ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXIX — nr 4
1970

Ultrastructure of the Rootcap in *Raphanus sativus*

F. KADEJ and A. KADEJ

The rootcap in *Raphanus sativus*, similar to many other plants, consists of two distinct parts (Fig. 1, 2): the columella, which extends from the quiescent centre of the root axis, and the lateral part encircling the columella and extending away from the rootcap over the root surface, where it diminishes rapidly to a single cell layer. Both regions of the rootcap are composed of several hundred cells. The columella consists of about 350 cells arranged in 7—10 horizontal layers. Within the small rootcap area extensive structural and ultrastructural changes occur in both horizontal and vertical directions.

The ultrastructure of rootcaps has been studied by several workers in different plants: in *Zea* by Whaley, Kephart and Mollenhauer (1959, 1964), Whaley, Mollenhauer and Leech (1960), Clowes and Juniper (1968), in *Linum* by Deschamps (1967), in *Ranunculus* by Fineran (1966), in *Allium* by Clowes and Juniper (1964), in some epiphytic and aquatic species by Mollenhauer (1967), and in *Plantago* nucleolar changes in the rootcap were studied by Hyde (1967).

MATERIALS AND METHODS

The tips of the primary roots of *Raphanus sativus* were used for all observations. Seeds of *Raphanus* were grown in tap water at 25°C. From roots 4 cm in length the tips were cut off and fixed in phosphate buffered (pH 6.9) 4% glutaraldehyde for 12 hours. This was followed by phosphate buffered (pH 6.9) 1% OsO_4 and then the tips were immersed for 5 min. in 1% $KMnO_4$. The specimens were embedded in Vestopal.

Ultramicrotome preparations were stained with uranyl acetate and lead citrate according to Reynolds (1963). Preparations were examined with a Philips 100 electron microscope. For light microscopy the root tips were fixed in a solution of chromic acid, glacial acetic acid, and formalin (Cr AF 0,5—1—20). For determination of polysaccharides in the microtome preparations, the PAS (periodic acid Schiff) reaction was performed.

RESULTS

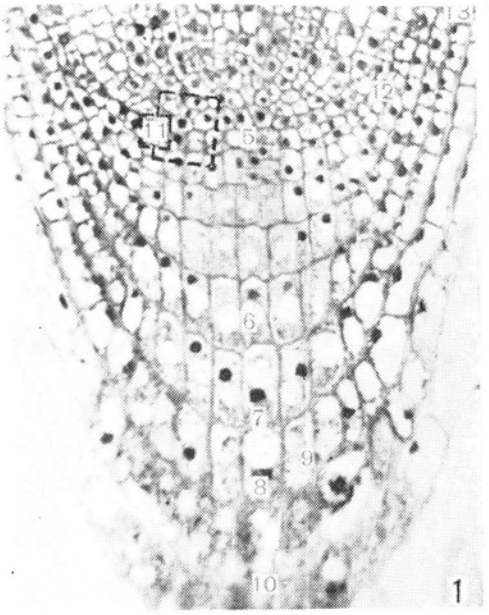
The layers of rootcap cells covering the apex of the root axis arise by periclinal divisions of the rootcap initials. The cells in layers are displaced to the apex and to the sides by newly formed cell layers. Columella cells mostly grow in a perpendicular direction and form regular vertical and horizontal layers. All cells in a given vertical column are of a common origin. Within the columella area it is possible to distinguish at least three separate regions of differing cell types. First, adjacent to the quiescent centre is the region of 1—3 layers of meristematic cells. Second, the 4th to 6th layers form an intermediate region of differentiating cells. Third, the 7th and following layers form a region of finally differentiated cells. The number of cell layers in each region may vary in different roots.

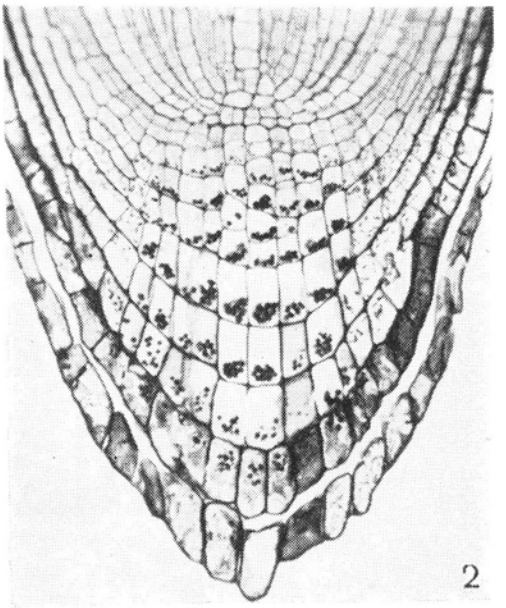
Cells of the initial layer remind one of the cells of the root meristem situated above the quiescent centre, but they differ distinctly in structure from the neighbouring cells of the quiescent centre (Fig. 4). The initial cells have relatively dense cytoplasm with all typical organelles: mitochondria of round profile and with well developed cristae as well as leucoplasts, sometimes with small starch grains. Rough endoplasmic reticulum (ER) occurs in the form of short, single cisternae. Dictyosomes with very few accompanying vesicles are also found. Vacuoles are not numerous, but in some cells reach fairly large dimensions. As a rule, the cells of the initial region have comparatively less starch than the cells of the quiescent centre, however, they have longer cisternae of ER (Fig. 5).

Lower, in the intermediate region of the columella the cells are larger. The most striking features are the large plastids packed with statolithic starch. The cells are becoming more parenchymatic in character due to their vacuolization. Cell organelles differ from those of the above region: dictyosomes are accompanied by numerous vesicles, and the structure of the mitochondria is more dense. ER in the form of long cisternae is often arranged in more or less parallel groups, sometimes near the cell walls (Fig. 6).

Figure 7 shows parts of two cells, one belonging to the intermediate region and the lower to the differentiated region. In the upper cell there are a few larger vesicles, however, the main feature of the lower cell is the occurence in the cytoplasm of numerous groups tightly bound vesicles. Each vesicle is limited by single membrane.

In further layers of cells (Fig. 8) it is possible to see differences in shape and contents of these vesicles. In a cell from the 6th layer flattened vesicles form stacks. Fig. 9 a cell of the 7th layer, has more inflated vesicles, and finally, in the external layer there form stacks of inflated vesicles with irregular profiles and also many scattered single circular vesicles randomly scattered (Fig. 10). These circular vesicles are so nume-





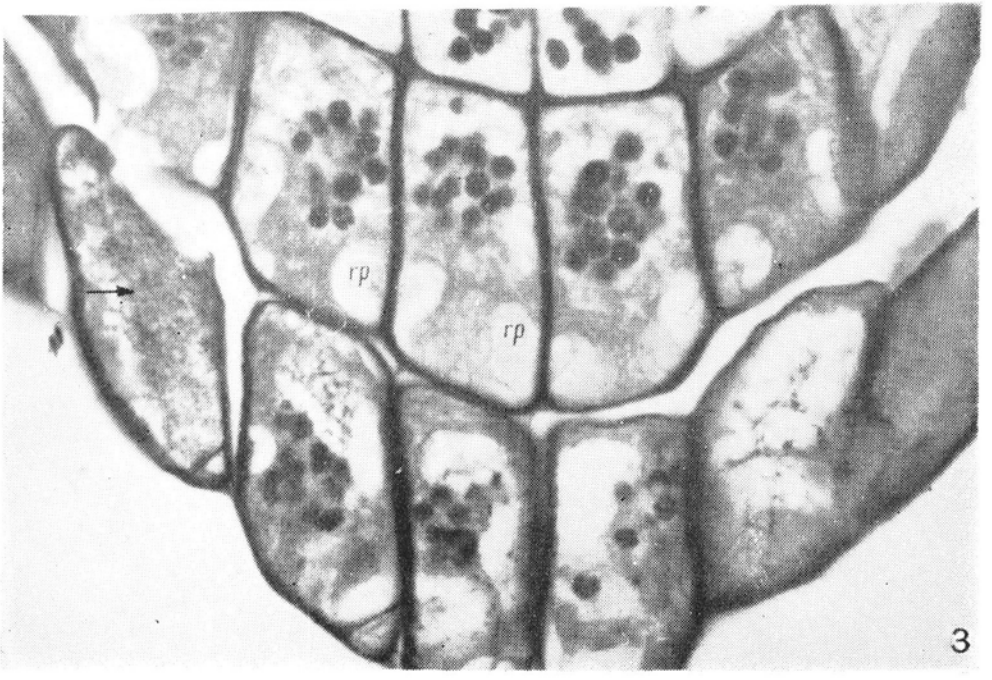


Fig. 1. Root tip of *Raphanus sativus*, indicating the location of electron micrographs (100 ×)

Fig. 2. Root tip after PAS reaction for polysaccharides (100 ×)

Fig. 3. Outer layer of the rootcap apex after PAS reaction. Small granules of PAS positive materials are in the cytoplasm of the outer layer cells (500 ×)

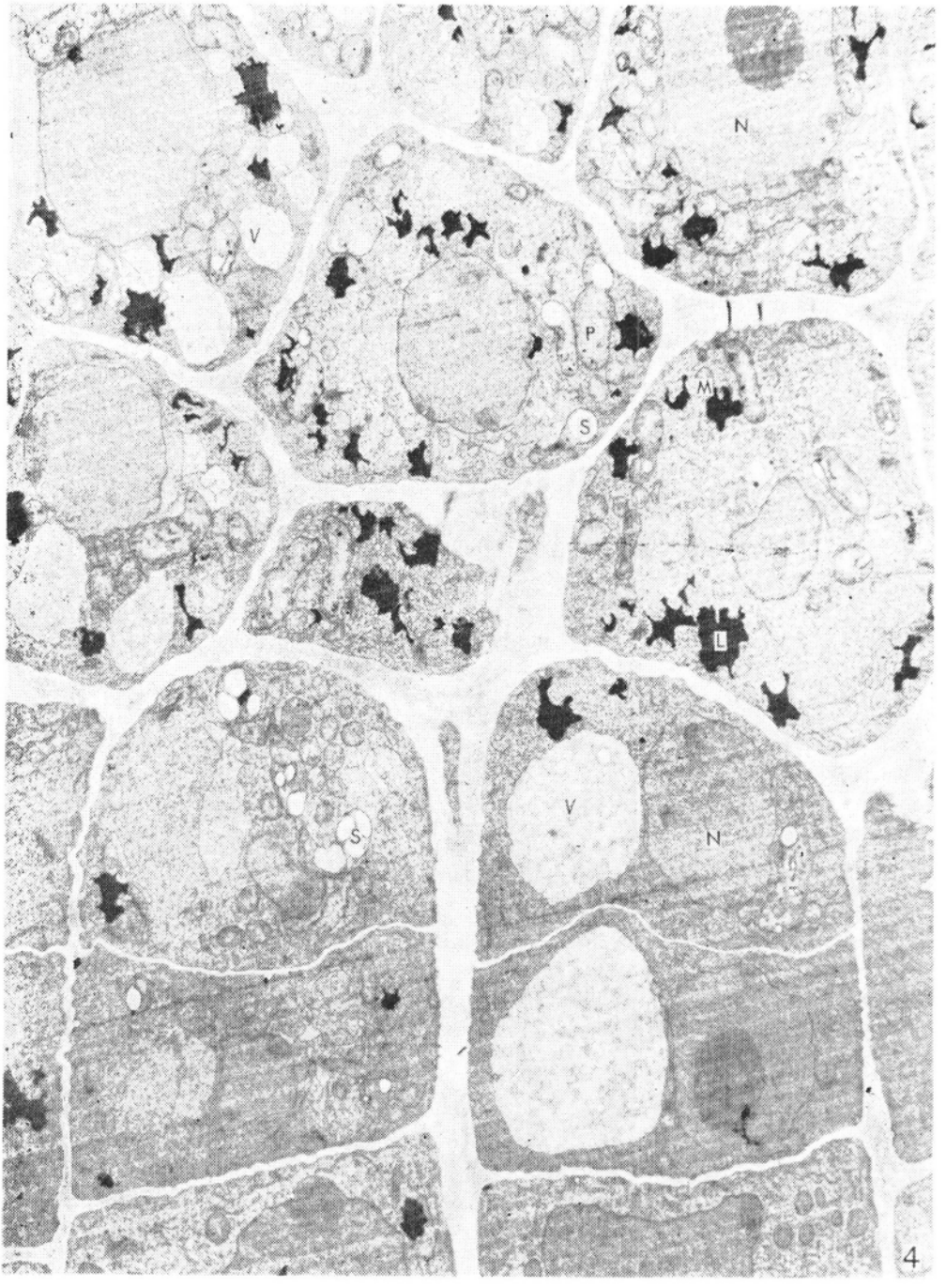


Fig. 4. In the upper part of the picture are cells of the quiescent centre. Underneath are rows of rootcap initial cells (ca 5000 ×)

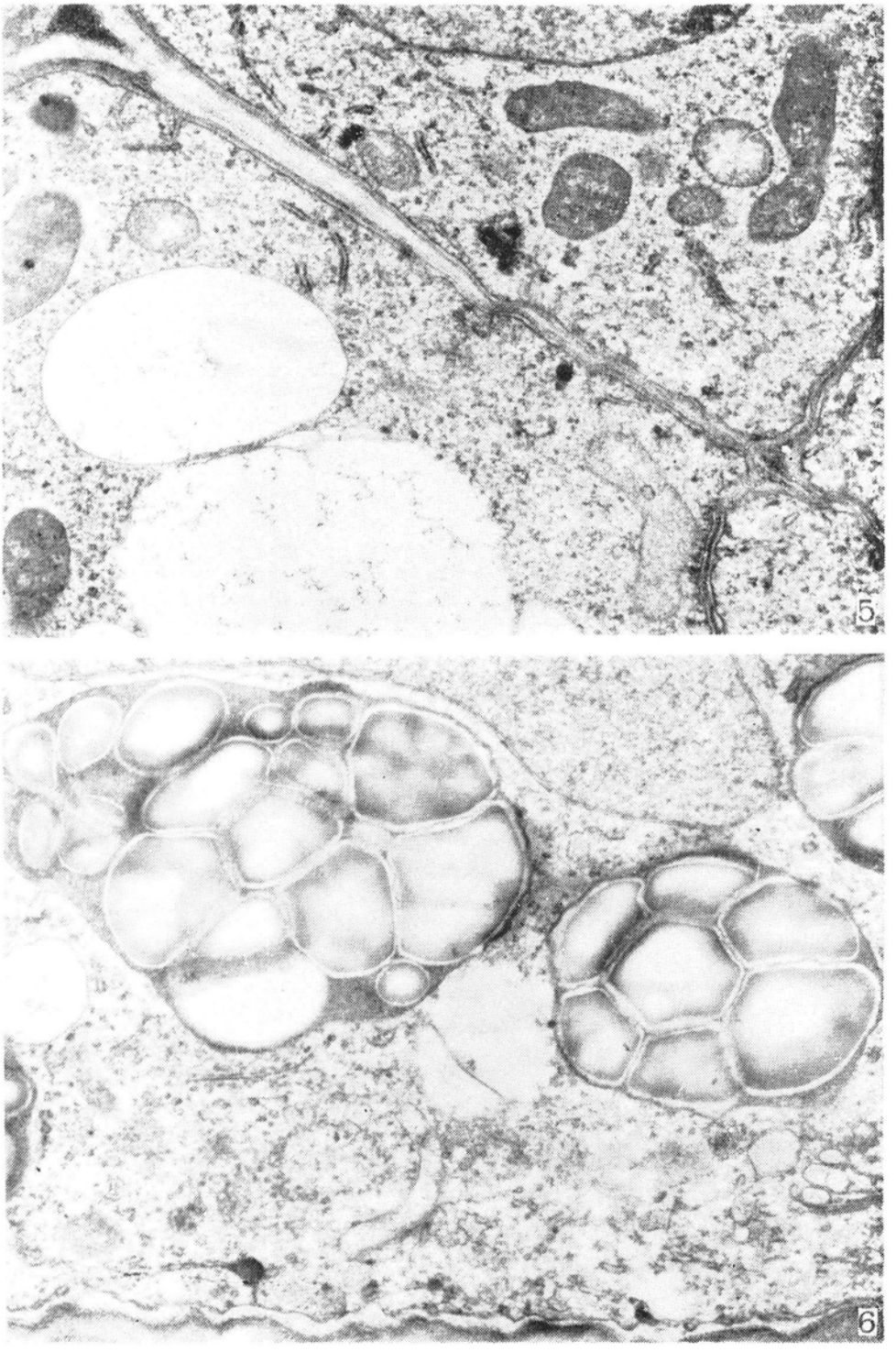
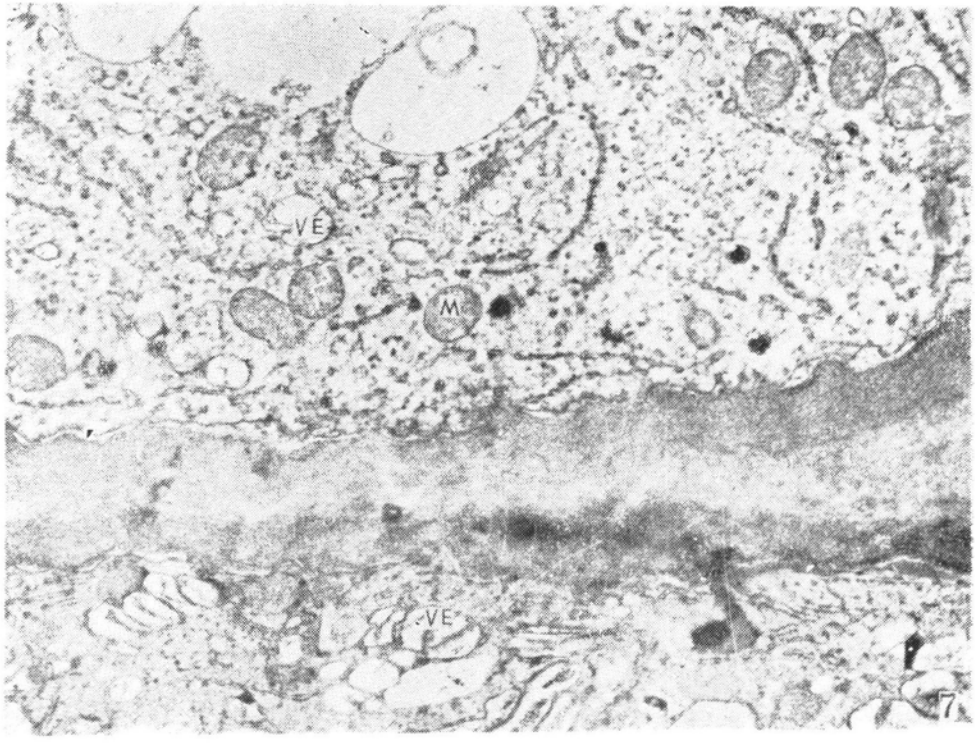


Fig. 5. Cell from the initial cell zone of the rootcap (20 000 ×)

Fig. 6. Cell fro mthe intermediate region of the columella with statolithic starch (20 000 ×)



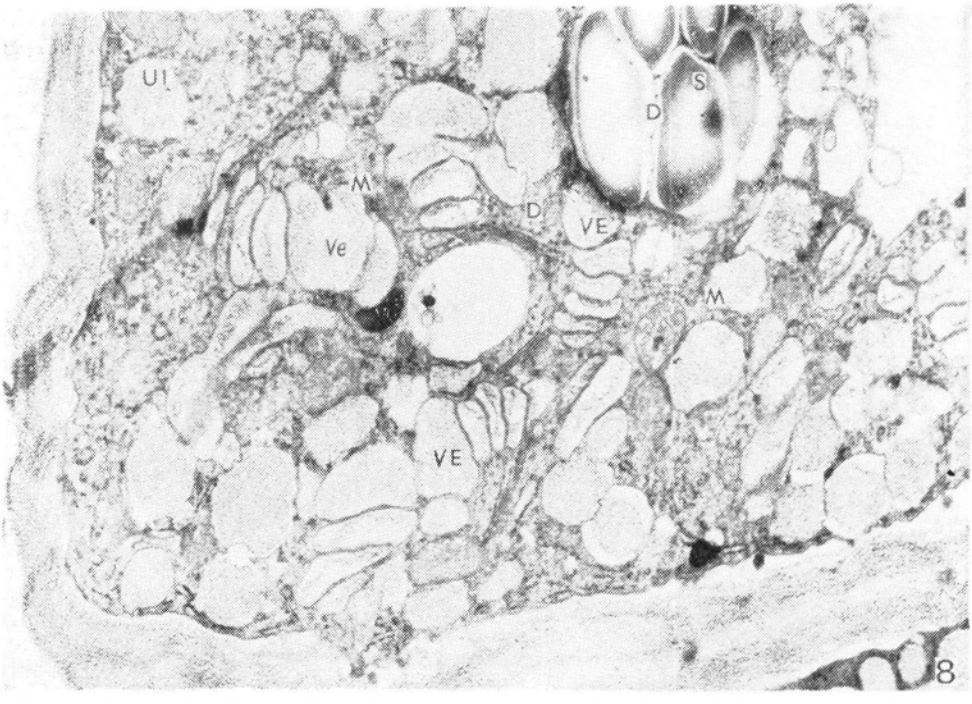


Fig. 7. Parts of cells in the 5th and 6th layers of the columella. Stacks of vesicles can be seen in the lower cell (2 000 ×)

Fig. 8. Cell from the 7th layer of the columella, showing stacked vesicles and unidentified bodies (20 000 ×)

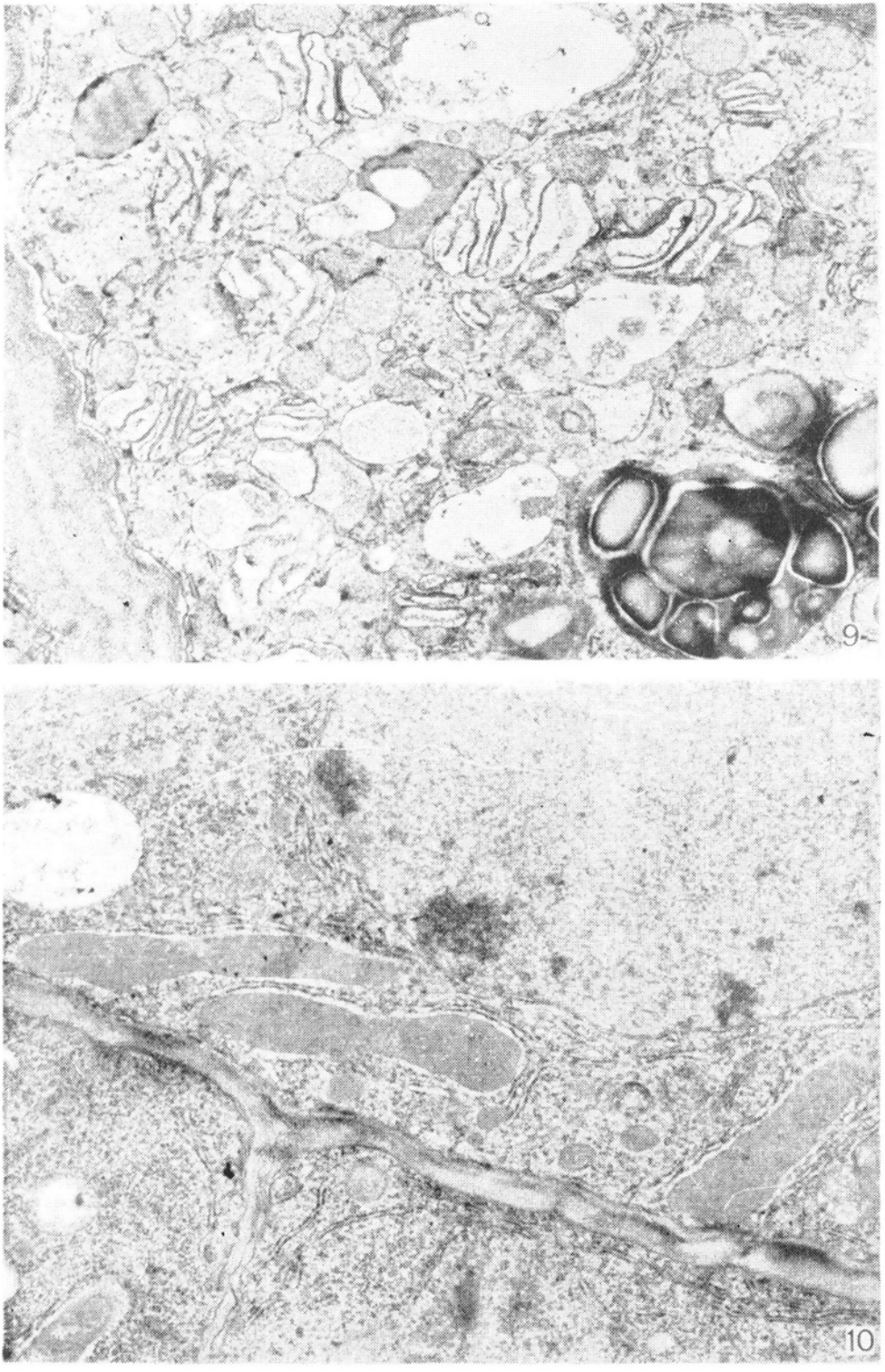


Fig. 9. Cell from the last but one layer of the columella. Vesicles with contents are visible (20 000 ×)

Fig. 10. Cell from the lateral part of the rootcap, adjacent to the initial zone. Elongated plastids and long cisternae of ER are visible (25 000 ×)

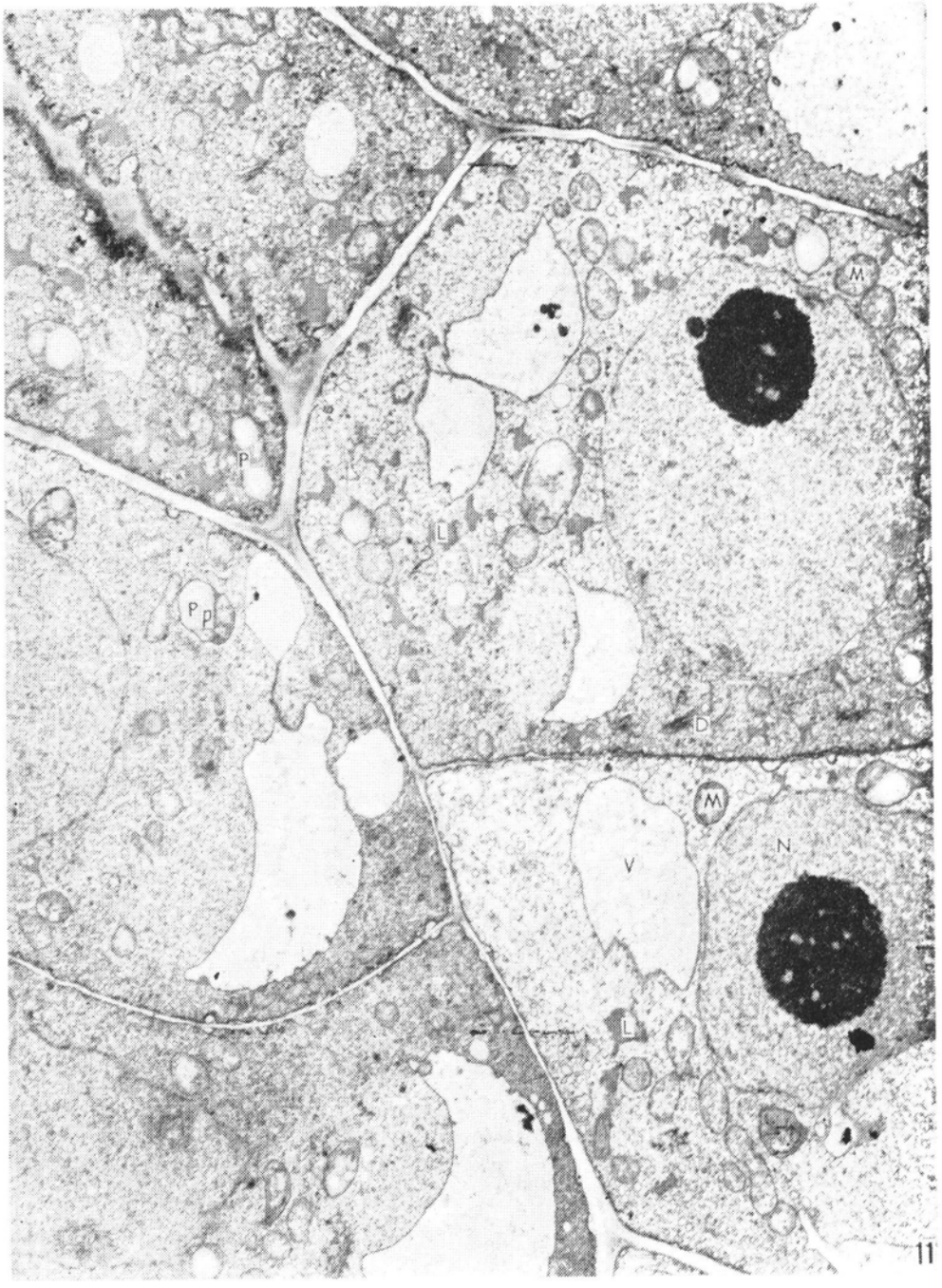


Fig. 11. Cells from the lateral part of the rootcap near the initial zone (10 000 ×)

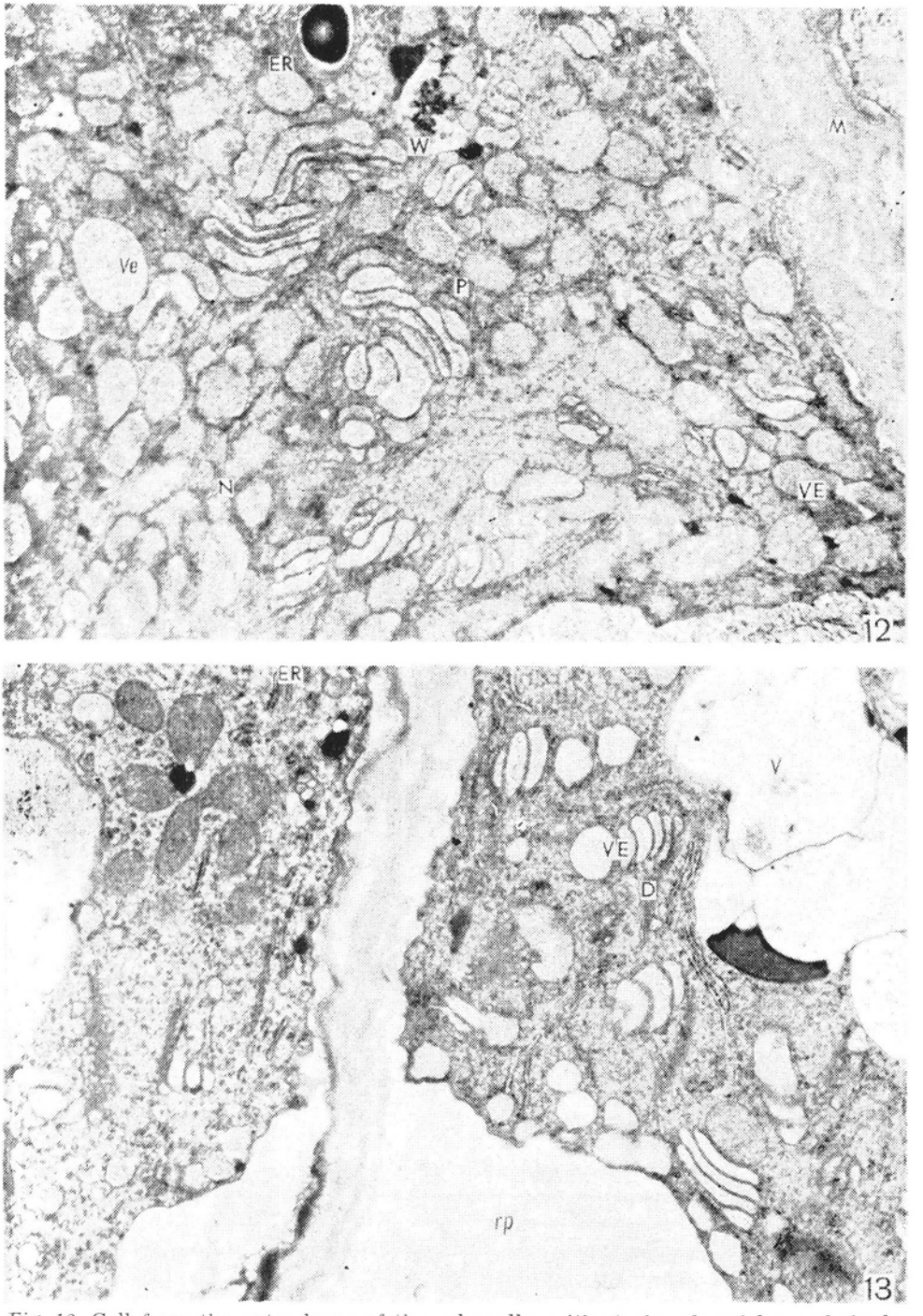


Fig. 12. Cell from the outer layer of the columella, with stacks of vesicles and single visicles (20 000 ×)

Fig. 13. Cells from the lateral part of the rootcap showing stacks of vesicles (20 000 X)

Legend: *L* — lipids *P* — plastids *S* — starch *V* — vacuole *N* — nucleus *D* — dictyosomes *ER* — endoplasmic reticulum *UI* — unidentified bodies *rp* — result of plasmolysing *VE* — vesicles *M* — mitochondria *W* — cell wall

rous, that they fill up almost completely the cytoplasmic space and are deformed due to their tight compaction. All vesicles in this region have fairly dense contents. The membrane of many vesicles seems to disintegrate. Rough ER still occurs in the form of long cisternae, but they are less easily to be seen as the vesicles filling up the cytoplasm. In the inner layers mitochondria are still visible and have a dense structure, but it is not possible to see them in the outer layers. There are also unidentified bodies with dense contents and of irregular shape. Dictyosomes are few, built of narrow cisternae in stacks of 3—5 and accompanied by thick vesicles. Statolithic starch is well preserved in the layer lying to the last one, but undergo disintegration in the outer layer.

The lateral parts of the rootcap are formed by initial cells similar in structure to those of the columellar region (Figs. 11, 12, 13). Differentiation in this region is generally similar to that in the columella. At first, the cells have dense cytoplasm, many ribosomes, and a large amount of rough ER in the form of long waved cisternae. The plastids are devoid of starch, optically dense and elongated. In further cells they contain small starch granules. The process of vesiculation is similar to that in the columellar region with characteristically stacked vesicles.

DISCUSSION

Extensive differentiation processes occur in the rootcap tissues. Over a span of several cell layers, meristematic cells are transformed into mature cells of parenchymatic character and then into cells considered to be secretory, producing slime substances. In the lateral region occur processes different from those described for the columella. These two regions have similar initial and final stages, but in the columella the first stage consists of the formation of tissue with statolithic starch, which is not present in the intermediate region of the lateral part. In connection with this, large plastids develop in the columella intermediate region. Initial cells of both regions have dense cytoplasm with profuse ER and ribosomes. This structure is changed gradually in further cells. Most striking in the course of these changes is the occurrence of a great number of large vesicles, characteristically grouped 3—6 together. The main visible element in the external layer are these vesicles containing some material.

At least some of these vesicles seem to arise from the expansion of the Golgi apparatus cisternae. This is indicated by the arrangement of these vesicle systems in rows or stacks similar to the Golgi cisternae. Also, the number of vesicles in a stack is relatively the same as the number of cisternae in a dictyosome. Many authors (Whaley, Kephart and Mollenhauer 1959, Mollenhauer 1967, and Spink and Wil-

s o n 1968) have seen a similar arrangement of vesicles directly related to the narrow cisternae of the dictyosomes. These pictures allow the supposition, that the vesicles are of Golgi origin. In *Raphanus* root cap a direct relationship between cisternae and vesicles has not been observed. This may be dependant on a different type of hypertrophy or other factors, such as the age of the root. It is not denied that the numerous single vesicles outside the stacks are of various origin.

Vesicle contents become denser in successive layers. We can presume the character of these contents after comparison of electron microscope and light microscope pictures of preparations with the PAS reaction for polysaccharides.

In the cytoplasm of the outer layer of cells there is a strong PAS reaction, and beside the large starch granules numerous small granules are visible (Fig. 3). These small granules may correspond to the vesicles seen in the electron microscope. (Figs 7, 8). Some authors, after using the PAS reaction and autoradiography in the electron microscope, have shown the polysaccharide character of the vesicle contents and their part in the formation of the cell wall and slime secretions covering the outer rootcap. Some amount of secretion material is reportedly accumulated between the plasmalemma and the cell wall (Morré, Jones and Mollenhauer 1967). In *Raphanus* rootcap accumulation here was not observed. However, optically empty PAS negative spaces were seen between the cell wall and the plasmalemma (Fig. 3). These spaces are considered to be the result of plasmolysis.

SUMMARY

The rootcap of *Raphanus sativus* displays various ultrastructural aspects within the columella and lateral regions. It is possible to differentiate 3 horizontal regions. The initial region contains meristematic cells with dense cytoplasm showing ER and other typical organelles. The intermediate region has large plastids filled with statolithic starch, long ER cisternae, large vacuoles, and a few vesicles. The region of final differentiation is characterized by the occurrence of numerous vesicles both in stacks of 3—6 and singly. It is assumed, that the stacked vesicles containing polysaccharide material originate from the expanded Golgi cisternae. These vesicles cause the characteristic appearance of secretory tissue which produces polysaccaride slime substances.

The authors wish to thank doc. dr B. R o d k i e w i c z for his interest and helpful discusion. They are also grateful to doc. dr L. Ż e b r o w s k i for the provision of working facilities in the Veterinary Institute in Pulawy, and Mr D. B i e b e r for his help in translating.

Department of Plant Anatomy and Cytology
M. Curie-Skłodowska University, Lublin, Poland

(Entered: July 9, 1970)

LITERATURE

Clowes F. A. L. and Juniper B. E., 1964, The fine structure of the quiescent centre and neighbouring tissues in root meristems, J. Exper. Bot. 15: 622—630.

Clowes F. A. L. and Juniper B. E., 1968, Plant Cells, Blackwell Scient. Publ. Oxford and Edinburgh, 177—194.

Deschamps R., 1967, Etude histologique, cytologique et ultrastructurale du méristème radiculaire du Lin, *Linum usitatisimum* L., Rev. Gén. Bot. 74: 159—175.

Fineran B. A., 1966, Fine structure of meristem and differentiating rootcap cells in *Ranunculus hirtus* Hook F., Phytomorphology 16: 1—16.

Hyde B. B., 1967, Changes in nucleolar ultrastructure associated with differentiation in the root tip, J. Ultrastr. Res. 18: 25—54.

Mollenhauer H. H., 1967, A comparison of rootcap cells of epiphytic terrestial and aquatic plants, Amer. J. Bot. 54: 1249—1259.

Morré D. J., Jones D. D. and Mollenhauer H. H., 1967, Golgi apparatus mediated polysaccharide secretion by outer rootcap cells of *Zea mays*, Planta 74: 286—301.

Spink G. C. and Wilson G. B., 1968, Golgi apparatus in tip cells of *Pisum sativum* primary roots, Trans. Amer. Microsc. Soc. 87(4): 516—521.

Whaley W. G., Kephart J. E. and Mollenhauer H. H., 1959, Developmental changes in the Golgi apparatus of maize root cells, Amer. J. Bot. 46: 743—751.

Whaley W. G., Kephart J. E. and Mollenhauer H. H., 1964, The dynamics of cytoplasmic membranes during development, 135—174. In Michael Locke (ed) 22 nd Symp. Soc. Study Devel. Growth Cellular Membranes in Development, Academic Press. New York.

Whaley W. G. and Mollenhauer H. H. and Leech J. H., 1960, The ultrastructure of the meristematic cell, Amer. J. Bot. 47: 401—449.

Ultrastruktura komórek kolumelii i części bocznych czepka korzenia Raphanus sativus

Streszczenie

Czepek wykazuje odmienną ultrastrukturę na terenie kolumelli i w częściach bocznych. Wzdłuż podłużnej osi czepka można wyróżnić trzy strefy. W strefie inicjalnej komórki mają gęstą cytoplazmę z dobrze rozwiniętym systemem ER, oraz inne organelle typowe dla wszystkich komórek merystematycznych. W strefie pośredniej komórki mają duże plastydy ze skrobią statolityczną, długie cysterny ER, duże wakuole i drobne pęcherzyki. W strefie ostatecznej dyferencjacji liczne, duże pęcherzyki wypełniają całe wnętrze komórek. Przypuszcza się, że powstają one z cystern Golgiego i są przejawem ich działalności wydzielniczej.