

AMERICAN JOURNAL OF Botany

The Ultrastructure of the Quiescent Center in the Apex of Cultured Roots of *Convolvulus arvensis* L.

Author(s): Harry L. Phillips, Jr. and John G. Torrey

Source: *American Journal of Botany*, Vol. 61, No. 8 (Sep., 1974), pp. 871-878

Published by: [Botanical Society of America](#)

Stable URL: <http://www.jstor.org/stable/2441623>

Accessed: 23/08/2011 15:44

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.



Botanical Society of America is collaborating with JSTOR to digitize, preserve and extend access to *American Journal of Botany*.

<http://www.jstor.org>

THE ULTRASTRUCTURE OF THE QUIESCENT CENTER IN THE APEX OF CULTURED ROOTS OF *CONVOLVULUS ARVENSIS* L.¹

HARRY L. PHILLIPS, JR. AND JOHN G. TORREY²

Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT

Cultured roots of the common bindweed, *Convolvulus arvensis* L. growing at the rate of 15-30 mm/day in sterile nutrient medium were fixed for electron microscopic analysis. The ultrastructure of the quiescent center, the initials of the ground meristem, and the initials of the procambium were studied in order to determine whether sequential structural changes could be correlated with models for specifying the mechanisms by which cell differentiation and cell division might be controlled. The differentiation of cells in the root proper occurs very gradually in linear files from the site of the quiescent center proximally into the different tissue regions. Major structural changes, such as the orientation and subsequent elongation of cells along the longitudinal axis of the root and cell wall changes, indicate that the control of differentiation and perhaps cell division occurs in radial gradients outwardly from the quiescent center.

PREVIOUS EXPERIMENTAL STUDIES on the apical meristem of *Convolvulus arvensis* showed that the terminal apex could be subdivided into physiologically distinct cell populations based primarily on the utilization of tritiated thymidine, which in turn reflected the relative degree of cell division in each region (Phillips and Torrey, 1971a, b). Further studies on the apical meristem with colchicine indicated that these cell populations differed in the duration of their cell cycle times and that this difference explained the differential uptake of tritiated thymidine (Phillips and Torrey, 1972). These experimental findings generated interest in ultrastructural differences among separate cell populations in the root apex.

Only a few ultrastructural studies on the apical meristem of roots have been reported. The early studies (Clowes and Juniper, 1964; Griffiths and Audus, 1964; Leech, Mollenhauer, and Whaley, 1963; Northcote and Pickett-Heaps, 1966) were performed on roots which were fixed with permanganate salts. As pointed out by Ledbetter and Porter (1970), this type of fixative destroys the integrity of membrane systems and disintegrates structures in the cytoplasmic ground substance. Nevertheless, the early studies did provide some information on ultrastructural differences among cells in the apical meristem of roots.

In later studies, investigators used buffered

osmium tetroxide, acrolein, glutaraldehyde, and combinations of these compounds as fixatives. All these fixatives preserved tissues far better than had permanganate salts. Hyde (1967) reported that glutaraldehyde in combination with osmium tetroxide preserved tissues better than osmium tetroxide alone—probably by preserving a broader range of proteins and thereby preventing extraction of these proteins by the buffer.

Our studies on the ultrastructure of cultured roots of *Convolvulus arvensis* L. employed the more recently developed techniques of fixation for electron microscopy in an attempt to provide additional information on the physiologically distinct cell populations in the apical meristem of roots and to correlate such information with previous research. In particular, an effort was made to characterize the structural nature of the cells comprising the quiescent center of the root.

MATERIALS AND METHODS—Cultured roots of *Convolvulus arvensis* L., obtained from a clone of roots maintained in a modified Bonner-Devirian medium (Torrey, 1954) for a period of 18 years by subculturing root tips and root segments, were used in these ultrastructural investigations. Root tips elongating at a rate of 15-30 mm per day were fixed, dehydrated, and embedded according to the procedures previously described for preparing roots for other studies (Phillips and Torrey, 1971b). The only differences were that the root tips were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer (pH 6.8) either for 2-2½ hr at room temperature or overnight in the refrigerator and were postfixed with 2% aqueous osmium tetroxide (pH 6.8) for 2 hr at room temperature.

¹ Received for publication 24 September 1973.

This research was supported in part by a fellowship GM-41021 to H.L.P. from the National Institutes of Health, USPHS, by U. S. Public Health Service Grant GM-06637 to the Electron Microscope Laboratory at Harvard University, and by the Cabot Foundation of Harvard University.

² Permanent mailing address: Cabot Foundation, Harvard University, Petersham, Massachusetts 01366.

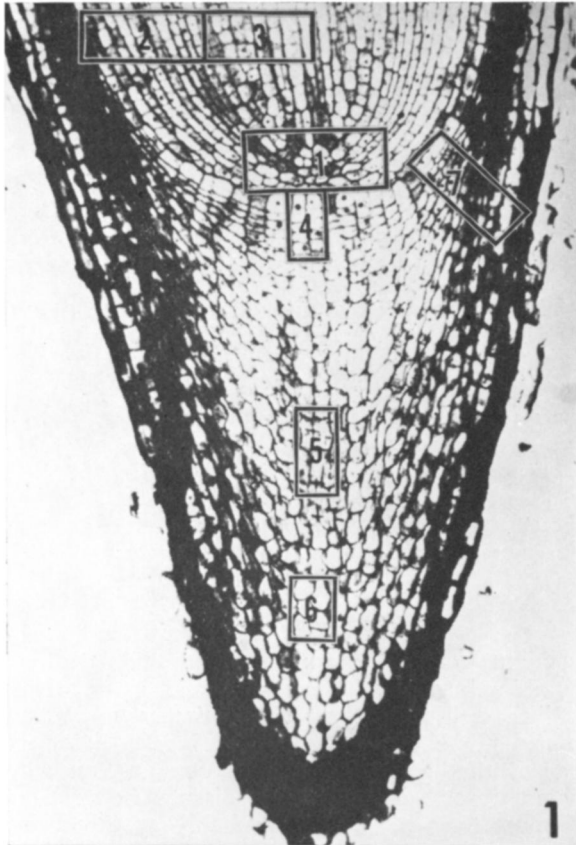


Fig. 1. Median longitudinal section of a cultured root of *Convolvulus arvensis* L., showing the different regions of the apical meristem which were examined under the electron microscope: (1) quiescent center, (2) ground meristem, (3) procambium, (4) root cap initials and their immediate derivatives in the first four tiers of the columella, (5) root cap columella cells, (6) columella cells displaced toward the root tip, and (7) initials of the root cap periphery and their derivatives. $\times 99$.

Longitudinal sections ranging between 50–100 μm in length were obtained from trimmed Epon-Araldite blocks with a diamond knife in the Sorvall Porter-Blum Ultramicrotome. The sections were picked up on grids of mesh size (150, 100, and 75 grid bars/inch) such that a large proportion of the section would be visible between the individual copper grid bars. Using these grids was important in order to know what region of the meristem was being examined and to understand the structural relationships between adjacent regions. The sections were stained for 5 min in 2% aqueous uranyl acetate and for 3 min in lead citrate solution (Reynolds, 1963). Stained and dried sections were examined with an RCA EMU-3D electron microscope.

The regions of the root proper which were examined in the terminal one millimeter of the root

apex were the undifferentiated cells constituting the ground meristem, the procambium, and the quiescent center (Fig. 1). The term ground meristem is used to denote the primary meristematic tissues which give rise to the cortex, and the term procambium denotes the primary meristematic tissues which give rise to the central cylinder. The quiescent center consists of the population of cells immediately distal to the initials of the ground meristem and the procambium and proximal to the root cap initials (Phillips and Torrey, 1971a, b).

The ground meristem was delimited from the procambium by the presence of a slightly thicker cell wall boundary between the two regions, the presence of intercellular air spaces in the ground meristem and the absence of such spaces in the procambium, and the absence of cellular connections between the two regions. The latter condition is due to the lack of periclinal cell divisions in the files of cells delimiting these two regions. The position of the quiescent center was based on previous experimental evidence provided by autoradiography (Phillips and Torrey, 1971a, b).

RESULTS—The Ground Meristem—The cells in this region, having elongated in the direction of the longitudinal axis of the root, are primarily rectangular when viewed in median longitudinal section (Fig. 2). Intercellular spaces (ics) of varying sizes are present between cells. Such spaces may be air spaces which form through the separation of longitudinal walls between adjacent cells or they may be artifacts of fixation. However, these spaces are observed only in the ground meristem.

The longitudinal (lw) and transverse walls (tw) between cells are generally of uniform thickness. Some cells show an uneven thickening of the primary wall and the middle lamella of the longitudinal wall. Cell walls which border intercellular spaces between cells are slightly thicker. The wall boundary between ground meristem cells and cells located in the periphery of the root cap is much thickened (rcj).

Plasmodesmatal connections between cells cross both the longitudinal and transverse walls (Fig. 3, single arrows). Occasionally a portion of the cell wall is less intensely stained than the rest of the wall or shows no staining at all. Such areas have several or many plasmodesmata. Sections cut tangential to a portion of the cell wall show that plasmodesmata are indeed found predominantly in clusters in these less intensely stained regions.

The cytoplasm of the cells contains numerous free ribosomes (r). Occasionally, such ribosomes appear in clusters (rc) and might represent polysomes. Only fragmentary profiles of endoplasmic reticulum with attached ribosomes (er) are found in the cytoplasm. Golgi bodies (G), mitochondria

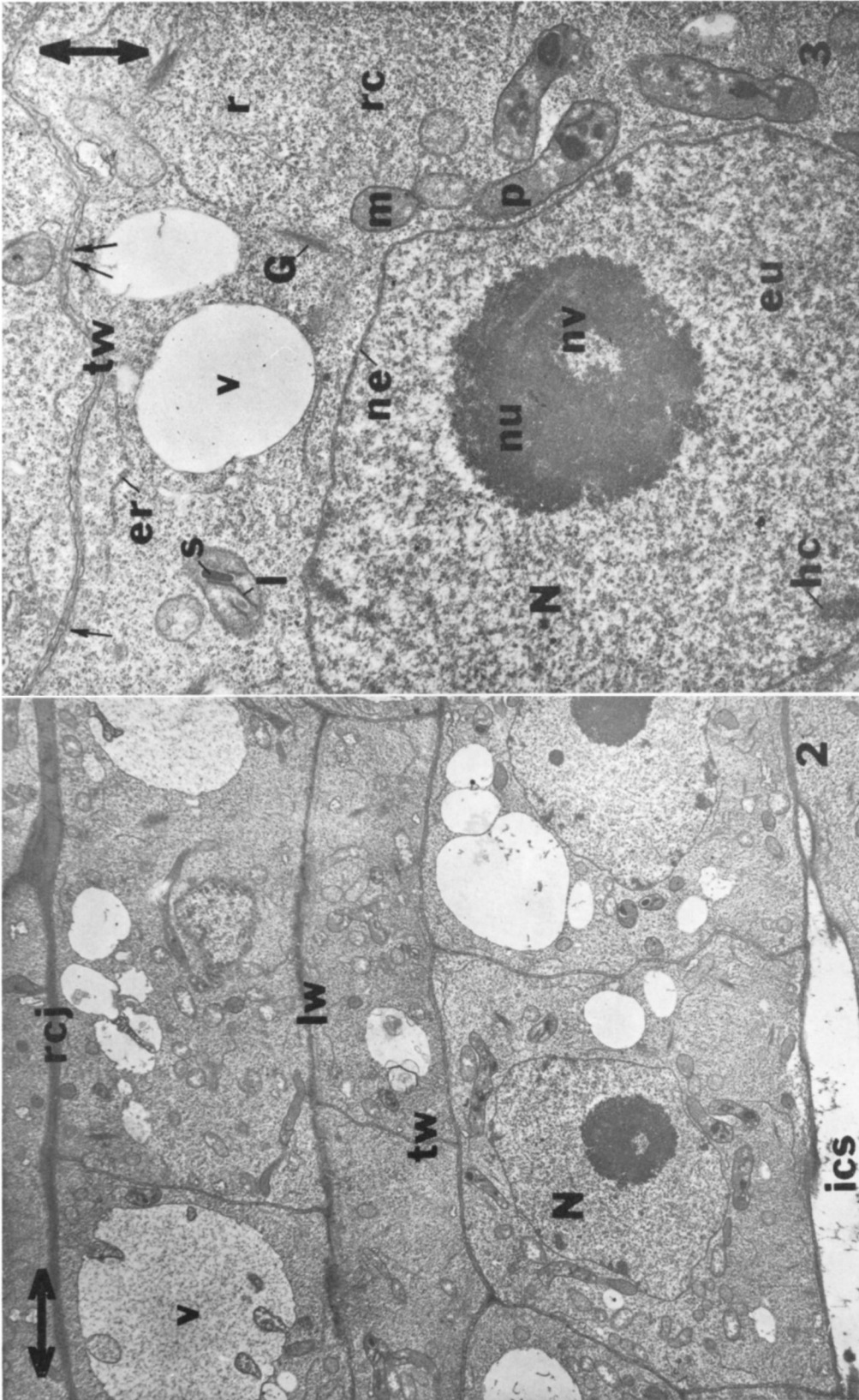
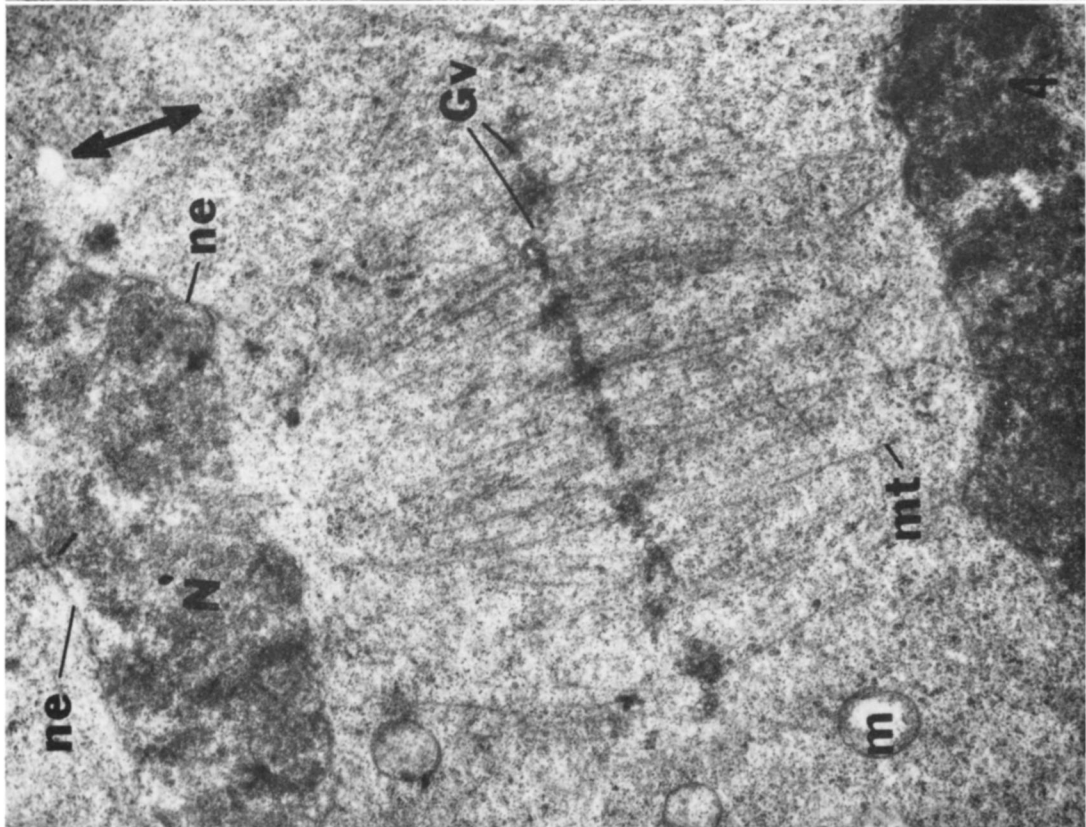
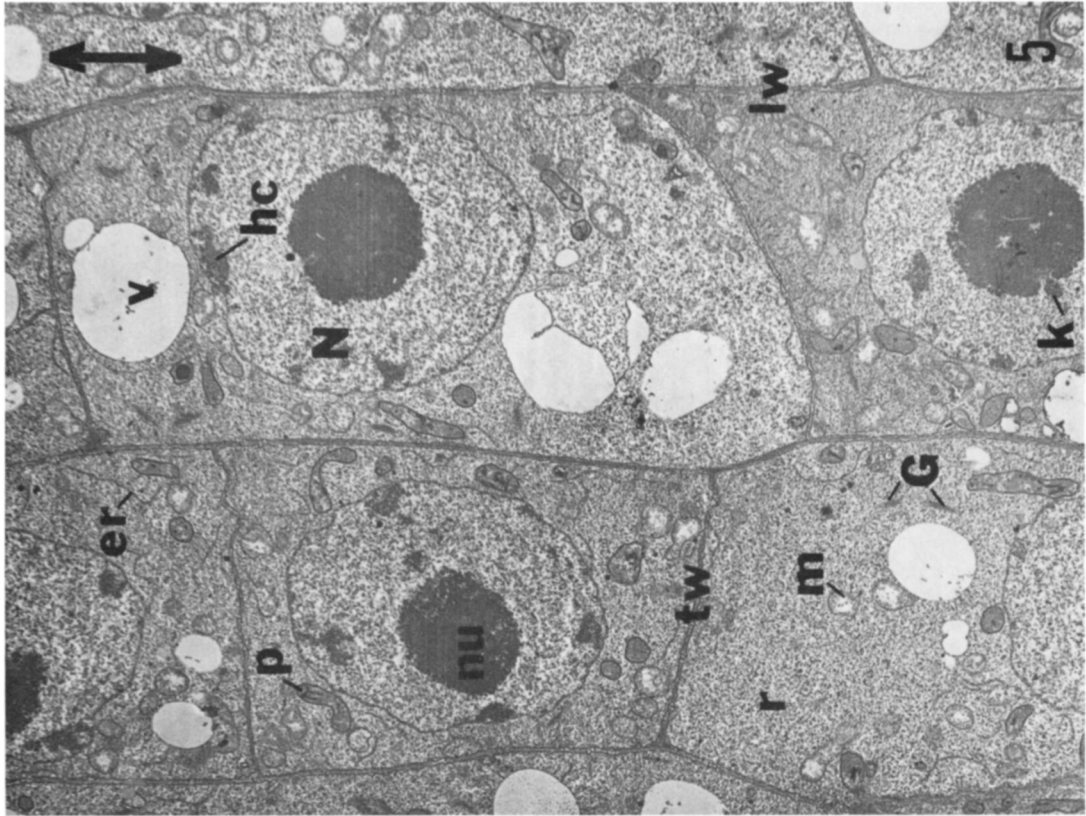


Fig. 2-3. Electron micrographs of the ground meristem. 2. Micrograph of the peripheral portion of the ground meristem. Note the unevenly and highly thickened root cap junction (rcj), the presence of a large intercellular air space (ics) forming along a longitudinal wall (lw), and the preferential elongation along the longitudinal axis of the root. $\times 4800$. 3. Section of a ground meristem cell, showing the typical cell structures. $\times 14,400$. Single arrows, plasmodesmata; eu, euchromatin; er, endoplasmic reticulum with attached ribosomes; G, Golgi body; hc, heterochromatin; l, lamellae; m, mitochondrion; N, nucleus; ne, nuclear envelope; nu, nucleolus; nv, nucleolar vacuole; p, plastid; r, ribosomes; rc, ribosome clusters; s, starch grain; tw, transverse wall; v, vacuole. The double-headed arrow indicates the direction of the longitudinal axis of the root.



(m), and plastids (p) are the other cytoplasmic organelles usually observed in these cells. The elongated and occasionally lobed plastids contain no or few lamellae (l) and one or two starch grains (s).

Nuclei (N) of these cells are primarily spherical, though elongated nuclei can be found in elongated cells. Most of the nucleus consists of lightly staining, dispersed euchromatin (eu), although several areas of densely staining, compact heterochromatin (hc) are present in every section. The heterochromatin is not restricted to the periphery of the nucleus (Fig. 2). The most distinctive feature of the nucleus is the darkly staining nucleolus (nu), which contains nucleolar vacuoles (nv), regions of less intensely staining material, and a mass of densely stained material adjacent to the periphery of the nucleolus—the karyosome.

Vacuolation is quite variable in ground meristem cells. Large vacuoles (v) are not infrequent although many cells have numerous small or medium sized vacuoles. Occasionally, cytoplasmic extensions into a vacuole or across a vacuole are evident.

Cell division continues to occur in this region of the ground meristem for some distance from the apical initials bordering the quiescent center (Fig. 4). As is typical of most cell divisions, cytoplasmic organelles are excluded from the region containing the division figure. The only organelles present between the forming nuclei are microtubules (mt) and Golgi vesicles (Gv) of varying sizes found at the site of the newly forming cell plate. The nuclear envelope (ne) forms around condensed chromatin at an early stage of cell plate formation during telophase.

Cells of the ground meristem further displaced from the root apex show the same general morphological features except that all the cells have elongated in the direction of the longitudinal axis of the root and have undergone radial cell expansion.

The Procambium—The cells in the region of the future central cylinder are rectangular and elongated in the direction of the longitudinal axis of the root (Fig. 5). Unlike the ground meristem, intercellular spaces are not found among such cells. Cell division continues to occur at this level of the procambium.

Both the transverse and longitudinal walls of

cells throughout the procambium are uniform in thickness. Plasmodesmatal connections are present on both the transverse and longitudinal walls and appear to occur in clusters in regions of the cell wall which show reduced staining.

The cytoplasm of these cells contains numerous unattached ribosomes which give the ground substance of the cells a fine granular appearance. Although Golgi bodies, mitochondria, and undeveloped plastids are relatively frequent in the cytoplasm, endoplasmic reticulum with attached ribosomes is present in only very small quantities. Vacuolation is variable; most cells have many small and medium-sized vacuoles. The nuclei and nucleoli of these cells are similar in morphology to those in ground meristem cells.

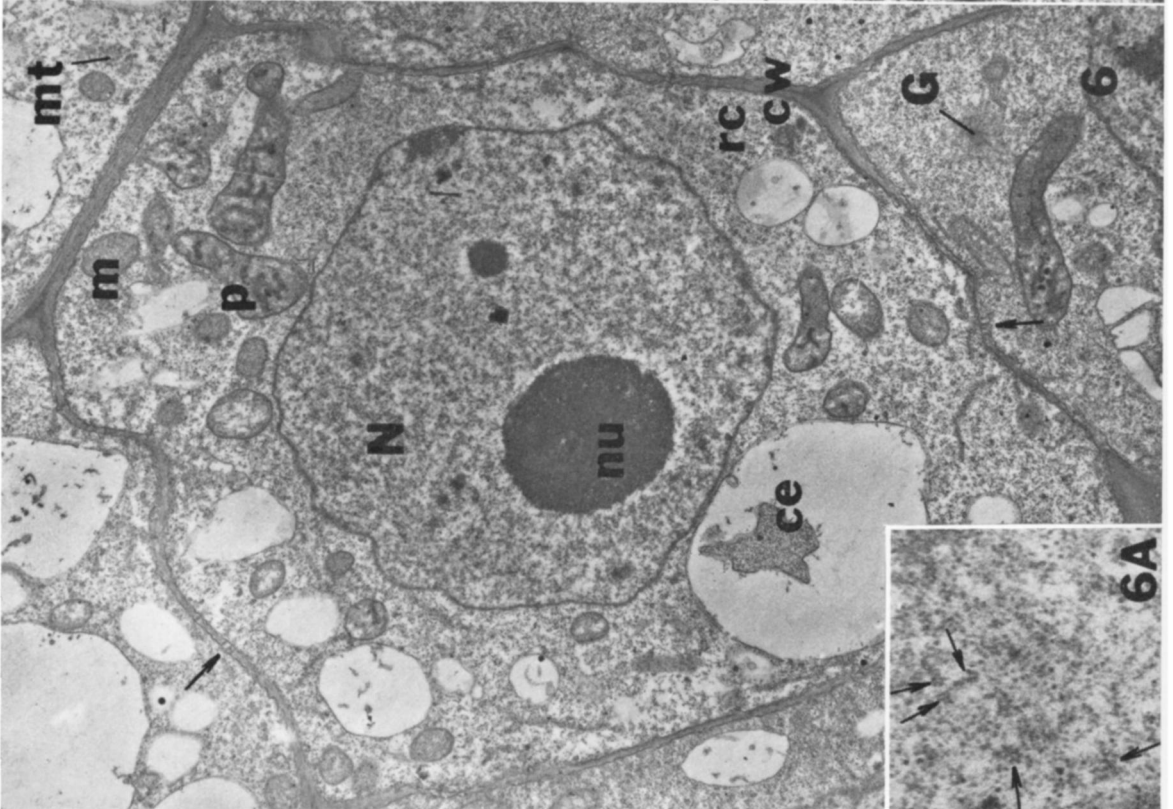
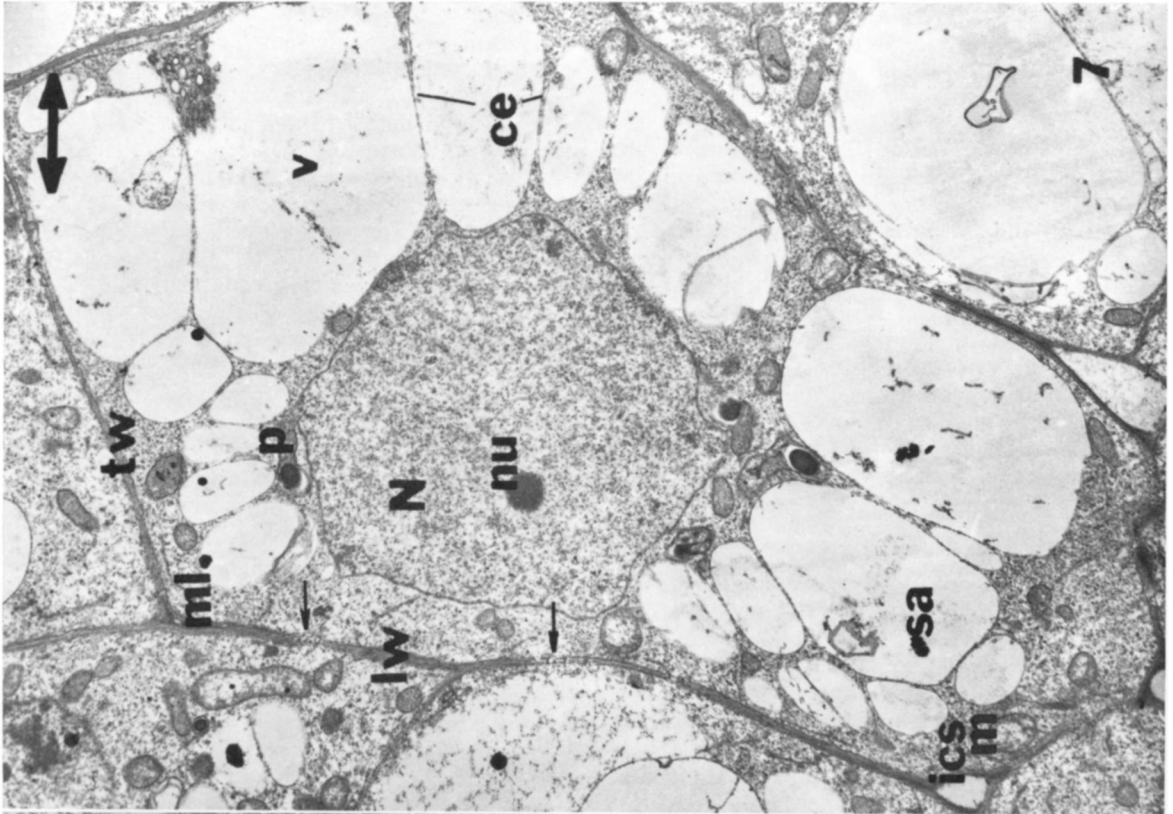
The Quiescent Center—The central cells of the quiescent center are more or less isodiametric (Fig. 6), whereas cells in the quiescent center located adjacent to the cortical initials show elongation along what would be equivalent to the longitudinal axis in ground meristem and procambial cells (Fig. 7). The walls (cw) of the central cells are irregular in thickness but tend to be thicker than the walls of surrounding meristematic cells. Intercellular spaces are apparent among cells located adjacent to the cortical initials—about six to eight cells radially outward from the center of the quiescent center.

Plasmodesmata are found traversing the cell walls either singly or in groups. The region of the cell wall where groups of plasmodesmata are found shows less intense staining than the regions of the cell wall lacking plasmodesmata. The plasmodesmata may be found in branched patterns; usually, the endoplasmic reticulum found in quiescent center cells is associated with such plasmodesmata.

Unattached ribosomes are abundant in the cytoplasm, and clusters of ribosomes, or polysomes, occur (Fig. 6A). Plastids with prolamellar bodies and associated lamellae as well as starch grains occur frequently. Golgi bodies are also present. The central cells are not highly vacuolated, although they do contain small and medium-sized vacuoles. Cells near the periphery of the quiescent center and adjacent to the cortical initials show increased vacuolation.

The major volume of the central cells is occupied by the nucleus, whereas, in peripheral cells where cell expansion begins, the nucleus occupies

<
Fig. 4-5. Electron micrographs of cells of the ground meristem and procambium. 4. Ground meristem cell at telophase. Note the well-defined microtubules (mt) extending from the future nuclei (N) to the region of the future phragmoplast where Golgi vesicles of different sizes are coalescing and the nuclear membranes (ne) are forming around the condensing chromatin. $\times 21,000$. 5. Electron micrograph of cells in the inner procambium. Note the finely granular appearance of the cytoplasm caused by numerous ribosomes, the many small vacuoles, and the lack of intercellular air spaces. See legend to Fig. 2-3 for key to labels. $\times 6200$.



considerably less of the cell volume. Nuclei have small isolated regions of heterochromatin and one or two prominent nucleoli. The nucleoli have nucleolar vacuoles and regions which stain with slightly different intensities.

DISCUSSION—The general morphology of cells in the ground meristem and procambium immediately derived from the apical initials of the root proper is basically similar. The main difference between these cells is the possible presence of intercellular air spaces which are found only in the ground meristem. If these spaces are indeed air spaces and not artifacts of fixation, then the difference in the thickness of longitudinal walls in the two regions is related to their development. In *Zea mays*, Leech et al. (1963) reported that air spaces occurred in the more proximal regions of the root proper but not among cells of the quiescent center. They also reported that the longitudinal walls bordering air spaces were thicker than longitudinal walls where no air spaces developed.

The cells of the quiescent center differ structurally from surrounding meristematic cells in two major ways. First, they are small, isodiametric or irregular in shape, and have a nucleus that occupies most of the cell volume. In contrast, other meristematic cells are large, rectangular cells which have undergone expansion by elongating primarily in the direction of the longitudinal axis of the root. Expansion occurs in those still within the quiescent center, as shown by the presence of cells at the periphery of the quiescent center which have elongated preferentially along one axis. Associated with this expansion and elongation in cells extending radially outward from the quiescent center is increased vacuolation. Secondly, the walls of cells in the quiescent center are generally much thicker than those of cells in the ground meristem and procambium. This difference reflects in part the lack of division in the quiescent center and the rapid division which occurs in the meristematic cells. Leech et al. (1963) and Clowes and Juniper (1964) reported that the walls of cells in the quiescent center of roots of *Zea mays* were uniformly thicker than those of meristematic cells. Furthermore, the transverse walls of meristematic cells were thinner since divi-

sions in this zone were predominantly anticlinal.

Plasmodesmata are distributed individually and in distinct groups in the walls of cells of the quiescent center and in those of meristematic cells in the root proper. The few apparent profiles of endoplasmic reticulum are associated with these plasmodesmata. Clowes and Juniper (1964) found in *Zea mays* that endoplasmic reticulum was sparse in the cells of the quiescent center and in the meristematic cells of the stele and cortex. Juniper and Barlow (1969) reported that the number of plasmodesmata per unit area on the transverse walls exceeded the number on the longitudinal walls in the root apex of *Zea mays*. The only exception to this finding was the quiescent center where roughly equal numbers of plasmodesmata occurred on all walls. They observed that the number of plasmodesmata per unit cell volume was considerably lower in cells of non-dividing tissue than in cells of dividing tissue. Furthermore, Juniper and Barlow (1969) suggested that the asymmetric distribution of plasmodesmata—that is, their preferential distribution on the transverse walls—was the means by which cell differentiation and cell division were controlled in the longitudinal files of cells diverging from the quiescent center.

Cells of the quiescent center and meristematic cells both show an abundance of free ribosomes. Clowes and Juniper (1964) noted in *Zea mays* that bound ribosomes occurred only in meristematic cells and in differentiating cells, although free ribosomes were found in all cells. The presence of polyribosomes in the cytoplasm of cells in the quiescent center of *Convolvulus* indicates that these cells either have constituted but nonfunctional polysomes or that these cells are engaged in synthetic activity.

The central cells of the quiescent center are structurally distinct from the cells at the periphery of the quiescent center and from the cells located in the meristematic regions. In a radial gradient outward from the center, the cells of the quiescent center show preferential elongation along one axis—the axis which predominates in cells in the meristematic zones. In addition, quiescent center cells become structurally more similar to meristematic cells along a radial gradient. These observations suggest that some factor or factors might be medi-

←

Fig. 6–7. Electron micrographs of cells of the quiescent center. **6.** Central cell of the quiescent center. Note the irregular shape of the cell; the highly thickened cell walls (cw); the small amount of vacuolation; the presence of plastids, mitochondria, ribosomes, and clusters of ribosomes; the cytoplasmic extension (ce) in the largest vacuole; the forming face of a Golgi apparatus (G); and a band of microtubules (mt) near the cell wall on the upper right. $\times 9700$. **6A.** Inset of a portion of the cytoplasm of a quiescent center cell, showing clusters of ribosomes, or polysomes (single arrows). $\times 33,100$. **7.** Highly vacuolated and elongated cell located in the peripheral portion of the quiescent center near the apical initials of the ground meristem. Note the thinner longitudinal and transverse cell walls, the appearance of small intercellular air spaces in the more darkly stained middle lamella (ml), and the fine cytoplasmic extensions between vacuoles. $\times 14,600$. See legend to Fig. 2–3 for key to labels; sa, stain artifact.

ating changes in cells along a gradient from the center of the quiescent center outward into the ground meristem and procambium. Webster and Langenauer (1973) suggest for *Zea mays* that the initial cells surrounding the quiescent center impose restrictions on the activity of its cells by acting as sites for the production of factors limiting cell division and differentiation. A gradient within the cells of the quiescent center would also be expected from this model.

Since the quiescent center is in close proximity to the root cap as well as the ground meristem and procambium, it is important to consider the rapidly dividing and differentiating cells of the root cap in models describing how cell division and differentiation are controlled in the root apical meristem. This relationship will be described in a separate paper.

LITERATURE CITED

- CLOWES, F. A. L., AND B. E. JUNIPER. 1964. The fine structure of the quiescent centre and neighboring tissue in root meristems. *J. Exp. Bot.* 15: 622-630.
- GRIFFITHS, H. J., AND L. J. AUDUS. 1964. Organelle distribution in the statocyte cells of the root tip of *Vicia faba* in relation to geotropic stimulation. *New Phytol.* 63: 319-333.
- HYDE, B. B. 1967. Changes in nucleolar ultrastructure associated with differentiation in the root tip. *J. Ultrastruct. Res.* 18: 25-54.
- JUNIPER, B. E., AND P. W. BARLOW. 1969. The distribution of plasmodesmata in the root tip of maize. *Planta* 89: 352-360.
- LEDBETTER, M., AND K. R. PORTER. 1970. Introduction to the fine structure of plant cells. Springer-Verlag, Berlin, Heidelberg.
- LEECH, J. H., H. H. MOLLENHAUER, AND W. G. WHALEY. 1963. Ultrastructural changes in the root apex, p. 74-84. *In: Symposia of the Society for Experimental Biology.* Academic Press, New York.
- NORTHCOTE, D. H., AND J. D. PICKETT-HEAPS. 1966. A function of the Golgi apparatus in polysaccharide synthesis and transport in the root cap cells of wheat. *Biochem. J.* 98: 159-167.
- PHILLIPS, H. L., JR., AND J. G. TORREY. 1971a. Deoxyribonucleic acid synthesis in root cap cells of cultured roots of *Convolvulus*. *Plant Physiol.* 48: 213-218.
- , AND ———. 1971b. The quiescent center in cultured roots of *Convolvulus arvensis* L. *Amer. J. Bot.* 58: 665-671.
- , AND ———. 1972. Duration of cell cycles in cultured roots of *Convolvulus*. *Amer. J. Bot.* 59: 183-188.
- REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-212.
- TORREY, J. G. 1954. The role of vitamins and micronutrient elements in the nutrition of the apical meristem of pea roots. *Plant Physiol.* 29: 279-287.
- WEBSTER, P. L., AND H. D. LANGENAUER. 1973. Experimental control of the activity of the quiescent centre in excised root tips of *Zea mays*. *Planta* 112: 91-100.