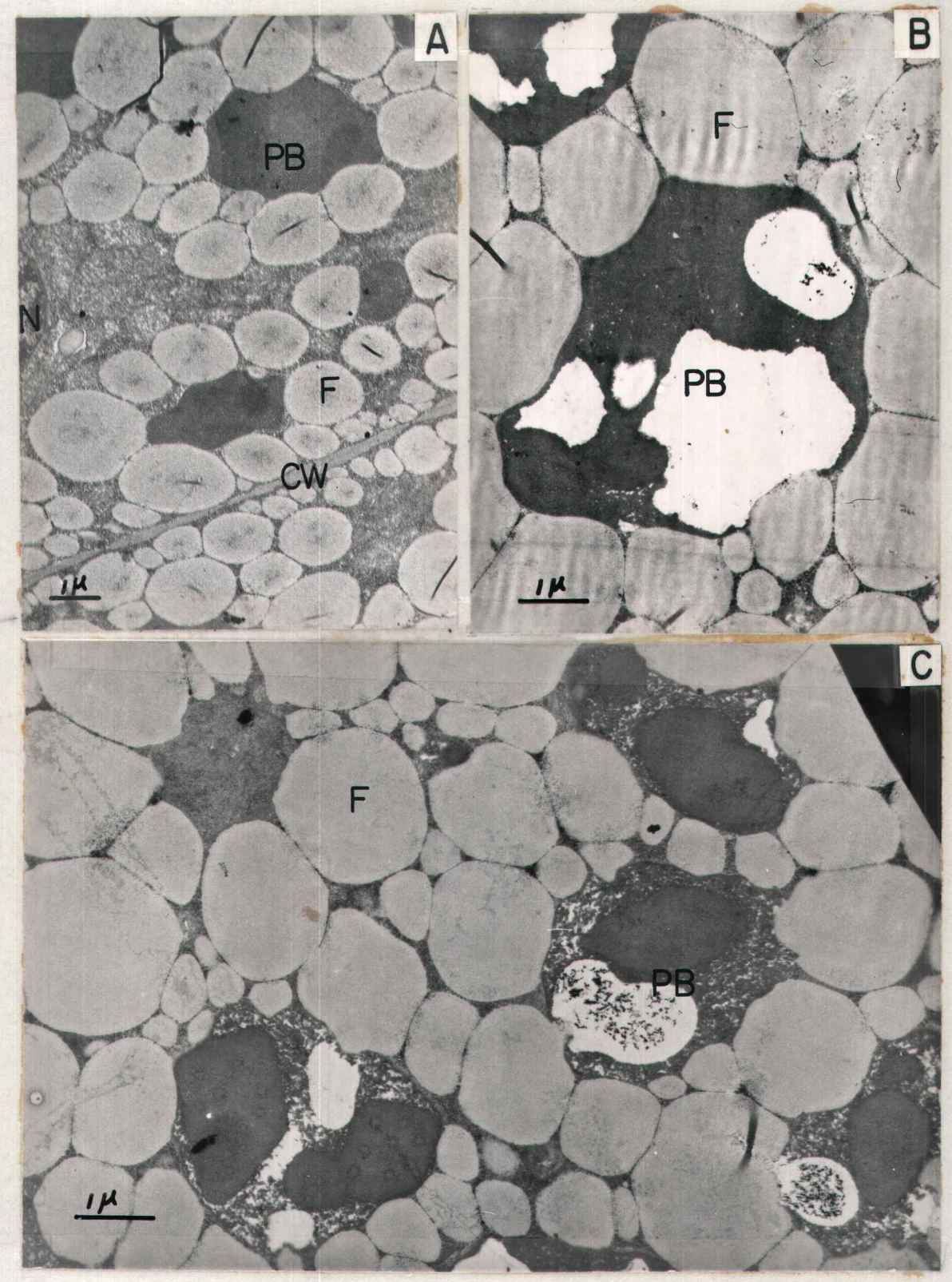
1μ
C
F
A
N
NO
PB
1μ

Plate 13. (A) Undegraded protein bodies in the cotyldeon of a stratified seed fixed with glutaraldehyde and Dalton's fixative.

(B) Protein bodies start to degrade at several places in a protein body in the cotyledonary tissue fixed with glutaraldehyde and Dalton's fixative.

(C) A portion of cotyledonary cell in a stratified seed fixed with glutaraldehyde and osmium. Different protein components seem to be deposited in these protein bodies.


A
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1μ
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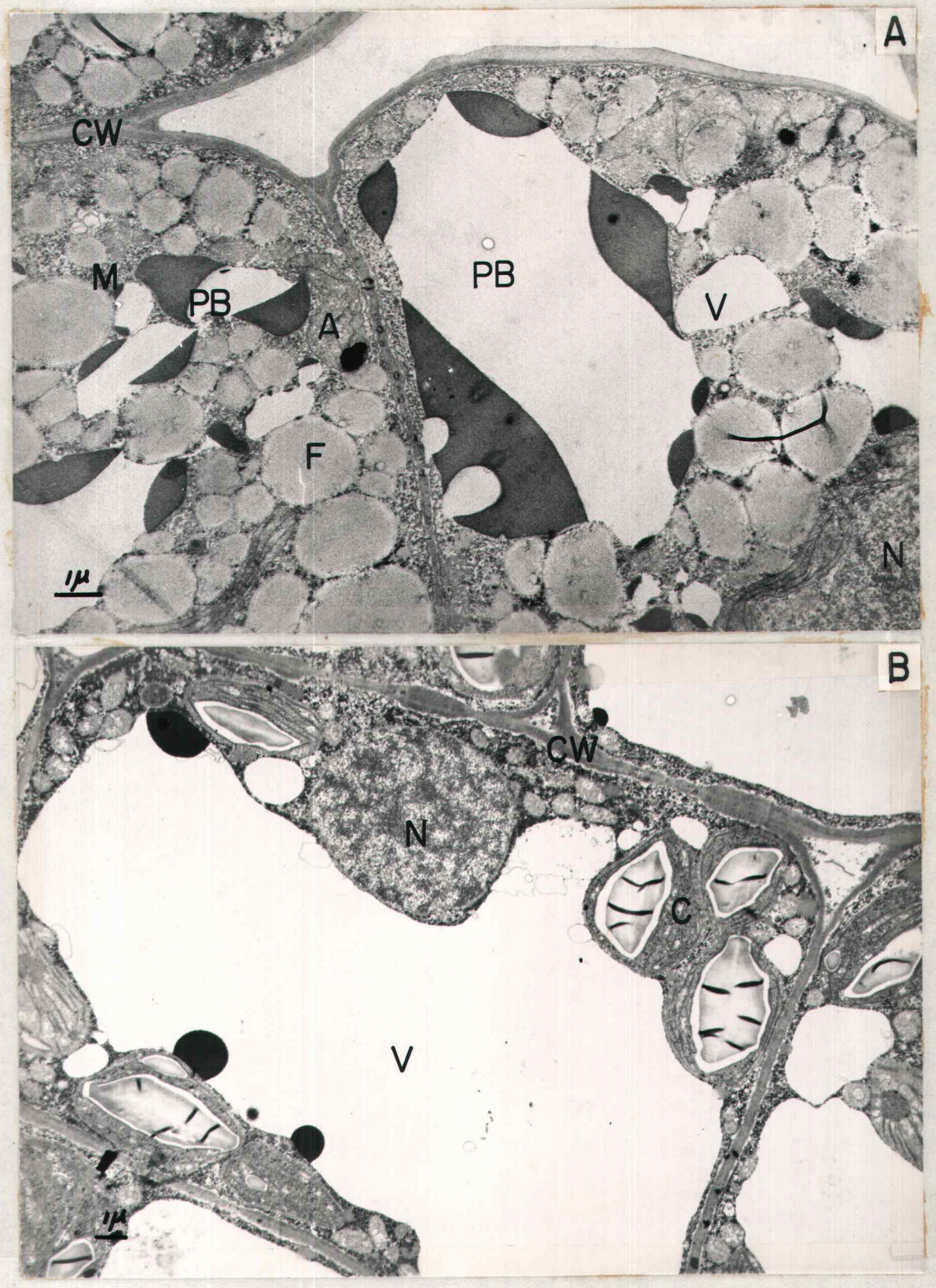
1μ
C
F
PB
1μ

Plate 14. (A) A portion of three cotyledon cells in a germinated seed fixed with glutaraldehyde and Dalton's fixative. Protein bodies are being degraded to form vacuoles.

(B) A mature cotyledonary cell in a germinated seedling fixed with glutaraldehyde and Dalton's fixative. No protein or fat bodies are observed. A large vacuole is formed indicating the fussion of individual small ones.


A
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A
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1μ
B
CW
N
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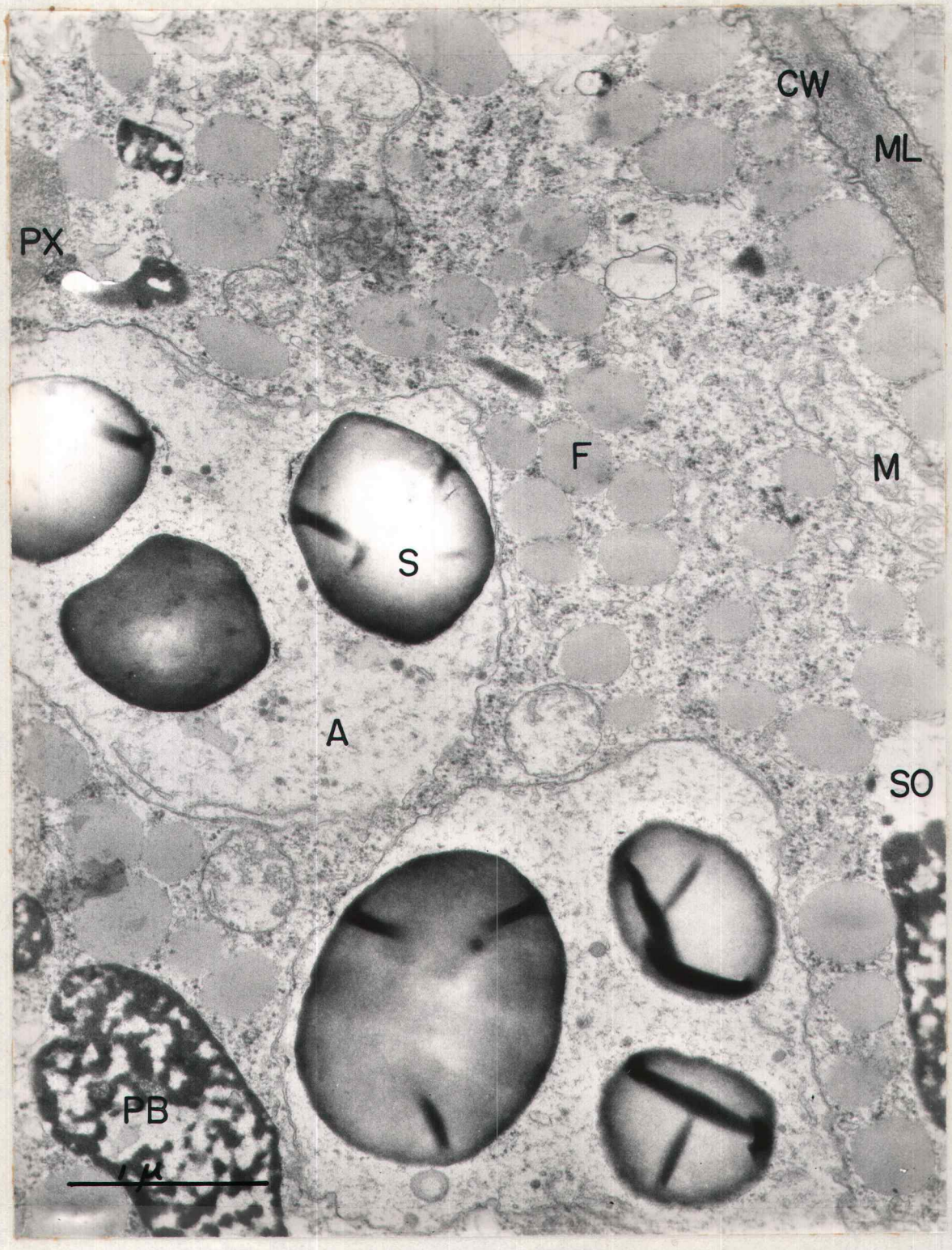
1μ

Plate 15. A portion of a root cell in a soaked seed fixed with glutaraldehyde and Dalton's fixative. Protein bodies start to convert to soluble form. The structure of amyloplasts and single membrane proplastic like structure are defined.


CW
ML
PX
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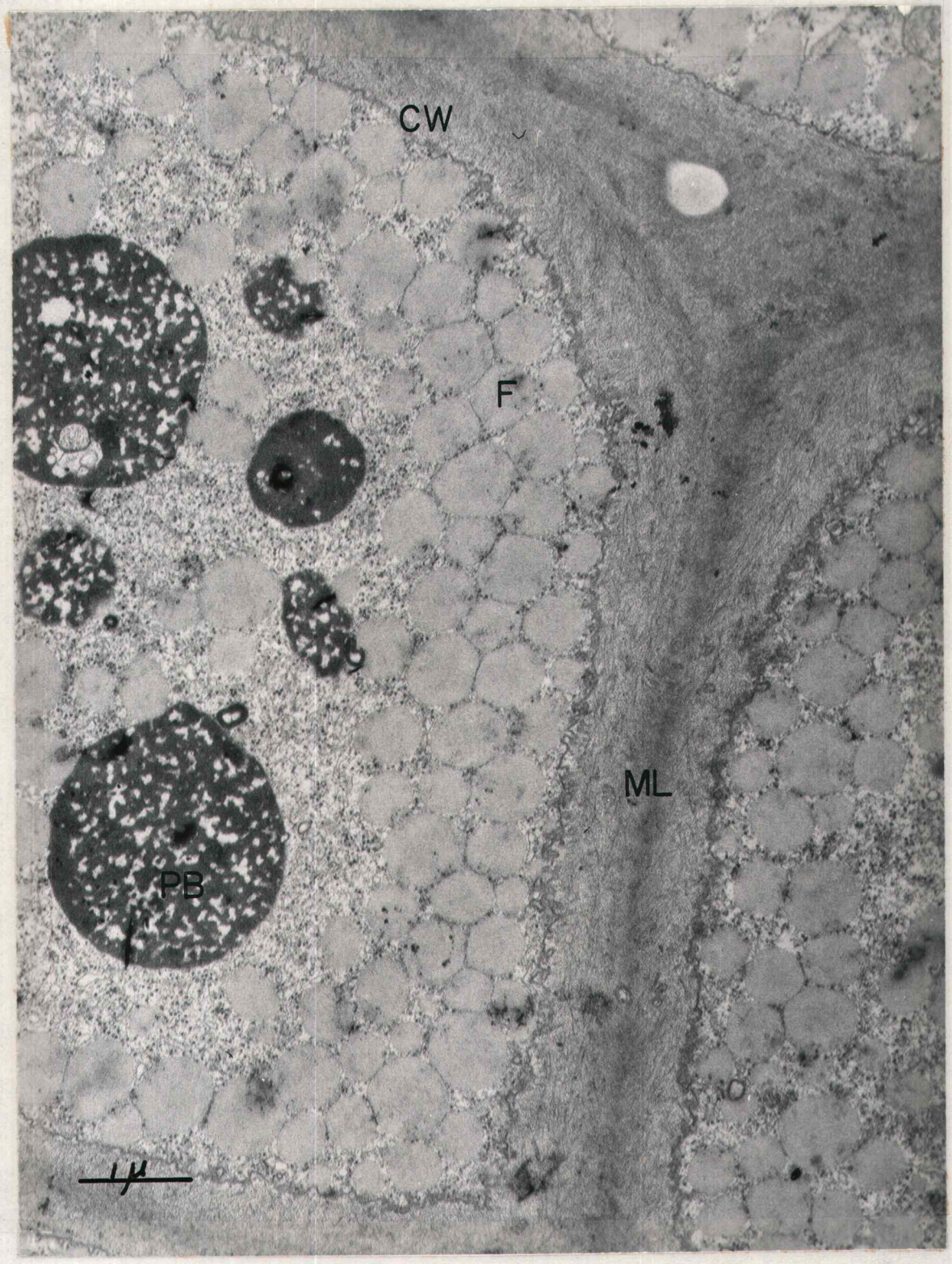
M
S
A
SO
PB
1μ

Plate 16. A portion of two root cells in a soaked seed fixed with glutaraldehyde and Dalton's fixative. Fibrous primary cell wall is conspicuous.


CW
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1μ

Plate 17. A portion of a cotyledon cell in a stratified seed fixed with potassium permanganate. Nuclear membrane with nuclear poles and other membrane systems are well defined.

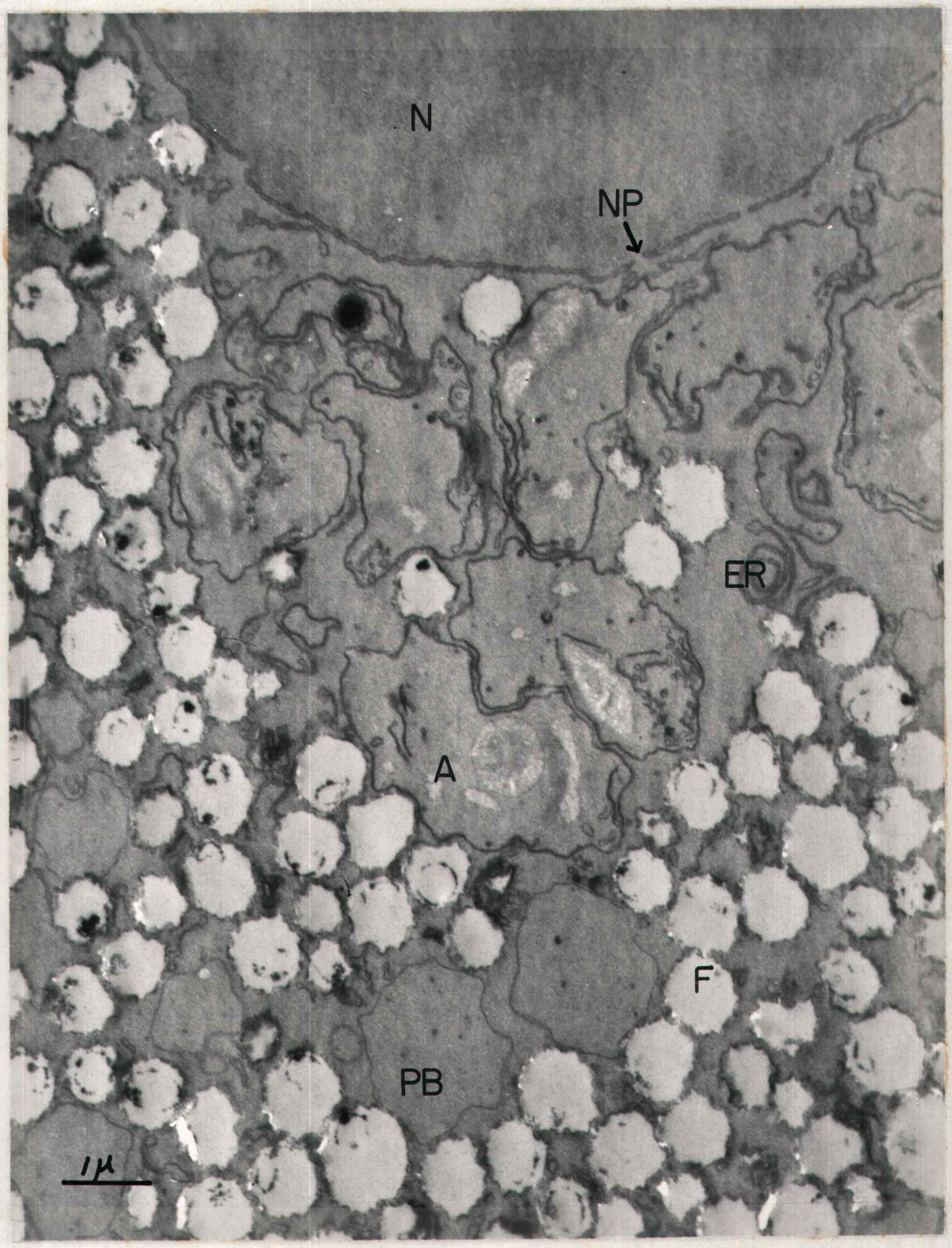
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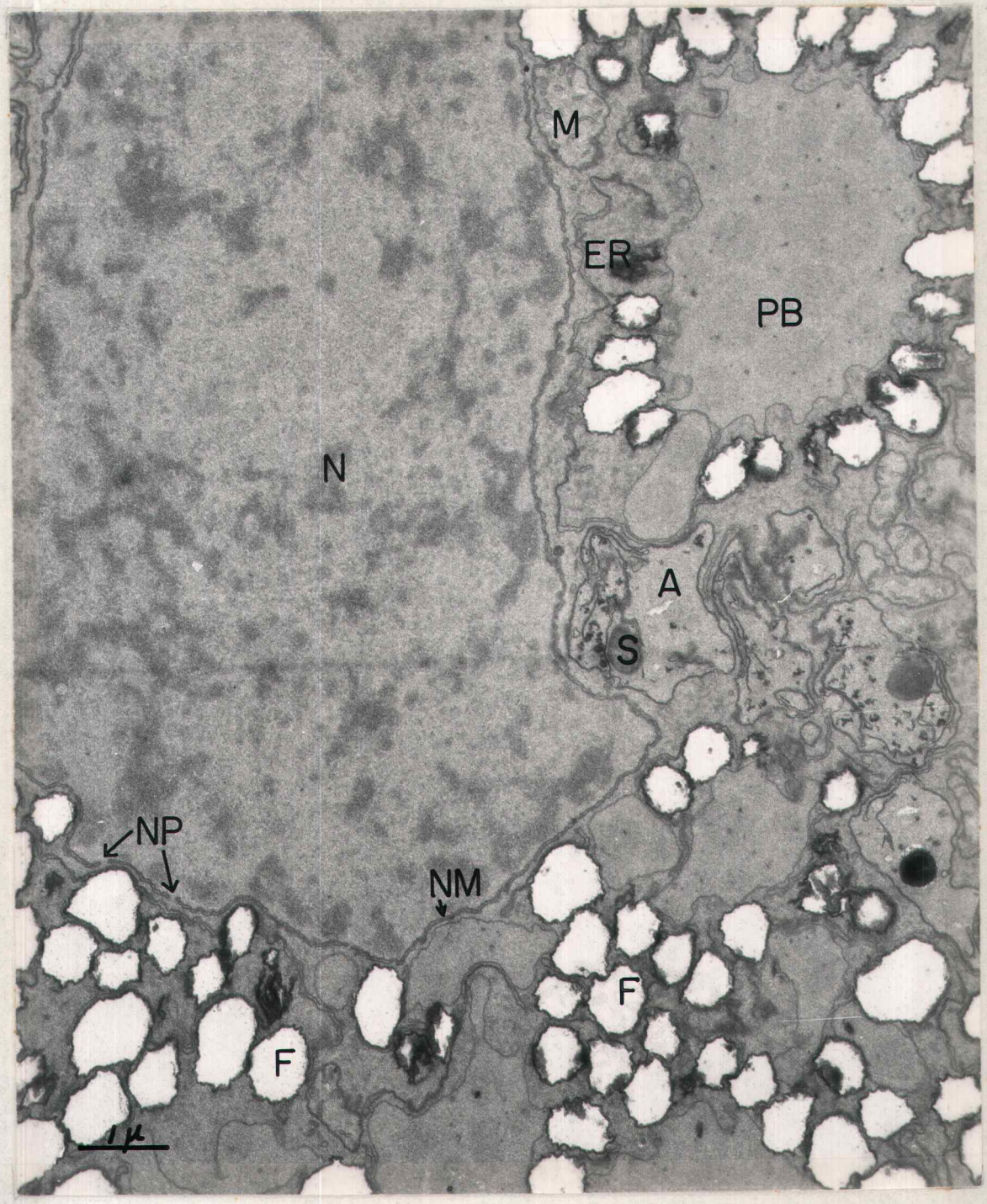
Plate 18. A portion of a root cell in a stratified seed fixed with potassium permanganate. Mitochondria and endoplasmic reticulum are more common in root material.


M
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1μ

Plate 19. (A)(B)(C) Development of chloroplasts from proplastids during germination. Lamella structure formed first and then starch granules are deposited.

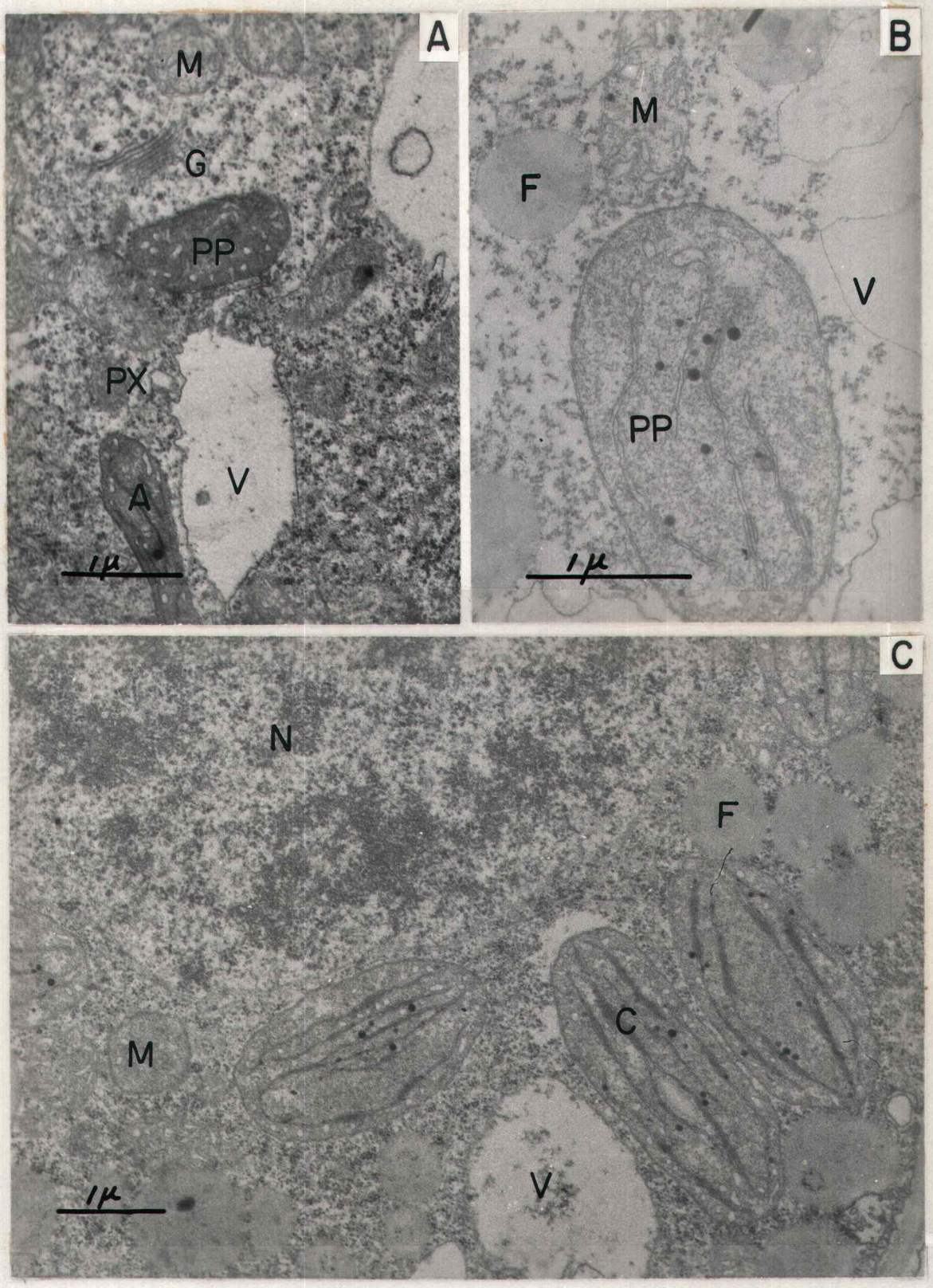
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Plate 20. (A) A portion of a cotyledonary cell at stage E showing different stages of chloroplast formation.

(B) A portion of a cotyledonary cell at stage F showing completely developed chloroplast.

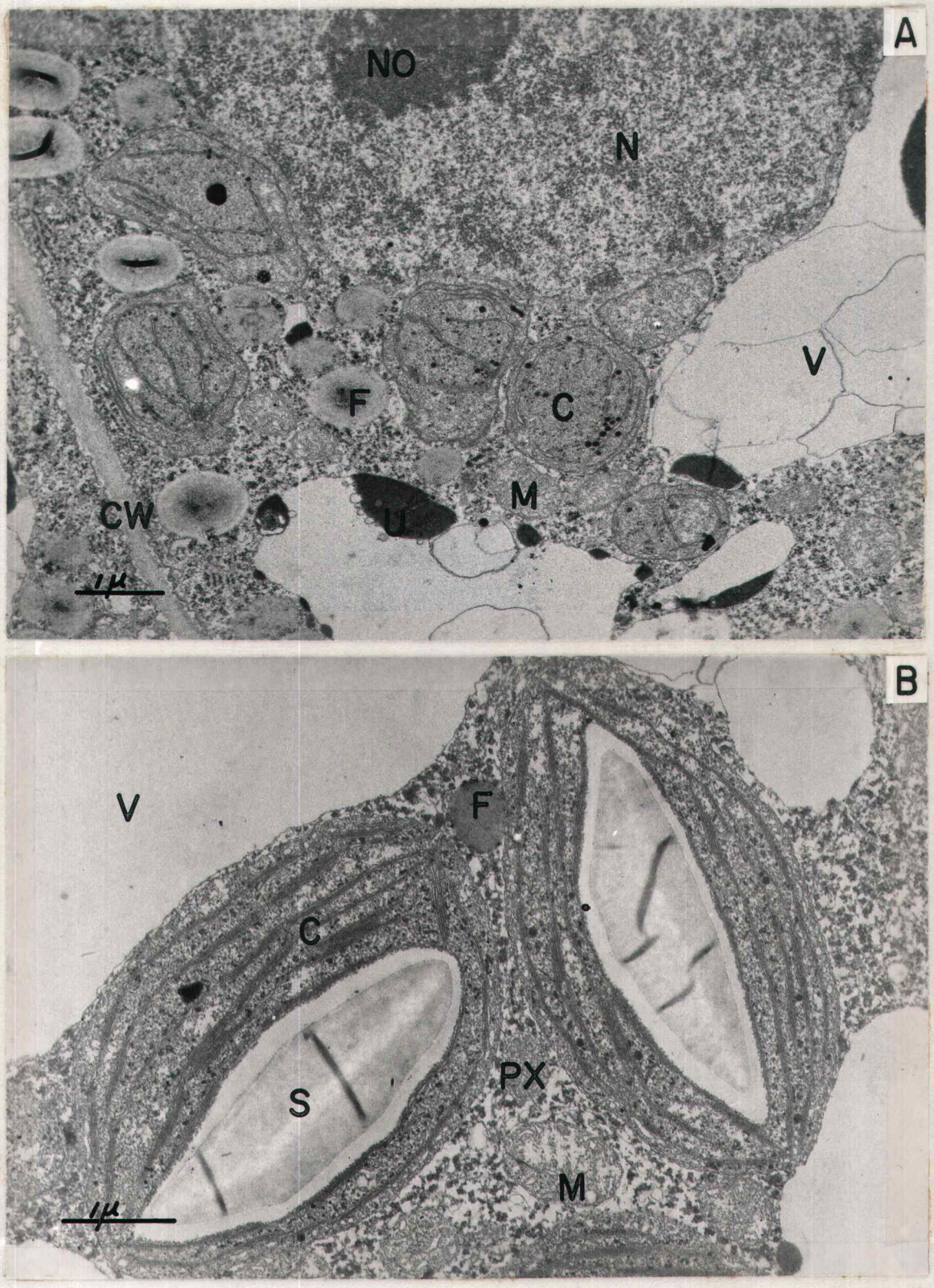
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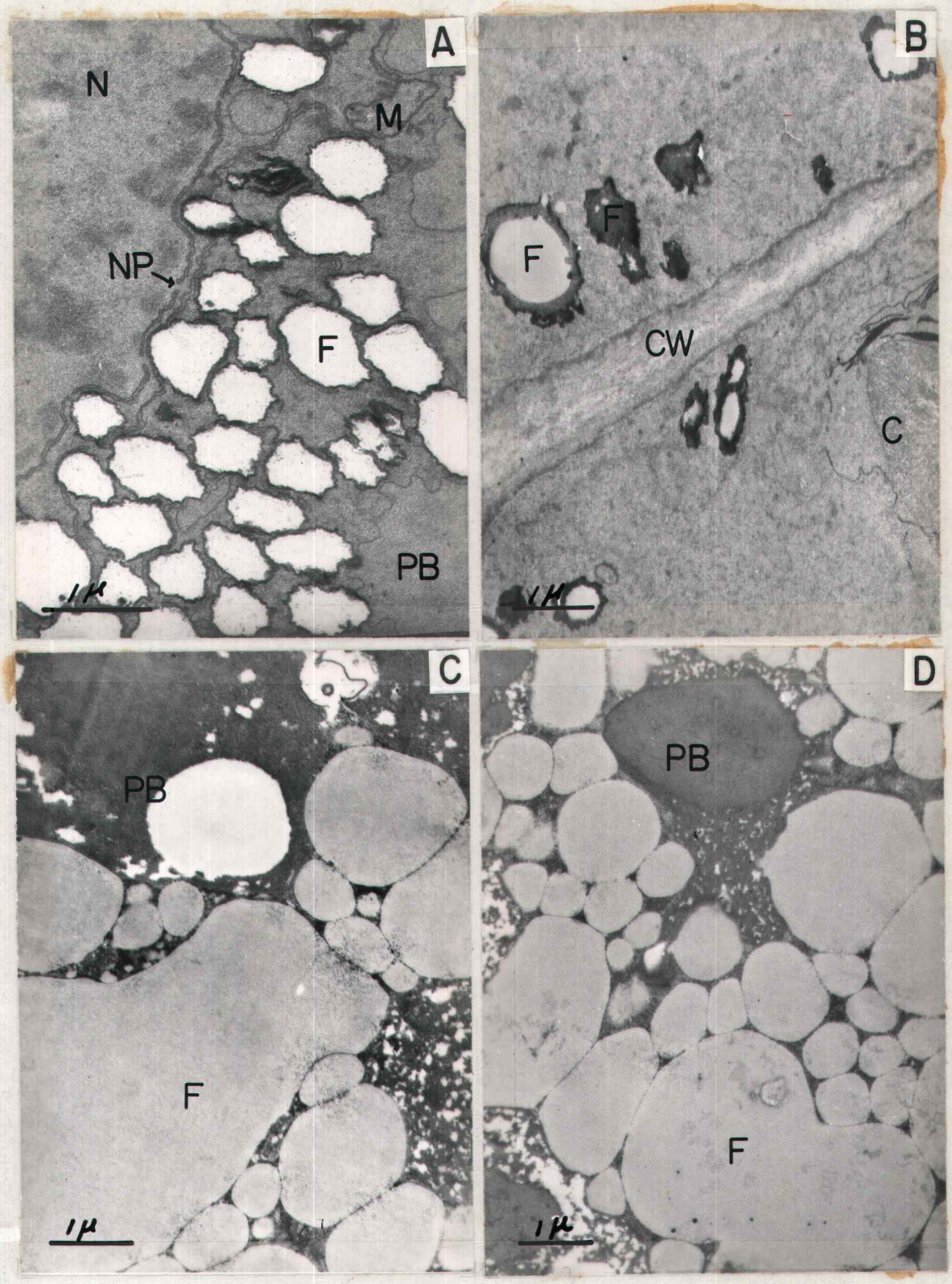
Plate 21. (A) A portion of a root cell fixed with potassium permanganate showing irregular shaped fat bodies.

(B) A portion of a root cell fixed with potassium permanganate. Thick wall is formed surrounding the fat bodies during degradation.

(C)(D) A portion of a cotyledon cell in dry seed fixed with glutaraldehyde and osmium tetraoxide. Fat bodies coalesce together.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PB
1μ
B
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F
CW
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1μ
C
PB
F
1μ
D
PB
F
1μ

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