The structure of the endodermis during the development of pea (*Pisum sativum* L.) roots

JOANNA KOPCIŃSKA, WŁADYSŁAW GOLINOWSKI

Department of Botany, Institute of Plant Biology, Warsaw Agricultural University,
Rakowiecka 26/30, 02-528 Warsaw, Poland

Abstract

It is shown on the basis of cytological studies that during the development of the pea root endodermis, the following structures were formed (in order of appearance): proendodermis, Casparian strips, suberin lamellae and secondary cell walls. The proendodermis cells had, in addition to the commonly occurring cell components, small vacuoles filled with phenols. The Casparian strips developed in the radial walls and accounted for no more than 1/3 of their length. The suberin layer, found on all of the endodermis walls, was deposited last over the Casparian strips. The secondary cell wall was formed only in the cells located over the phloem bundles. Its thickness was uniform over the entire circumference of the cell.

Key words: root, endodermis, Casparian strips, suberin lamella, secondary cell wall

INTRODUCTION

The endodermis is made up of a single layer of cells which forms a continuous encirclement of the root stela, separating it from the cortical parenchyma. The position of this tissue in the root and the structural changes occurring in its cell walls indicate that it plays an active role in regulating the passage of water and aqueous solutions between the root stela and cortical parenchyma. Its important physiological role is the reason why studies on the structure and ontogeny of the endodermis are undertaken. Observations on the course of the developmental stages of the endodermis have been done mainly on monocotyledons (Clarkson et al. 1971, Karas and McCully 1973, Robards et al. 1973, Haas and Carothers 1975). From among the representatives of dicotyledons, only the ultrastructure of the first stage of development of *Convolvulus* (Bonnett 1968) and *Cucurbita* (Harrison-Murray and Clarkson 1973)

endodermis have been described; a full picture of the ontogeny of this tissue in *Ranunculus acris* has been presented by Scott and Peterson (1979a).

MATERIAL AND METHODS

Pisum sativum L. var. 'Nefryt' seeds were germinated in the dark at a temperature of approx. 26°C, then transferred to dishes filled with perlite. The plants were grown under greenhouse conditions, watered with Hoagland's medium. Roots were sampled from 10- and 21-day-old seedlings, fixed in FAA and embedded in paraffin. Series of transverse and longitudinal sections were stained with safranine and fast green, then analysed in a light microscope. Fresh, hand-cut samples were used when specific color reactions were done for phenolic compounds, using a solution of aniline-KIO_3 (Mace 1963), $FeCl_3$, Mitchell's reagent and the nitroso-reaction (Turowska et al. 1970). For the ultrastructural studies, fragments of roots were taken and fixed in a mixture of 3% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylan buffer, pH 7.2 (according to Karnovsky 1965) for 4 hrs at room temperature. Post-fixation in 1% OsO_4 for 2 hrs at 0°C was employed. The material was dehydrated in a series of increasing ethyl alcohol, acetone and propylene oxide concentrations, embedded in Epon 812 (according to Luft 1961) and sectioned on an LKB microtome. The sections were stained with uranyl acetate and lead citrate. The material was then viewed in a JEM 100C electron microscope.

RESULTS

In the root meristem, less than 0.1 mm from the root tip, a layer of cells elongated in the tangential direction, separating the small, intensly staining cells of the stele from the isodiametric cortical parenchyma cells (Fig. 1) could be distinguished. This was the proendodermis. It was characterized by the traits typical for meristematic tissues: large cell nuclei and a dense cytoplasm with numerous ribosomes. In the protoplasts of proendodermis cells, Golgi apparatuses, frequently dividing mitochondria, plastids containing plastoglobuli and starch grains of various sizes (Figs. 2, 3) were observed. Somewhat later, lipid bodies (Fig. 4) began to appear in the proendodermis. Tiny vacuoles were filled with an osmophilic, flocculent material. Plasmodesmata grouped together, were found in the thin, cellulose cell walls. At a distance of 5–6 mm from the root tip, significant changes occurred in the protoplasts of cells in the structure

of their walls. A large, central vacuole was formed in each cell. In the cytoplasm, Golgi apparatuses were observed along with distended ER cisterns positioned along the plasma membrane (Figs. 5, 6), often containing an osmophilic fibrilose material (Fig. 7). The lipid bodies typical for the proendodermis, now were seen only sporadically. In the walls of the radial cells, somewhat closer to the border with the pericycle, Casparian strips were formed (Figs. 5, 6, 7). They arose first in the connecting cells above phloem bundles, then over the xylem. The cell wall in the region of the strip was thickened and more homogenous (Figs. 6, 7). The plasma membrane adhered tightly to the wall in the place where the strip was (Figs. 6, 7). The ER cisterns often exhibited close contact with the plasma membrane in the neighbourhood of the Casparian strips (Fig. 6). At this stage of the development of the endodermis, osmophilic material and vesicular bodies arose in the space between the plasma membrane and cell wall on the ends of the strip. The plasmodesmata occurred mainly in the tangential walls, rarely in the radial ones; in several cases they were seen in the Casparian strips. Observations done in the electron microscope made it possible to determine that in the ontogeny of the pea root endodermis, further development stages, which until now have been described mainly in monocotyledons take place in this tissue. These are: the formation of a suberin layer on the internal side of the cell walls (Fig. 8) and the formation of a secondary cell wall (Fig. 13). The suberin lamellae, similarly as the Casparian strips, arose first in the endodermis laying across from the phloem. In these cells, the ER cisterns, as well as the membraned structures occurring between the plasma membrane and the suberin lamella being formed, were filled with a dark substance. In addition, osmophillic granules (Figs. 9, 10) were observed in the ER cisterns and outside of the plasma membrane. The suberin lamella was deposited on the tangential and radial walls simultaneously. Its regular outline and layered structure (Fig. 11) were disturbed in the vicinity of Casparian strips, which were the last to be formed (Fig. 12). The endodermis cells which were directly above the protoxylem, did not reach the suberin lamella stage in their development. These are the passage cells. In the studied material, only in the endodermis cells above the phloem bundles was the formation of secondary cell walls seen (Figs. 13, 14, 15). It was formed on the surface of the suberin lamella on both the tangential and radial walls, and its thickness was uniform over the entire circumference of the cell. It was characterized by a homogeneous structure (Fig. 15). In order to see if there was further development of the secondary wall in the endodermis, observations were done on sections of the root base of 21-day-old plants. It was not found, however, that the development of the secondary cell wall was any more advanced in older plants. It still remained thin, homogeneous and was found only in the cells positioned

PLATE I
Fig. 1. A cross section through a pea root at about 0.1 mm of its length. Already visible are differentiated proendodermis cells (arrow). 388 ×

Fig. 2. A diagram of a proendodermis cell. The dotted area denotes the cell wall. Nu — cell nucleus, Mi — mitochondria, Ga — Golgi apparatus, ER — endoplasmic reticulum, Pa — plastid, Va — vacuole, Li — lipid bodies

Fig. 3. A proendodermis cell; visible in the cytoplasm are: mitochondria (Mi), Golgi apparatuses (Ga), endoplasmic reticulum cisterns (ER), ribosomes. Vacuoles (Va) are filled with a fiberous, phenolic material. Nu — cell nucleus, Pd — plasmodesmata. 19000 ×

Fig. 4. Proendodermis cells with lipid bodies (Li) occurring in their cytoplasm. 5460 ×

PLATE II
Fig. 5. A diagram of endodermis cells having a Casparian strip in their radial walls. The cell wall area is dotted. Va — vacuole, Ga — Golgi apparatus, ER — endoplasmic reticulum

Fig. 6. The radial wall between neighbouring endodermis cells (En). The Casparian strip region is marked with an asterisk. Golgi apparatuses (Ga) with numerous vesicles are visible in the cytoplasm. Distended endoplasmic reticulum (ER) cisterns exhibit close contact with the plasma membrane (arrow). In the area between the plasma membrane and cell wall, fine osmophilic granules appear (arrow head). 19000 ×

Fig. 7. Casparian strip region (asterisks). The plasma membrane closely adheres to the cell wall; its three-layered structure is clearly visible (arrow). Endoplasmic reticulum cisterns (ER) are filled with a fiberous, osmophilic material (arrow head). 56000 ×

PLATE III
Fig. 8. A diagram of a cell wall (dotted area) between two neighbouring endodermis cells. On the surface of the cell wall of both cells, a suberin lamella (dark, thick line) which is being deposited, can be seen. Ga — Golgi apparatus, ER — endoplasmic reticulum

Fig. 9. The cell wall between an endodermis (En) and a cortical parenchyma cell (Co). A suberin lamella (arrow) being formed is visible. Fine osmophilic granules occur in ER cisterns and on the external surface of the plasma membrane (arrow head). 56000 ×

Fig. 10. A radial wall between neighbouring endodermis (En) cells, one of which has already formed a suberin lamella (arrow). In the distended ER cisterns (ER), a fiberous material is seen (arrow head). 38000 ×

PLATE IV
Fig. 11. A radial wall between endodermis cells (En). In one of the cells, a suberin lamella has been formed (arrow). 38000 ×
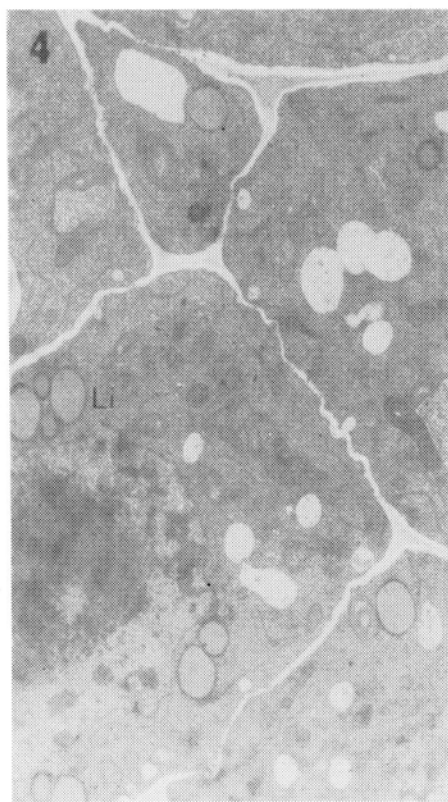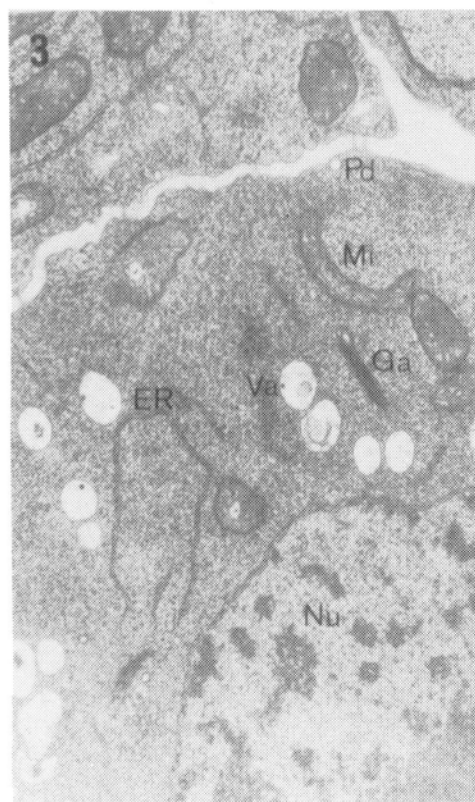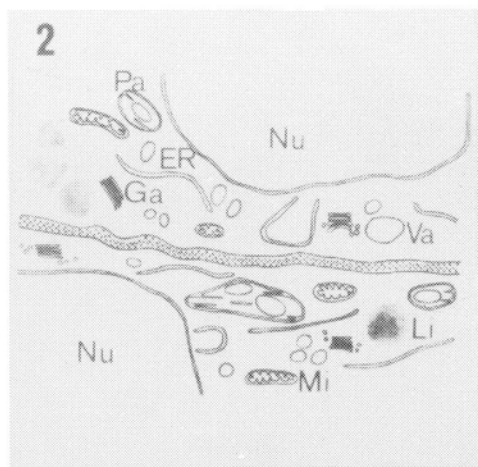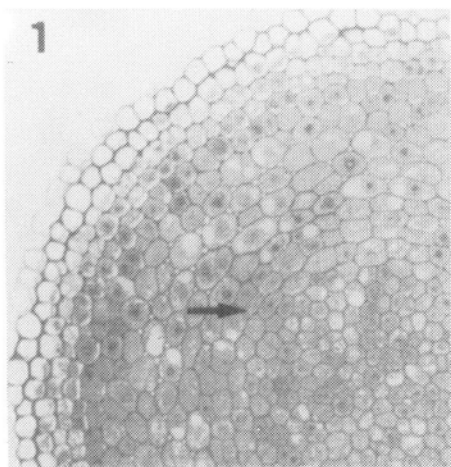
Fig. 12. A radial wall between neighbouring endodermis cells (En) in the vicinity of a Casparian strip. The regular outline of the suberin lamella has been disrupted, an osmophilic material is seen to have been built into it. 38000 ×
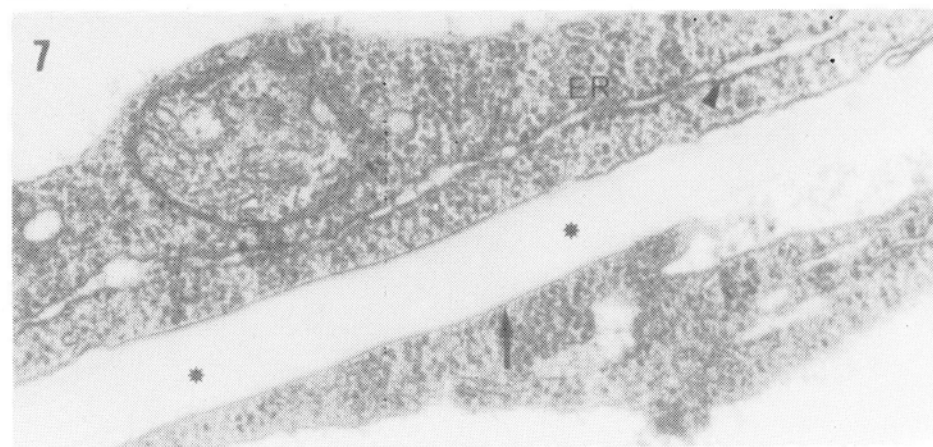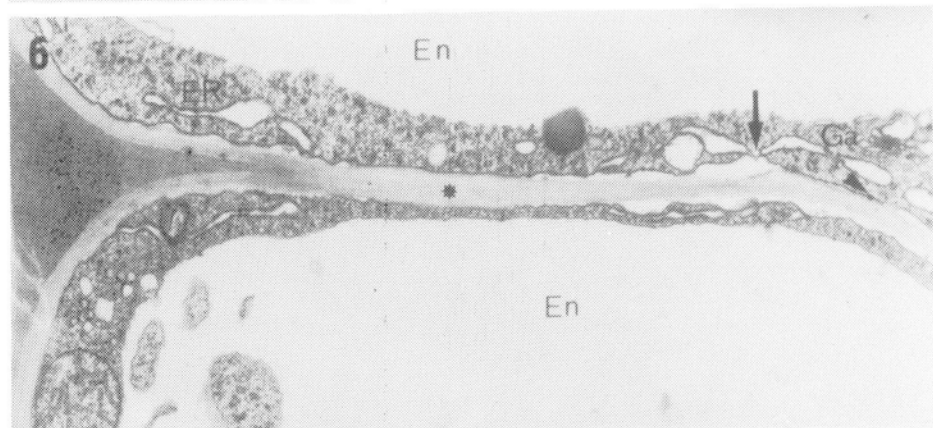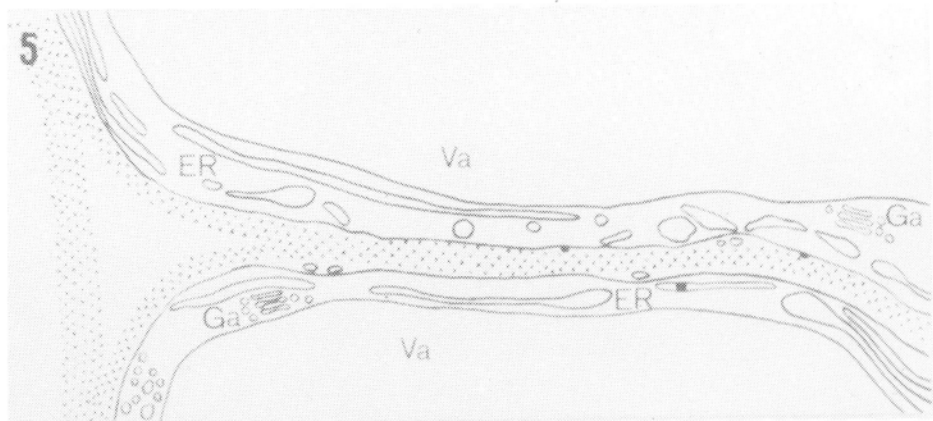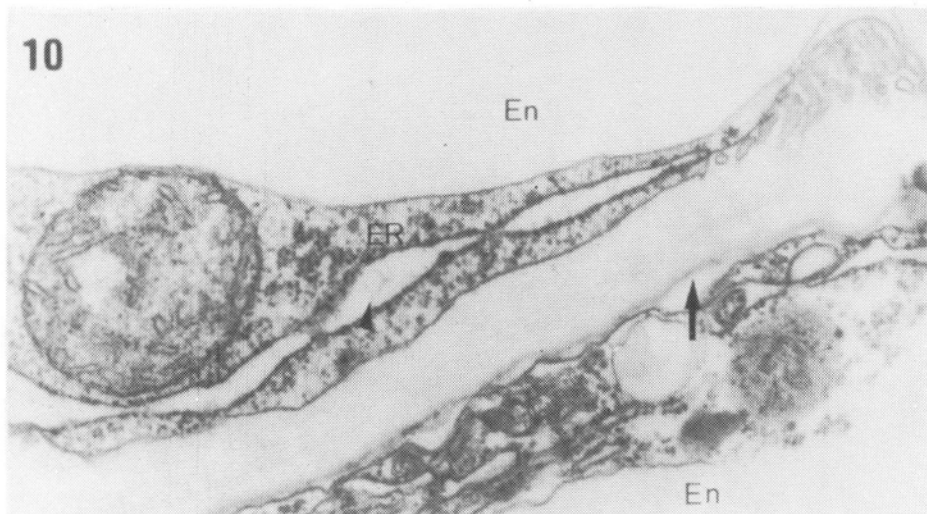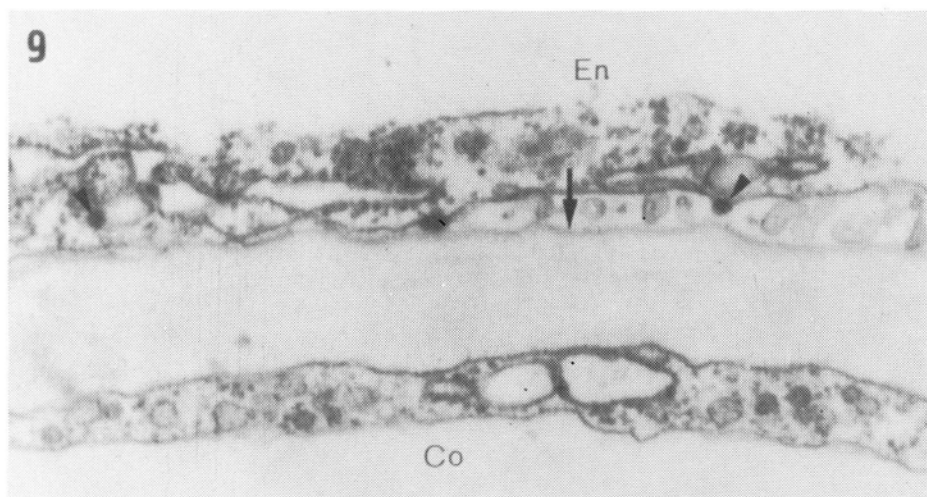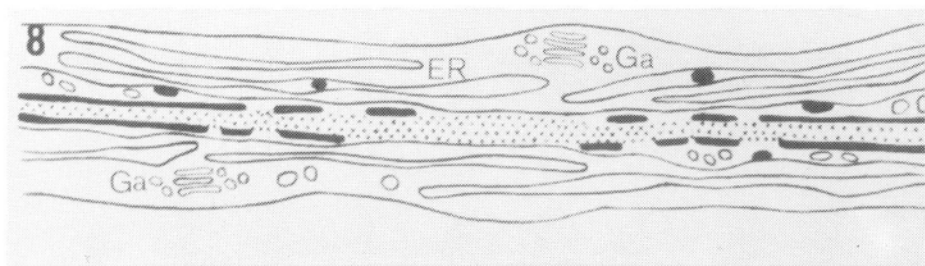
PLATE V
Fig. 13. A diagram of a radial wall between endodermis cells. In both cells, on the surface of the suberin layer (dark, thick line), a secondary cell wall is being formed (dotted area). Ga — Golgi apparatus, ER — endoplasmic reticulum

Fig. 14. A tangential wall between endodermis (En) and pericycle cells (Pe). The arrow in the endodermis cell points to the forming secondary cell wall. 38000 ×
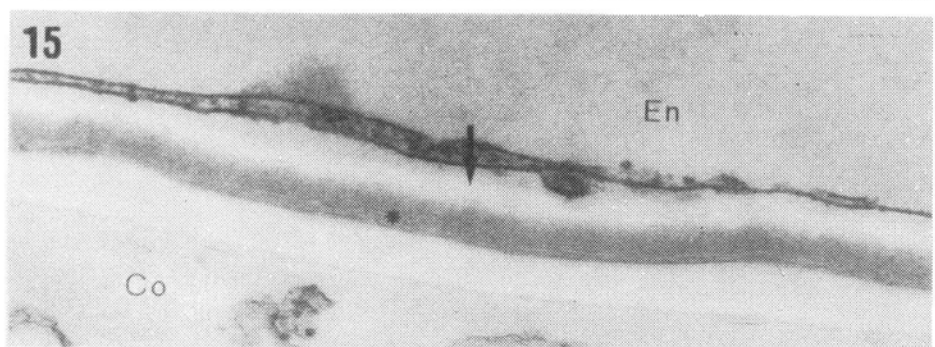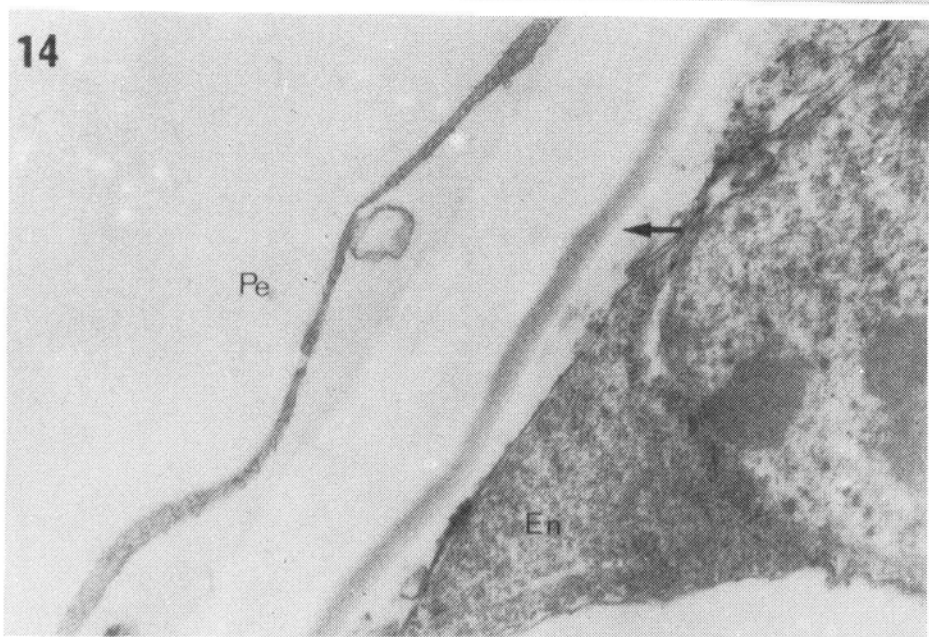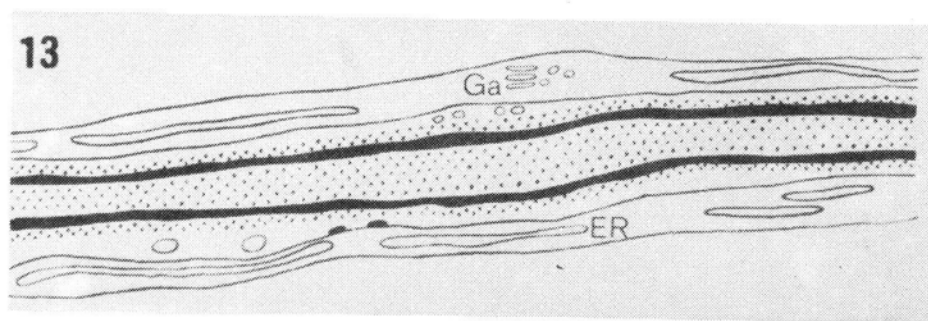
Fig. 15. A tangential wall between an endodermis (En) and cortical parenchyma cell (Co). The endodermis cell is in the third stage of development. On the surface of the suberin layer (asterisks) the secondary cell wall (arrow) is being deposited. 56000 ×

8

9

10

11

En

En

12

En

En

Figure 13 (labeled: Ga, ER)

Figure 14 (labeled: Pe, En, arrow)

Figure 15 (labeled: En, Co)
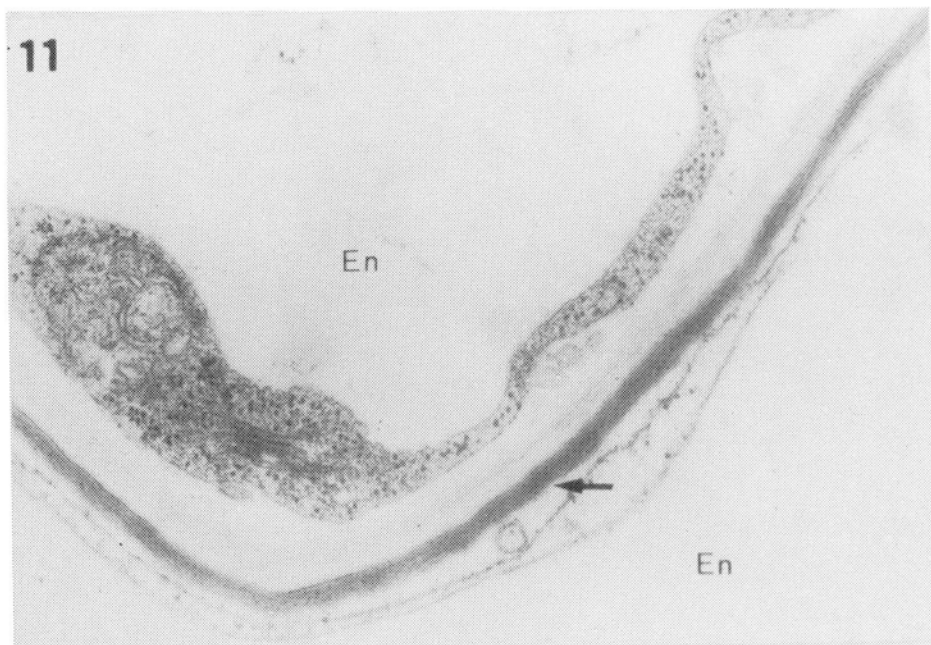
across from phloem bundles. The development of the pea root endodermis took place asynchronously, that is, the subsequent stages of development were always seen first in the cells above the phloem, then in those over the xylem.

DISCUSSION

On the basis of observations made with the light and electron microscopes, the following development stages of the pea root endodermis were determined: proendodermis, formation of Casparian strips, deposition of suberin lamellae, formation of secondary cell walls. The proendodermis had the characteristics of a typical meristematic tissue. The osmophilic, flocculent material found in its vacuoles is probably composed of substances phenolic in nature (Scott and Peterson 1979b). Van Fleet (1961) and Mueller and Beckman (1976) point to a high concentration of phenols as the most characteristic trait of the proendodermis and that which allows this tissue to be distinguished histochemically in the very early stages of root ontogeny. Our observations on pea roots do not support this thesis. The level of phenols in the vacuoles of proendodermis cells was low here, and could not be demonstrated by color reactions visible in the light microscope.

The characteristics of the Casparian strip region in the studied material — the thickening of the cell wall, its homogenous structure and tight contact with the plasma membrane are in agreement with data from literature (Bonnett 1968, Karas and McCully 1973, Robards et al. 1973, Haas and Carothers 1975, Scott and Peterson 1979a). Scott (1963) associates the fact of the strict adherence of the plasma membrane to the cell wall only with the occurrence of plasmodesmata in the Casparian strip, which would provide continuity between the protoplasts of endodermis cells, even under conditions of plasmolysis. Our observations indicate that the plasmodesmata in the region of the strips in pea roots occur sporadically, thus the explanation of the author cited above seems unconvincing. Taking into account the chemistry of the strip (Van Fleet 1961, Scott and Peterson 1979b), the hypothesis set forth by Bonnett (1968) that the increased affinity of the plasma membrane to the cell wall results from the interactions of the hydrophobic fatty material, built into the primary cell wall during the formation of the strip, with the membrane lipids, seems more probable. The facts that active dictyosomes have been observed in the immediate vicinity of the Casparian strips and that the vesicles arising from them have been found to accumulate, point to, along with the appearance of membranous structures in the space between the cell wall and plasma membrane, the participation of these structures in the

expansion of the strips. It seems that ER cisterns also take part, this because they exhibit close contact with the plasma membrane only in the vicinity of the Casparian strips. The osmophilic contents of the ER cisterns may be the materials used in the growth of the cell walls in the area of the strips.

The location in the cell where the suberin lamella is initiated is different for different plants. In the endodermis of *Abies procera* (Wilcox 1954), they were first found on the tangential internal walls; in the mestome sheaths of wheat, on the external tangential walls (O'Brien and Kuo 1975). In the pea endodermis they develop, similarly as in *Ranunculus acris* (Scott and Peterson 1979a) and pumpkin (Haas and Carothers 1975), simultaneously on both tangential and radial walls. The fiberous material, and also the osmophilic granules occurring in ER cisterns at this stage of endodermis development, also appeared between the plasma membrane and the suberin lamella which was being formed. These observations indicate that the suberin precursors may be contained in the ER cisterns. The participation of these structures in the synthesis of suberin precursors and in their transport has already been suggested by Barckhausen and Rosenstock (1973), Wattendorff (1974) and Scott and Peterson (1979a).

A problem giving rise to many controversies is the origin of the membrane fragments observed by many authors (Robards et al. 1973, Karas and McCully 1973, Haas and Carothers 1975) in the suberin lamella. They are of the opinion that the plasma membrane which, in the region of the strip tightly adheres to the cell wall, is engulfed when the suberin lamella is formed, and synthesized in these places *de novo*. Analysis of the microphotographs of the studied material seems to indicate that these could rather be the remnants of the membranous structures taking part in the formation of the suberin layer.

The process of the formation of the secondary cell wall in the endodermis of pea roots, in comparison with other mono- and dicotyledons (Clarkson et al. 1971, Harrison-Murray and Clarkson 1973, Robards et al. 1973, Haas and Carothers 1975, Scott and Peterson 1979a), was very weakly expressed. The wall developed exclusively in the cells positioned across from the floem bundles and was only a thin layer on the surface of the suberin lamellae. No impregnation of the wall with osmophilic material was found, as had been in barley (Clarkson et al. 1971, Robards et al. 1973), maize (Haas and Carothers 1975) and buttercup (Scott and Peterson 1979a). These authors have suggested that the impregnating material, which may be lignin, suberin or polyphenols, limits the permeability of the wall. In view of the fact that the secondary cell wall in the pea root endodermis retains its cellulose nature, it can be presumed that it is no barrier to apoplastic transport.

REFERENCES

Barckhausen R., Rosenstock G., 1973. Feinstructurelle beobachtungen zur traumatogenen suberinisierug beim knollparenchym von Solanum tuberosum L. Z. Pflanzenphysiol 69: 193–203.

Bonnett H. T., 1968. The root endodermis: fine structure and function. J. Cell Biol. 37: 199–205.

Clarkson D. T., Robards A. W., Sanderson J., 1971. The tertiary endodermis in barley roots: fine structure in relation to rapid transport of ions and water. Planta 96: 292–305.

Haas D. L., Carothers Z. B., 1975. Some ultrastructural observations on endodermal cell development in Zea mays roots. Amer. J. Bot. 62: 336–348.

Harrison-Murray R. S., Clarkson D. T., 1973. Relationship between structural development and the absorption of ions by the root system of Cucurbita pepo. Planta 114: 1–16.

Karas I., McCully M. E., 1973. Further studies of the histology of lateral root development in Zea mays. Protoplasma 77: 243–269.

Karnovsky M. J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 25: 137A.

Luft J. H., 1961. Improvements in epony resin embedding methods. J. Biophys. Biochem. Cytol. 9: 409.

Mace M. E., 1963. Histochemical localization of phenols in healthy and diseased banana roots. Physiol. Plant. 16: 915–925.

Mueller W. C., Beckman C. H., 1976. Ultrastructure and development of phenolic-storing cells in cotton roots. Can. J. Bot. 54: 2074–2082.

O'Brien T. P., Kuo J., 1975. Development of the suberized lamella in the mestome sheath of leaves. Aust. J. Bot. 23: 783–794.

Robards A. W., Jackson S. N., Clarkson D. T., Sanderson J., 1973. The structure of barley roots in relation to the transport of ions into the stele. Protoplasma 77: 291–311.

Scott E. M., 1963. Root hair zone of soil-grown roots. Nature 199: 1009–1010.

Scott M. G., Peterson R. L., 1979a. The root endodermis in Ranunculus acris. I. Structure and ontogeny. Can. J. Bot. 57: 1040–1062.

Scott M. G., Peterson R. L., 1979b. The root endodermis in Ranunculus acris. II. Histochemistry of the endodermis and the synthesis of phenolic compounds in roots. Can. J. Bot. 57: 1063–1077.

Turowska I., Kohlmünzer S., Molik-Węgiel J., 1970. Skorowidz fitohistochemiczny. AM, Kraków.

Van Fleet D. S., 1961. Histochemistry and function of the endodermis. Bot. Rev. 27: 165–220.

Wattendorff J., 1974. The formation of cork cells in the periderm of Accacia senegal Willd. and their ultrastructure during suberin deposition. Z. Pflanzenphysiol. 72: 119–134.

Wilcox H., 1954. Primary organization of active and dormant roots of noble fir Abies procera. Amer. J. Bot. 41: 812–821.

Struktura endodermy w rozwoju korzenia grochu (Pisum sativum L.)

Streszczenie

Na podstawie badań cytologicznych stwierdzono, że w rozwoju endodermy korzenia grochu pojawiają się kolejno: proendoderma, pasemka Caspary'ego, lamelle suberynowe oraz wtórna ściana komórkowa. Komórki proendodermy mają, oprócz normalnie występujących składników komórkowych, małe wakuole wypełnione związkami fenolowymi. Pasemka Caspary'ego rozwijają się w ścianie radialnej i stanowią nie więcej niż 1/3 jej długości. Warstwa suberynowa, obserwowana na wszystkich ścianach endodermy, najpóźniej odkłada się nad pasemkami Caspary'ego. Wtórna ściana komórkowa ma jednakową grubość na całym obwodzie komórki.