THE ROOT ENDODERMIS: FINE STRUCTURE AND FUNCTION

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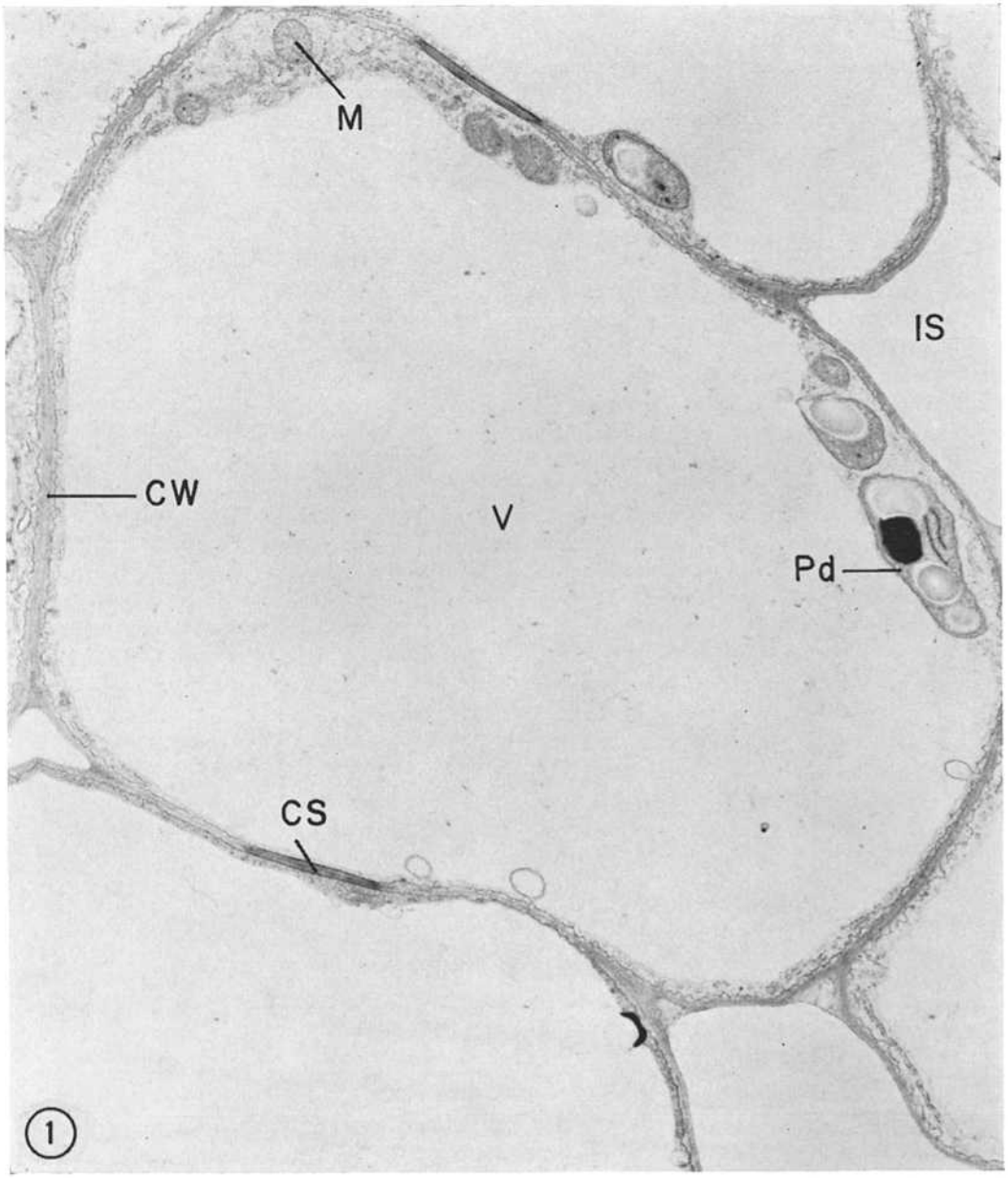
INTRODUCTION

The topology of the root endodermis, a tissue surrounded by peripherally located parenchyma cells and central conducting cells, suggests that it has a regulatory role in radial movement of water, minerals, and hormones. Each endodermal cell is characterized by a narrow band extending along the radial and transverse walls; this band, termed the Casparian strip, is chemically distinguishable from the remainder of the wall by stains specific for suberin and lignin. Suberin, a lipid substance, causes this portion of the wall to be hydrophobic and thus prevents passage of water and aqueous solutes. Each band is shared by the adjacent endodermal cells so that a network of suberized cell wall surrounds the conducting region of the root. At or before this network, water and aqueous solutes (such as hydrated ions) must move from the intercellular space system of the cortical parenchyma into the cytoplasm of the endodermal cell, in order to reach the center of the root.

Despite extensive research on uptake and movement of water, minerals, and hormones, the specific role of the endodermis in transport is not defined.



Abbreviations

CS, Casparian strip
CW, cell wall
ER, endoplasmic reticulum
IS, intercellular space
M, mitochondrion
Mt, microtubule
P, plasmalemma
Pd, plastid
T, tonoplast
V, vacuole

FIGURE 1 Transection of an endodermal cell showing the position of the Casparian strip on the radial walls. Portions of cortical parenchyma cells appear at the right margins; portions of pericycle cells appear at the left margin. × 10,000.

Hypotheses concerning ion uptake and movement suggest either that the endodermis actively absorbs ions from the cortical intercellular space system and secretes them into the central cylinder cells or that the endodermis acts merely to prevent extensive backflow once water and ions have reached the conducting tissue (6). In the latter case, active ion uptake occurs in cortical parenchyma cells. Once across the plasmalemma of a cortical parenchyma cell, ions could move to the central conducting cells through the symplasm (a cytoplasmic continuum across the root which has resulted from plasmodesmatal connections between adjacent cells). A critical examination of cellular and subcellular structure may distinguish between these alternatives.

MATERIALS AND METHODS

Roots of *Convolvulus arvensis* (field bindweed) have been maintained in continuous culture for 16 yr (2). Short segments were excised from mature regions of the root axis and fixed in 3% glutaraldehyde for 1.5 hr; they were then washed, postfixed in 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, and embedded in Araldite-Epon (8). Fixation, rinsing, and postfixation were carried out at room temperature in 0.05 M phosphate buffer at pH 6.8. Silver sections were cut on a Servall MT-1 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed in a Siemens Elmiskop 1A at 80 kv.

RESULTS

The wall of each endodermal cell is shared with three types of cells: other endodermal cells, pericycle cells to the inside, and cortical parenchyma cells to the outside. Fig. 1 shows an entire endodermal cell in transverse view. The Casparian strip is positioned on the radial walls and occupies a near median position. Most of the cell content is taken up by a large central vacuole limited by the tonoplast (vacuole membrane). The cytoplasm forms only a narrow peripheral layer between the plasmalemma and the central vacuole. A magnified view of a portion of the radial wall shows the structure of the Casparian strip (Fig. 2) which measures approximately 2 μ in length and slightly less than 0.2 μ in width. The cell wall in the region of the Casparian strip is slightly thickened; its staining reaction is more intense and homogeneous than that in other regions of the wall. The plasmalemma is thicker at the Casparian strip and more regular in outline than elsewhere in the cell. Although the cytoplasm contains all the organelles generally found in higher plant cells, it comprises only a thin parietal layer. Free and bound ribosomes, dictyosomes, endoplasmic reticulum, plastids, mitochondria, and microtubules, all are present, but none occurs with any great frequency. No preferential disposition was observed of any cell organelles with respect to the Casparian strip.

Aside from the distinguishing appearance of the Casparian strip, the plasmalemma in this region also demonstrates a remarkable affinity for the cell wall. The failure, following plasmolysis of endodermal cells, to achieve separation of the plasmalemma and wall in the Casparian strip region was described many years ago by Behrisch (1) and termed "band plasmolysis." Fig. 3 is an electron microscopic illustration of this response which is achieved by treating the root tissue with a 20% aqueous solution of glycerin for 15 min prior to fixation in glutaraldehyde. Plasmolysis has occurred in all regions except in the region of the Casparian strip.

Fig. 4 shows the Casparian strip region at one end of an endodermal cell which was probably cut across at the other end during glutaraldehyde fixation. All cell contents have disappeared during the preparative steps except for the plasmalemma and the microtubules. The adjacent cell sharing the same radial wall appears normal. This cell provides another example of the wall-membrane association in the Casparian strip region. In this region the plasmalemma adheres to the wall. From the margin of the Casparian strip, it departs from the wall at a right angle course and traverses what would have been the central vacuole. Both at the margin of the Casparian strip and at one point well into the strip, the plasmalemma is cleaved, with the inner portions of the characteristic dark lines of the unit membrane adhering to the wall. The affinity of this portion of the membrane for the wall appears stronger than the forces holding the membrane intact. (The same conclusion is warranted from close examination of Fig. 3). A microtubule, seen in transverse view, occupies its normal position with respect to the plasmalemma. The inset shows another region where the peripheral microtubules are more frequent. The association of the plasmalemma and microtubules following the loss of all other cytoplasmic components suggests that the microtubules may be anchored to the plasmalemma. A direct physical connection between the plasmalemma and peripheral microtubules has been demonstrated recently in tobacco

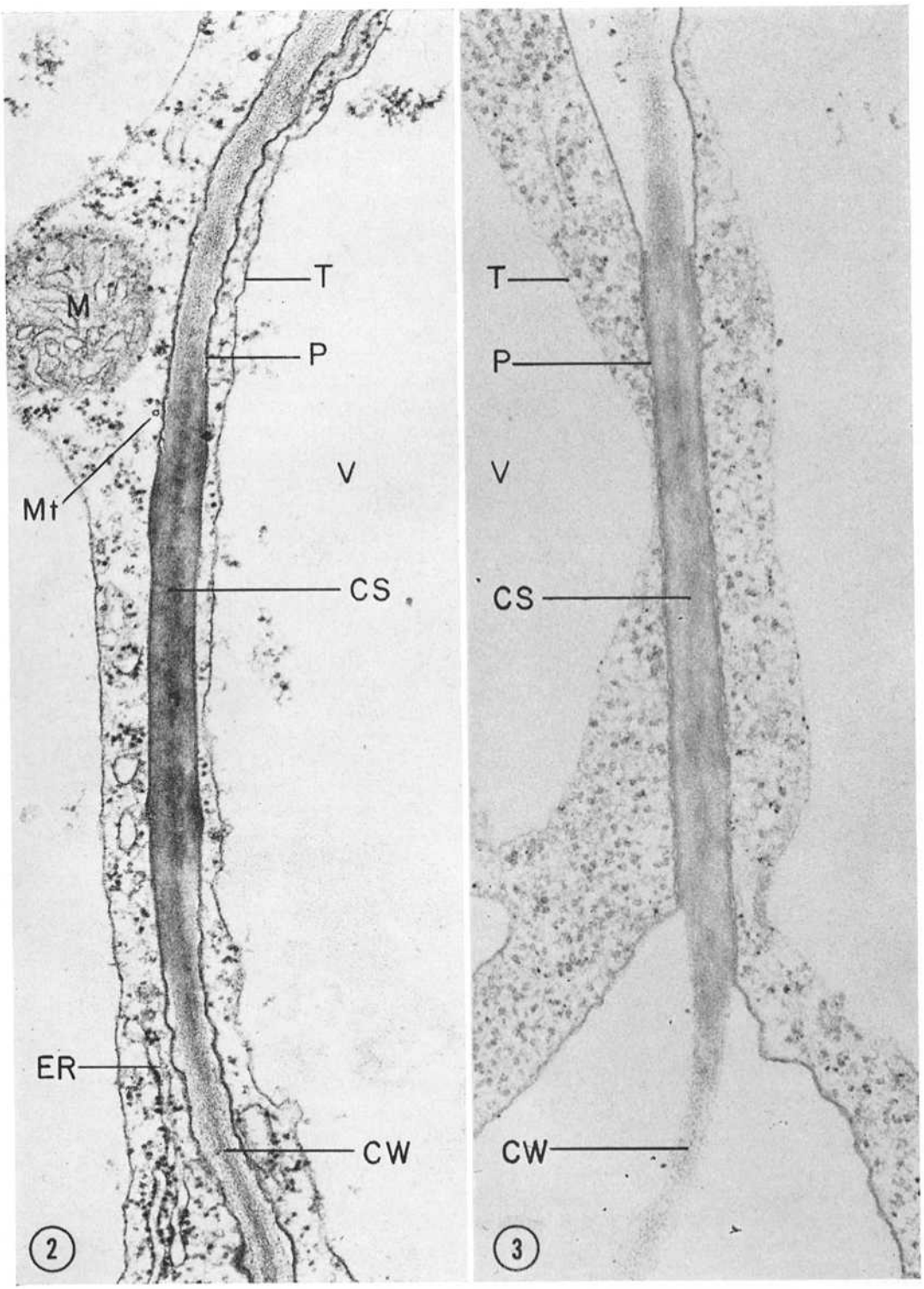


FIGURE 2 Transection of the radial wall between two endodermal cells. The Casparian strip region of the wall is more intensely stained than other regions of the cell wall. A thin parietal layer of cytoplasm separates the plasmalemma from the large central vacuole in each cell. × 50,000.

FIGURE 3 A view similar to that in Fig. 2. The tissue was plasmolyzed by a 15 min exposure to 20% glycerin prior to fixation. The plasmalemma no longer adheres to the cell wall except in the region of the Casparian strip. × 64,000.

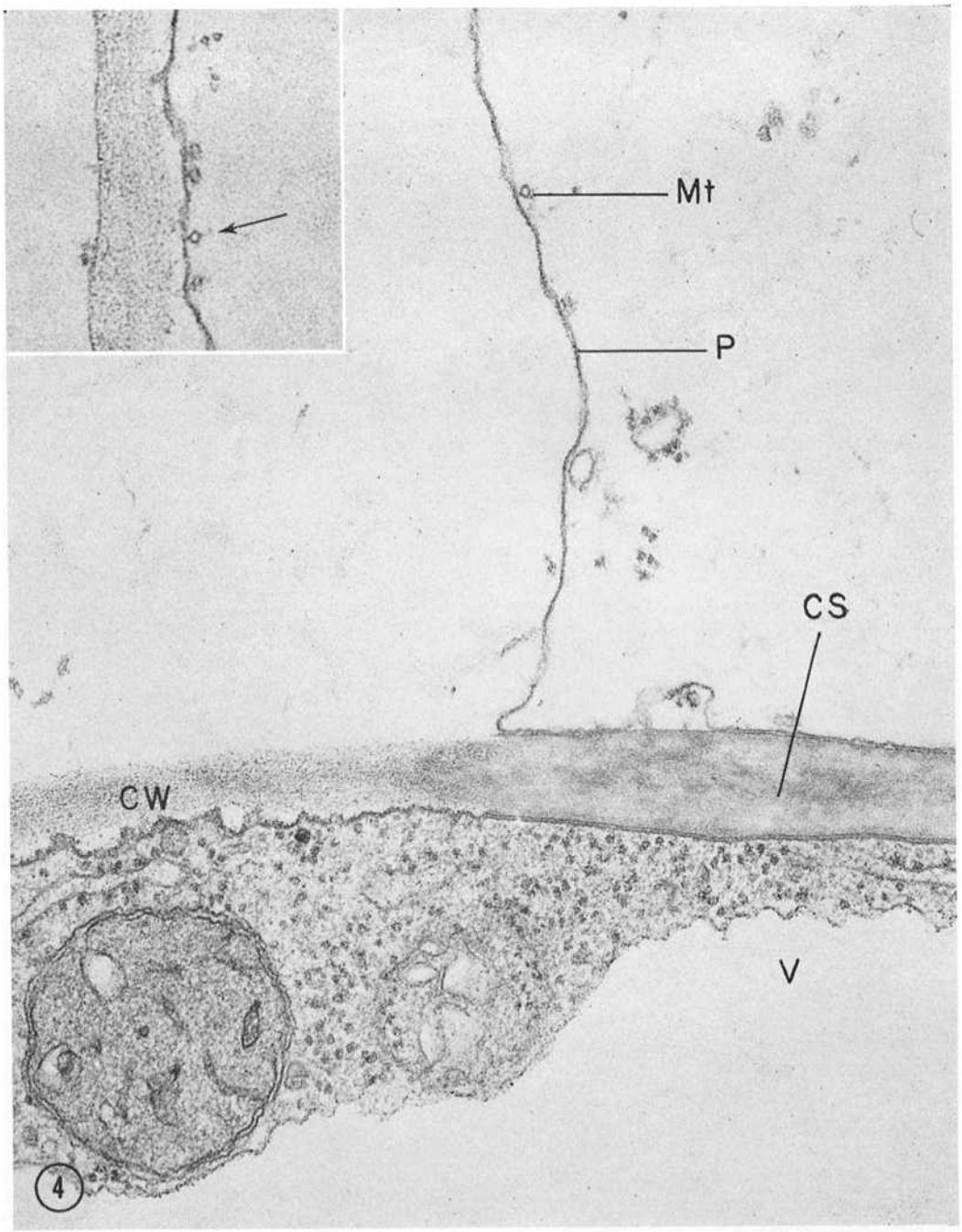


FIGURE 4 Transection of the radial wall between two endodermal cells. One cell was presumably cut open during fixation. Of the cytoplasmic components once present, only microtubules consistently remain. The plasmalemma still adheres to the cell wall in the region of the Casparian strip. The inset, a portion of a similar cell, shows a group of microtubules along the plasmalemma in a region of the cell opposite an intercellular space. The arrow indicates a possible connection between the plasmalemma and a microtubule. × 80,000. Inset × 100,000.

by Cronshaw (3), and some indication of such a connection is visible in the inset.

Band plasmolysis is an anatomical expression of a physiological barrier to radial movement exclusively through the intercellular space system. If active ion uptake into cortical parenchyma cells occurred, radial movement could proceed from cortical parenchyma cells to the endodermis and on to pericycle cells, provided that there were direct protoplasmic connections (plasmodesmata) between these cells. In these elongate cells, plasmodesmata are sufficiently separated so that they occur in low frequency in thin sections. Approximately 25% of the transverse views of entire endodermal cells show no plasmodesmata, e.g., Fig. 1. The mean number of plasmodesmata visible in a section of an entire endodermal cell is 3.2. Comparisons of the presence and frequency of plasmodesmata in walls shared with adjacent endodermal, cortical, or pericycle cells have shown that these intercellular connections are present in approximately equal frequency between all three cell types and thus provide direct cytoplasmic contact between all neighboring cells. The Casparian strip occupies approximately one-third of the radial wall shared by adjacent endodermal cells. Hundreds of thin sections of radial walls were examined. No plasmodesmata were ever observed in the region of the Casparian strip. Serial sections were not attempted; sections showing surface views of the Casparian strip were attempted but were unsuccessful.

DISCUSSION

A comprehensive study of the endodermis of two plant species (*Acorus calamus* and *Clivia nobilis*) has been published by Falk and Sitte (4) who noted that plasmodesmata could be found on all walls of the endodermal cell, except in the region of the Casparian strip. Scott (9) suggested that band plasmolysis may result from a concentration of plasmodesmata in the Casparian strip and thus may prevent plasmolysis in this region. The absence of plasmodesmata in the Casparian strip region in three plant species makes this explanation unlikely. The tenacious association must be based on other factors, such as interactions generated between the hydrophobic suberized wall of the Casparian strip and the membrane lipids or hydrophobic portions of membrane proteins.

Falk and Sitte found an irregularity which appeared as a ledge in the wall at the edge of the Casparian strip. They suggested that this irregularity resulted from continued wall deposition in all regions of the cell except the Casparian strip. No similar wall organization was observed in *Convolvulus*, although there was a transition from an irregularly coursing plasmalemma throughout most of the cytoplasm-wall interface to an even, straight plasmalemma in the region of the Casparian strip. This irregular course of the plasmalemma may be due to slight plasmolysis, during fixation, all along the cell surface, or it may accurately represent the living condition.

To date, fine structure studies of endodermal cells have not been combined with physiological studies of active ion uptake. However, based on the structure of the endodermis reported here and also observed by Falk and Sitte, the cell structure does not appear to be specialized for active uptake and secretion. Mitochondria are not numerous, nor are their cristae well developed. A high level of ATP production by endodermal cells would be required for active uptake and secretion by endodermal cells into the central conducting region. The endodermal cell structure is more compatible with active ion uptake by cortical parenchyma cells and transport through the symplasm. Direct cytoplasmic contact, via plasmodesmata, is assumed to exist all the way from peripheral cortical parenchyma cells, through the endodermis, to central parenchyma cells (5, 7). However, this assumption is based on work published a number of years ago in which staining procedures were used to render plasmodesmata visible at the light microscope level.

Despite the high degree of differentiation of the endodermis occupying an interfacial position between ion absorbing and transporting tissue, endodermal cells may be stimulated, as a normal event during lateral root formation, to resume meristematic activity and to assume a function totally unrelated to ion, water, or hormone transport. A subsequent report will describe the effect of this meristematic activity on the Casparian strip and the role of the tissue derived from the endodermis in cortical cell death during lateral root protrusion.

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REFERENCES

1. BEHRISCH, R. 1926. Zur Kenntniss der Endodermiszelle. *Ber. Deut. Botan. Ges.* **44:** 162.

2. BONNETT, H. T., JR., and J. G. TORREY. 1965. Chemical control of organ formation in root segments of *Convolvulus* cultured in vitro. *Plant Physiol.* **40:** 1228.
3. CRONSHAW, J. 1967. Tracheid differentiation in tobacco pith culture. *Planta.* **72:** 78.
4. FALK, H., and P. SITTE. 1961. Untersuchungen am Caspary-Streifen. *Proc. European Regional Conf. Electron Microscopy, Delft, 1960.* **2:** 1063.
5. LIVINGSTON, L. G. 1935. The nature and distribution of plasmodesmata in the tobacco plant. *Am. J. Botany.* **22:** 75.
6. LÜTTGE, U., and G. G. LATIES. 1966. Dual mechanisms of ion absorption in relation to long distance transport in plants. *Plant Physiol.* **41:** 1531.
7. MEEUSE, A. D. J. 1941. Plasmodesmata. *Botan. Rev.* **7:** 249.
8. MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* **39:** 111.
9. SCOTT, F. M. 1963. Root hair zone of soil-grown roots. *Nature.* **199:** 1009.