ULTRASTRUCTURE OF THE CELLS IN ROOT APICAL MERISTEM OF *PHASEOLUS* ASSOCIATED WITH GERMINATION

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Abstract: The cellular chnages in EM level occurring in the promeristem of root tip of *Phaseolus radiatus* Linn. associated with the germination were observed. The obvious changes were the rearrangement and reorganization of cytoplasmic organelles, and the increase in clarity of membrane system in this stage.

The following important changes were noted as germination time was prolonged: migration of lipid bodies from the periphery of cell to throughout much of cytoplasm, and a decrease in their number; starch-containing plastids became scarce; the dispersing of the Golgi vesicles from the edge of the dictyosome throughout the cytoplasm; the increase of the irregular or reticulated dictyosome. The possible role of some organelles such as: lipid bodies and plastids in the early stage of germination is also discussed.

INTRODUCTION

Studies at the cellular level of meristematic tissues have usually been centered on changes occurring during maturation and differentiation. Whaley, Mollenhauer and Leech (1960) described the general structure of the meristematic cells including the root cap region. Avers (1962), Hyde (1967), Mesquita (1969), Mollenhauer, Whaley and Leech (1961), Webster and Hof (1973) and Whaley, Kephart and Mollenhauer (1959) have reported on their studies of the developmental changes of various organelles in the root meristem. Esau (1963) mentioned the structural changes of the cells associated with the differentiation. There is no doubt but that the proinitial cells maintain their meristematic nature during root growth, and the number of cells and gross morphology alters as the root grows (Chiang and Tsou, 1974; Hayat, 1963; Popham, 1955; Seago, 1971).

Most workers have thought that few cellular changes occur in the initial cells of the root meristem, and so this region has received less attention than neighboring regions. Yoo's observation (1970) shows that cellular organelles such as: plastids, mitochondria and protein bodies in the root apical meristem of *Pisum sativum* change as the root begins to grow following germination.

The modifications occurring in anatomical zonation in the meristem during the early growth of the root in *Phaseolus radiatus* has been previously reported (Chiang and Tsou, 1974). The first indication of this dynamic change occurring in the root is about 8 hr after germination, and continuous changes take place until apical zonation was clearly defined about 24 hr after germination. This report deals with the changes in the central initial group of cells at the cellular level during this critical period as well as slightly before and after this period of growth, as observed with an electron microscope.

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MATERIALS AND METHODS

Seeds and seedlings of *Phaseolus radiatus* Linn. were used. The source of the materials as well as the methods of soaking and sampling were mentioned in the previous paper (Chiang and Tsou, 1974).

Excised root tips were preliminarily fixed in 3% glutaraldehyde. The materials were then divided into two groups. One was put in 2% $KMnO_4$ (Pease, 1964) and the other in Dalton's OsO_4 (Dalton, 1955) for post fixation, dehydrated in ethanol, and embedded in Epon. Thin sections were made with glass knives, and stained with uranylacetate—lead citrated combination (Reynolds, 1963). Electron micrographs were taken with a Hitachi-11 EM in the Veterans General Hospital, Taipei. We wish to thank all of the staff who work in the EM chamber at the Veterans Hospital, for their helpful suggestions during the preparation of the EM sections.

RESULTS

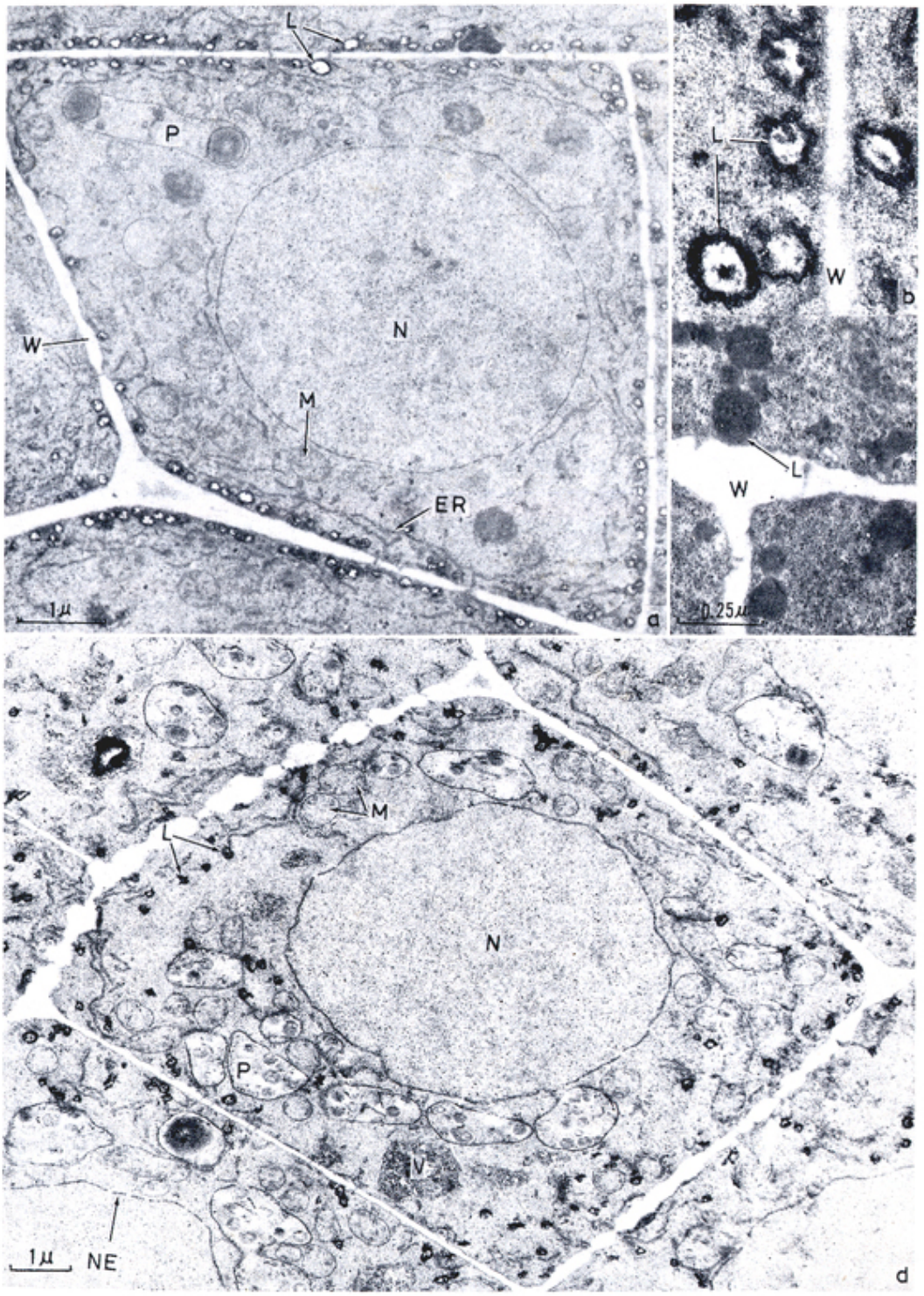
A. Central Initial Group.

Various stages of root growth including the resting radicle, 4 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr roots were prepared for EM sectioning. The authors were not able to obtain a satisfactory micrograph of the first stage i.e., the resting radicle without soaking. The sections obtained from the dry embryo lacked membrane clarity, the intracellular organelles were invariably compressed and damaged during the sectioning. Very little cell contents were preserved. The first stage in the present observation was the material 4 hr after soaking. The obvious changes occurring in the cells during the above stages at the EM level was the rearrangement and reorientation of the organelles, and the structure of some of the organelles. In general, the increase in the clarity of the membrane system and the cytoplasmic organelles was characteristic of the effect of germination.

Endoplasmic reticulum: Only rough endoplasmic reticulum was found in OsO_4 fixed material. In 4 hr roots, most of the endoplasmic reticulum was distributed in the periphery of the cell along the cell wall (Fig. 1a). Some was arranged close to the nuclear envelope, but not in the region away from both the nuclear envelope and cell wall. So that the cytoplasm in the region (termed the middle region of the cytoplasm in this report) appeared as a rather clear area with very little endoplasmic reticulum. The linear axis of the endoplasmic reticulum as shown in the section was always parallel with the nuclear envelope as well as the cell wall. Some of them formed direct continuity with the nuclear envelope, and others formed a ER-plasmodesma-ER connection with its cell wall and neighbouring cell (Fig. 3a). Endoplasmic reticulum gradually became more dispersed to the middle region of cytoplasm during root growth. Endoplasmic reticulum was seen evenly distributed throughout most of the cytoplasm in the roots of 24 hr or more hours after

Fig. 1a. Micrograph showing the distribution of the various cell organelles in 4 hr root ($KMnO_4$).
Fig. 1b. Enlarged view of lipid bodies fixed with $KMnO_4$ (8 hr root).
Fig. 1c. Enlarged view of lipid bodies fixed with OsO_4 (8 hr root).
Fig. 1d. Section from 12 hr root, note the migration of cell organelles, especially the lipid bodies ($KMnO_4$).

Key to labeling for electron micrographs: D—dictyosome; ER—endoplasmic reticulum; GV—Golgi vesicle; L—lipid body; M—mitochondrion; N—nucleus; NE—nuclear envelope; P—plastid; V—vacuole.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germination (Figs. 2, 3a). The parallel pattern of the endoplasmic reticulum orientation also disappeared. Some endoplasmic reticulum traversed the middle region of the cytoplasm from the outer surface of the nuclear envelope to the cell wall (Fig. 2b). Almost all of the endoplasmic reticulum in the cells of the 4 hr root were unbranched. A few branched endoplasmic reticula were seen in later stages of germination (Fig. 3b).

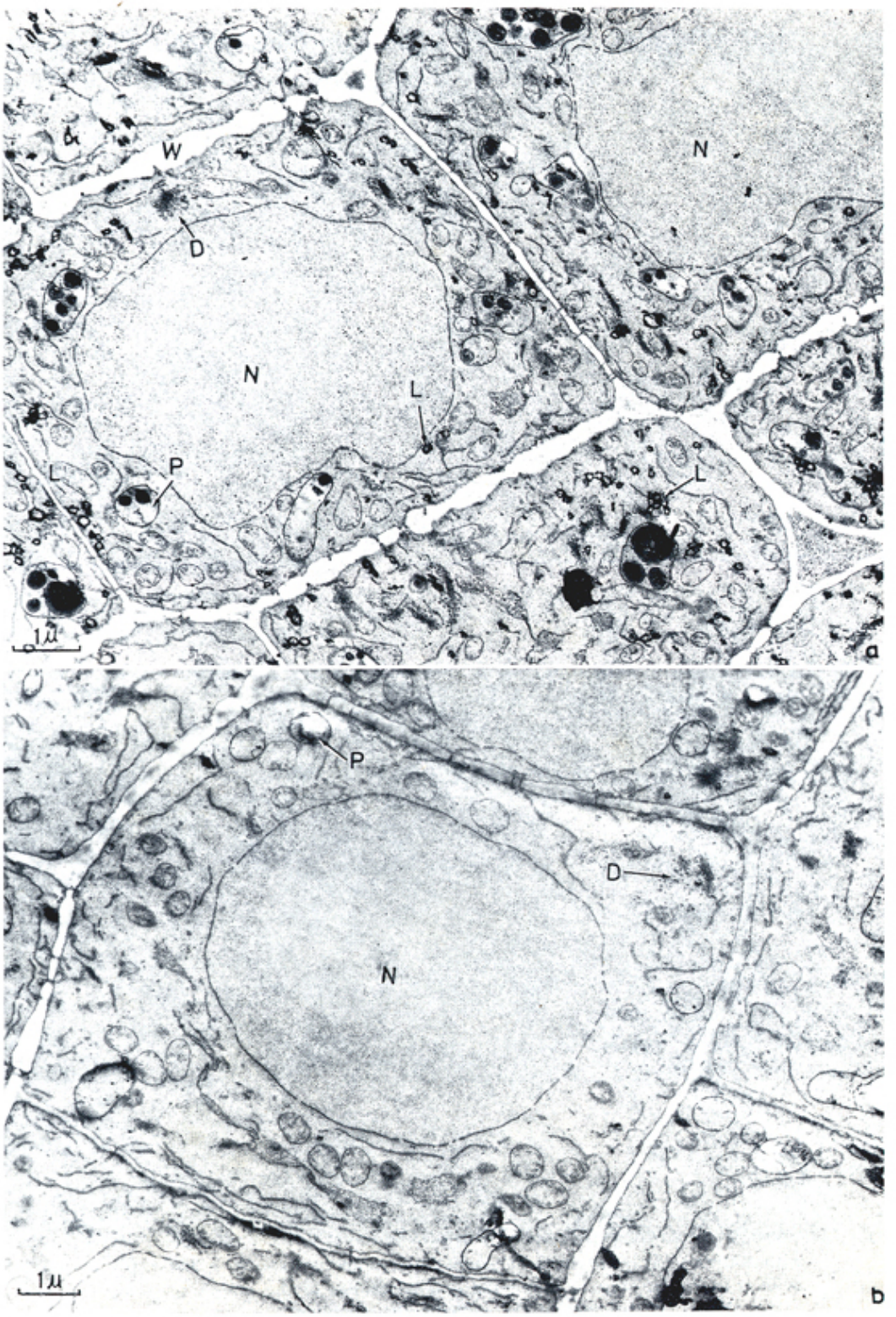
Lipid body: The cell organelles designated as lipid bodies or spherosomes fixed in OsO_4 showed great variation in appearance from those fixed in $KMnO_4$. They were circular and bounded by a smooth single membrane but not distinct (Figs. 1b, 1c). The lipid bodies were spherical and smooth in appearance, possessing a uniformly stained matrix in OsO_4 fixed material. But in $KMnO_4$ fixed members, they showed a more elaborated structure. They were surrounded by a thicker membrane which was not smooth on its outer surface and possessed two to several projections into both the matrix and cytoplasm internally and externally respectively. The matrix was rather uniform in electron transmission as seen in OsO_4 fixed cells. In addition to the internal projections of the bounding membrane, one to several densely stained structures were present in the central matrix in the $KMnO_4$ fixed cells (Figs. 1b, 3b). Though the detail structure of this organelle in OsO_4 fixed cells differed from that in $KMnO_4$ fixed ones, the distributional change in the cytoplasm during the developmental stage was identical in both materials fixed in different post fixatives.

The distributional change in each collected material showed a very peculiar pattern. The lipid bodies in 4 hr and 8 hr roots were numerous and were confined to the regions of cell periphery i. e., the cell surface. They more or less corresponded with the occurrence of the endoplasmic reticulum in this period of germination (Fig. 1a). When the germination time was prolonged these lipid bodies migrated inward through the cytoplasm (Fig. 1b). In 24 hr root, they were distributed throughout much of the cytoplasm. In 48 hr root very few lipid bodies were seen. Finally in the 72 hr material no lipid bodies were visible (Figs. 2b, 3a). The disintegrating lipid bodies were commonly seen in 24 hr roots (fig. 3b). They generally seemed to retain their individual structure during the process of germination.

Dictyosome: The distribution of dictyosomes does not show a regular migration pattern as that in ER. With a very few exceptions, dictyosomes are evenly distributed throughout cytoplasm in all the stages examined (Figs. 1, 2, 3a). Dictyosomes were more numerous in the 24 hr root than in younger roots, and reached their maximum number in the 48 hr root.

The structural variation of dictyosomes is seen associated with root growth. The number of Golgi cisternae in 4 hr, 8 hr and 12 hr roots ranged from three to five forming a stack associated with a few small vesicles which appear isodiametric as seen in section (Fig. 1d). The size of the sectional view of the cisternae and the number of dictyosomes increased as root growth proceeded. The number of the flattened cisternae in a stack ranged from 5 to 7, and the maximum length of the sectional view of a cisterna measures 0.55 μ whereas that in 4 hr root was about 0.45 μ. The distended edges of Golgi cisternae were not seen until the roots were 24 hr old (Fig. 5a). The width of Golgi cisterna was more or less uniform along the whole sectional surface in the earlier stages of the roots. The most interesting phenomenon in structural change of the dictyosome was the behavior of the Golgi

Fig. 2. Sections from the 24 hr (a) and 48 hr (b) roots, note the disappearance of lipid bodies as well as the starch granules within the plastids. Key to labeling see Fig. 1.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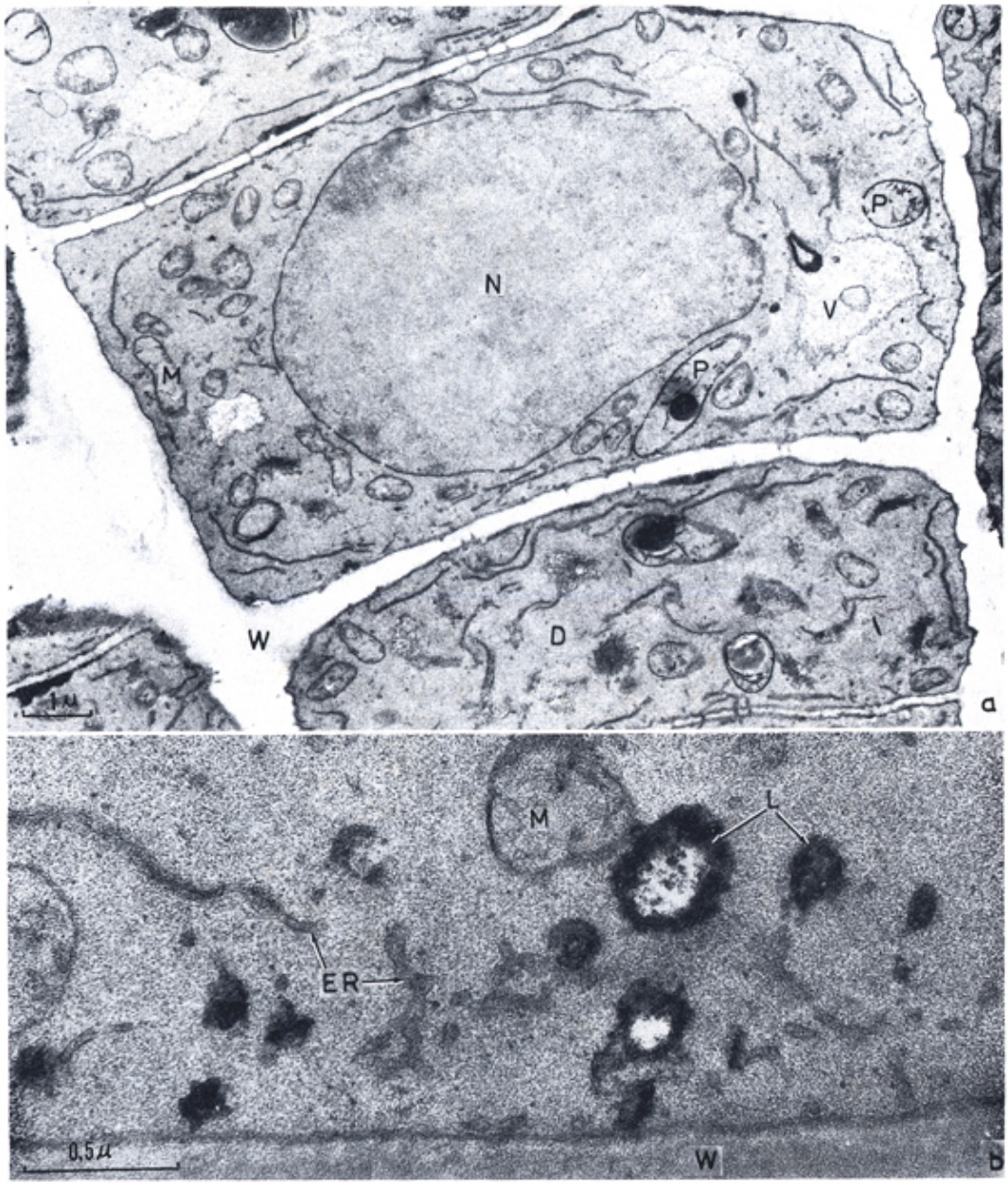
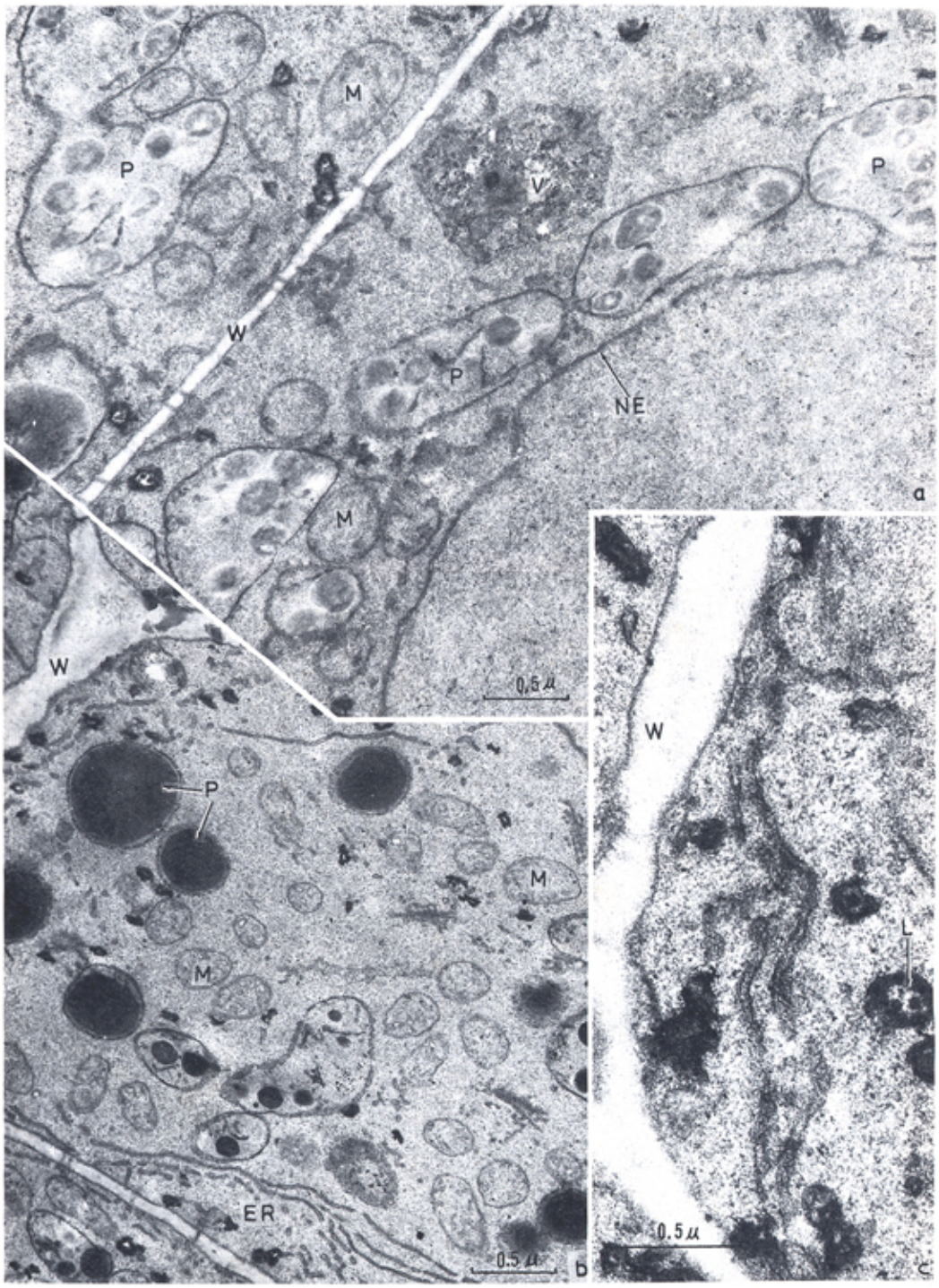


Fig. 3. Micrographs showing the cellular organelles in later stages of development (a, 72 hr root); and disintegrating lipid bodies (b, 24 hr root), both with $KMnO_4$.

Fig. 4. Micrographs showing the cellular structure in the 12 hr (a, c) and 24 hr (b) roots (fixed with $KMnO_4$). Polymorphism of plastids and numerous starch granules per plastid are evident in Fig. a. Fig. b. shows the gradual change of the starch granules in plastids. Fig. c shows the structure of the endoplasmic reticulum.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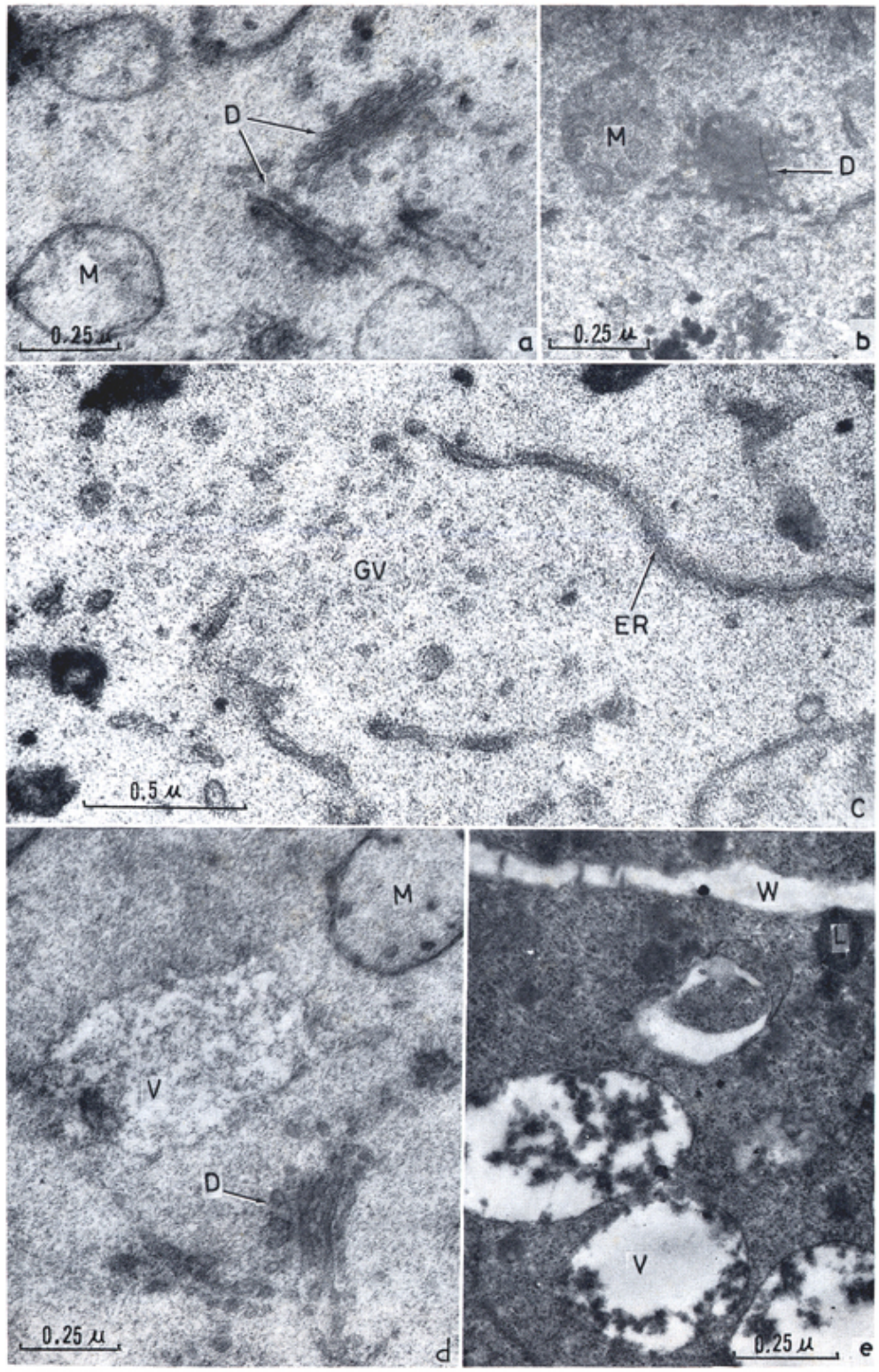
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vesicles. The Golgi vesicles in the cells of younger roots (4 to 8 hr roots) were less numerous, and always stayed around the Golgi cisternae. The Golgi vesicles showed little change in their size during germination, but they gradually left the edges of cisternae and were released into the cytoplasm and became dispersed throughout the cytoplasm (Figs. 2a, 2b, 5a, 5b, 5c, 5d). The dictyosomes in irregular or reticulate forms were also very common in stages after 24 hr old (Figs. 2, 3a, 5b).

Plastid: It is not easy to identify the presence of plastids in the cell of a root growing in the soil. In the present materials, roots were grown under light during the expermiment. The light made the identification of the plastids easier by the presence of their lamellated structure. On the other hand it must be remembered that this can not be considered a normal pattern of development of the root meristem which grows under natural conditions.

The plastids from the 4 hr and 8 hr roots showed a great range in both size and shape, being round, elliptic or irregular in section (Fig. 1a). The irregular-shaped pattern disappeared and the size became more or less uniform in the 48 hr roots. The thylakoids and starch granules were the most characteristic feature in the plastids. They were constantly separated from the cytoplasm by a double membrane. Starch granules in the early stage of the developing root were small and numerous. They decreased in number and increased in size as the germination time was prolonged. Some plastids in 24 hr roots contained only one starch granule (Fig. 4b). Then they decreased in size as germination proceeded. The starch-containing plastids became scarce in 48 hr—72 hr roots (Figs. 2b, 3a). Although the number of the thylakoids per plastid showed a slight increase, the organization of the granal system retained its juvenile structure. A well elaborated granal system was not seen in all stages examined.

Mitochondrion: Typical mitochondria were found in all stages. They had a double membrane, tubular internal membrane (cristae) and matrix, being round or elliptic. More abundant mitochondria were seen in the roots at later stages. In 48 and 72 hr materials, among the various cell organelles, the mitochondrion was the most numerous type (Fig. 3a). This structure did not appear to change during germination. The matrix was always denser than the plastids of the same cell. The outline of the membrane system of the mitochondria is better defined than that of the other organelles (except vacuoles) when fixed in OsO_4.

Vacuole: Among all the cell organelles, vacuoles were the fewest in number in all stages of development. They were seen as electron-opaque regions in both the OsO_4 and $KMnO_4$ fixed materials (Figs. 3b, 3e). The single membrane of the tonoplast was better defined in OsO_4 fixed cells. Neither conspicuous increase in vacuole number nor extensive vacuolation was seen during germination.

DISCUSSION

With the exception of the materials at the very earliest stage of germination, all the cellular organelles in the root fixed with $KMnO_4$ were well defined under the electron microscope. The clarity of the membrane system and cytoplasmic organelles increased as germination time increased during the early stages. This

Fig. 5. Micrographs showing the structures of the various organelles in different stages of development. a, c, d, 48 hr; b, 72 hr; e, 12 hr root.

fact indicates that the change of clarity is probably due to the structural nature of the material rather than any technical defect. Perner (1965) tried the vapor form of OsO_4, the cellular structure showed a great difference when fixed with aqueous fixatives; so in order to obtain a uniform series of preparations during germination, all materials were fixed with aqueous fixatives in the present investigation. Sections fixed with OsO_4 lacked membrane clarity. The membranes of the mitochondrion and vacuole were better preserved than any other organelles fixed by OsO_4. Apparently the membrane system in the mitochondrion and vacuole differ from those of other organelles, such as: ER, plastid, dictyosome and nuclear envelope in molecular constitution. Since we were unsuccessful in the preparation of a section of the resting radicle the present report does not give information concerning cellular changes in the very early stages until about 4 hr after soaking (Chiang and Tsou, 1974).

The variation in shape caused by different fixatives ($KMnO_4$ and OsO_4) of lipid bodies is also shown in other plant cells (Jacks, Yatsu & Altschul, 1967). The structure of lipid bodies in *Phaseolus radiatus* is similar with that in the meristematic cells of *Pisum sativum* root (Yoo, 1970); *Arachis hypogea* cotyledon (Jacks *et al.*, 1967); and *Hordeum vulgare* aleurone cells (Paley and Hyde, 1964). Lipid bodies in the promeristem of the present material are abundant in the root at early stages (24 hr before germination). The change in the pattern of distribution and disappearance of lipid bodies in the cell during germination also corresponds with that reported for *Hordeum* (Nieuwdorp and Buys, 1964) and *Pisum* (Yoo, 1970). The activity and function of the lipid bodies in the juvenile stage of the plant body have been suggested as the organelle for lipid storage (Jacks *et al.*, 1967); for the prevention of loss of moisture from the cells (Yoo, 1070); and for lipid supply to the ER membrane (Mollenhauer, 1967). The behavior of the lipid bodies in the early stage of germination revealed in the present investigation as well as the others (Nieuwdorp and Buys, 1964) show that lipid bodies in the promeristem and probably in other tissues serve as the main energy source utilized during the early stages of germination. Jack *et al.* (1967) also reported that more than half of the lipid content per cotyledon of *Arachis hypogea* was untilized during germination. The lipid bodies in the proinitial cells disappeared during the early stages of germination. But the authors were unable to trace the fate of the lipid bodies during germination. In general, lipid bodies are the organelles that show the most conspicuous change of all the organelles in the apical initial cells during the early stages of germination. In addition to the behavior of lipid bodies, morphological changes in the dictyosomes in the proinitial cells was obvious. The structural variation of the dictyosomes during germination included the increase of the number of cisternae; the size of the sectional view of cisternae the number of dictyosomes; the distending of cisternal edge; and the increase in the peripheral vesicles of the dictyosome. The apparent increase and the morphological changes in dictyosomes during the germination suggests a general activation of this organelle, presumably in preparation for cell wall synthesis. Mitosis in the proinitial cells was seen in both optical and electron micrographs during the corresponding stages (Chiang and Tsou, 1974; Fig. 6). The previous investigation (Chiang and Tsou, 1974) indicated that cell expansion occurs accompanying early development of the proinitial cells. Dictyosomes are thought to be responsible for wall formation in a variety of plant cells (Fowke and Pickett-Heaps, 1972).

The most important consideration related to the structural variation in plastid is the disappearance of starch granules within the plastids. The time of disintegration

of starch granules during germination corresponded with the structual modification of dictyosomes and the behavior of lipid bodies. Apparently both starch granules and the lipid bodies were involved in energy supply. Though the roots were exposed to the light during the experiment a well-established granal system was not seen in the stages examined. This can be explained as genetical rather environmental, since the proinitial cell group has never been thought to be the site of photosynthesis.

The mitochondria and vacuoles were uniformly present in all the stages examined. Only a slight increase in the number of mitochondria could be noted. It seems reasonable to believe that the increase of the activities of the cytoplasmic organelles are dominant in the germinating embryo. But most of the studies on this matter always refer to the storage part of embryo and not the extreme tip of the embryo i.e., prointial cells. From the present study, it is obvious that the developmental changes in ultrastructure of cytoplasmic organelle in the proinitial cells also take place during the germination. The proinitial cells act not only as the meristematic center but also as one of the energy sources for germination.

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