MICROSCOPIC AND SUBMICROSCOPIC STRUCTURE OF DEVELOPING ROOT CAP OF *CERATOPTERIS THALICTROIDES***

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ABSTRACT

The anatomy and some submicroscopic cellular structures in the root cap of developing adventitious root of *Ceratopteris thalictroides* were studied. The root cap initial originates from the single apical cell in the root meristem by means of sequencial segmentation. The root cap initial then follows an orderly cell division to give rise to a multicellular root cap since the cap cells are traceable to their common origin or to the apical cell. The cap cells increase considerably in size as they develop. During the course of cap cell development, the nucleus is still present with little increase in size and the nucleolus appears to be absent in older root cap cell. Most of the cytoplasmic organelles maintain rather complete figures even in the sloughing off cells except the plastids. The possible significance of wall structure in relation to the process of sloughing off is discussed. The root cap shows characteristic features which lead to the consideration that the root cap may be an acting or modified multiseriate epidermis.

INTRODUCTION

The root cap is commonly present in both land and water plants. The water plants are often considered to have a more extensive root cap (Esau, 1961). The cells of the root cap are commonly homogeneous being living parenchyma cells. The structure, development and the function of the root cap under natural as well as the controlled environments have been studied by several workers (Bartoo, 1929, 1930; Conard, 1908; Richardson, 1955). All the root tissues including the root cap in some pteridophytes arise from a common origin, *i.e.*, the single apical cell in root meristem. The origin and development of root cap in ferns have been studied in some ferns (Bartoo, 1929, 1930; Conard, 1908; Clowes, 1961; Pal and Pal, 1962). Although the root caps in these ferns originated from the single apical cells, the patterns of later development in these ferns are considerably different from each other. The present author intends to trace the sequence of root cap formation in the root of *Ceratopteris thalictroides*. The observations with the electron microscope will assist in getting a better understanding of the development of root cap in these ferns.

MATERIALS AND METHODS

The root tips of young adventitious roots (about 5 cm in length) of *Ceratopteris*

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thalictroides (L.) Brongn. growing in the tank of a green house were harvested and fixed immediately in Craf III for anatomical study. Customary methods of dehydration and paraffin embedding were followed using a tertiary butanol series. Serial sections of both transverse and longitudinal planes cut at 8–10μ were stained with safranin and fast green (Johansen, 1940).

For study with the electron microscope, roots were fixed in a solution consisting of one part 25% aqueous solution of glutaraldehyde and 8 parts 0.15 molar phosphate buffer at pH 7.2 and followed by buffered 1% $KMnO_4$ for two hours. The materials were dehydrated in an alcohol-propylene oxide series and embedded in Maraglass (Pease, 1964). Sections were cut with a diamond knife and were stained with lead citrate (Reynold, 1963). Sections were examined with a Hitachi HS–7S electrom microscope.

RESULTS

The apical meristem of the root of *Ceratopteris thalictroides* is conical which is always closely covered by a conical root cap of living parenchyma cells, usually in four to six layers at the region in the front of the apical cell and in successively fewer layers in the regions behind or away from the apical cell (Fig. 1). No intercellular spaces are found in the face between the root cap and the apical meristem. The root cap consists of a group of growing cells which are closely packed and have no intercellular spaces. The inner cells of the root cap, especially those cells near the apical cell, are smaller and younger, meristematic and somewhat tabular in shape. The outer cells of the root cap become progressively larger and older. The outer cells near the tip of the root cap are roughly isodiametric, whereas those cells away from the tip are very much elongated (Fig. 1). The cells of the root cap as well as the other tissues of the root are produced by successive divisions of the segments cut from the basal face (distal face) and three lateral faces of the apical cell. The immediate segments formed from three lateral faces of the apical cell give rise to various initials of the tissues of the root proper whereas the segment from the basal face gives rise to the root cap.

The new cells of the root cap are produced by successive divisions of the apical cell cut from the basal face, while the outer most cells of the root cap are constantly sloughed off. The root cap of *C. thalictroides* consists of two or three clearly distinguishable groups of cells (Fig. 1B). These cell groups, or constituting units are distinguishable because each of them has its own origin. In the following account these cell groups, or constituting units, of the root cap are 'sub-units of root cap' for convenience. The sub-unit of the root cap itself, is also conical in shape and its cells are arranged in two layers, except in the marginal region where the cells are uniseriate (Fig. 1B).

The immediate segments cut from the basal face of the root apical cell is the

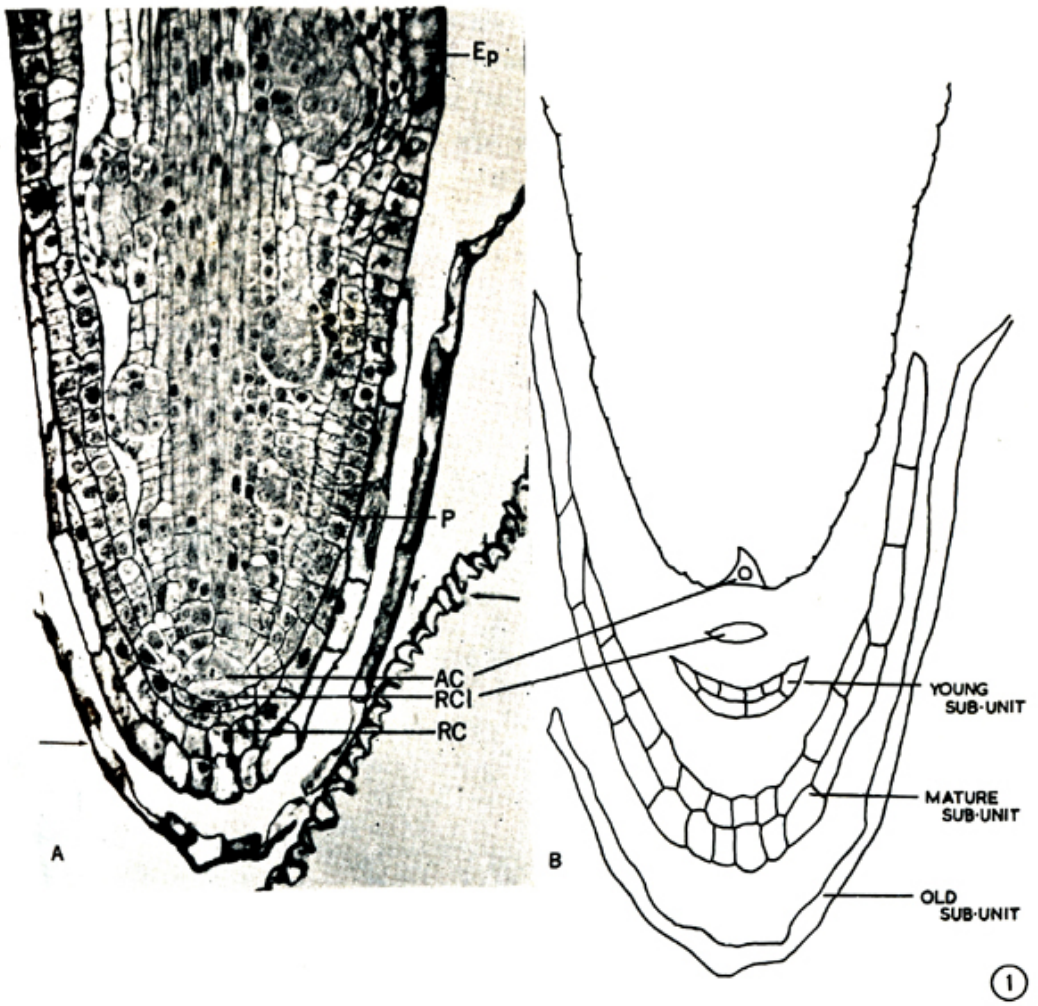

Fig. 1A. A microphotograph of median longitudinal section of the root tip of an adventitious root, showing apical cell (AC), root cap initial (RCI), root cap (RC), older layers of root cap that is sloughing off (arrows), protoderm (P) and epidermis (Ep), ×135.

Fig. 1B. A diagram showing the constitution of the root cap which is depicted in Fig. 1A. The root cap is composed of three constituting units. Each sub-unit of the root cap has its own initial cut from the basal face of the root apical cell. The sub-unit of the root cap is biseriate except at the margin where it is uniseriate. The sub-units are shown as separate entities for purposes of analysis.

root cap initial which is lenticular in shape (Figs. 3a, 3h). The first and second divisions of the root cap initial are anticlinal, *i.e.*, perpendicular to the basal wall of the apical cell, and also perpendicular to each other, resulting in a quadrant, a four-celled young sub-unit of the root cap (Figs. 3b, 3i). The wall resulting from the second division is distinguishable from that formed in the first division since it does not meet the first wall in the same plane (Figs. 3i, 3j). Each of these four cells then undergoes an anticlinal division, perpendicular to the first wall, to produce a uniseriate eight-celled sub-unit of the root cap (Figs. 3d, 3k).

As can be seen in a figure presented by Pal and Pal (1962, Fig. 13), this anticlinal division also occurs perpendicularly to the second wall of the quadrant. The four inner (median) cells of the eight-celled sub-unit of the root cap continues to divide by anticlinal divisions parallel to the first wall, resulting in a uniseriate twelve-celled sub-unit of the root cap (Fig. 3l). Four cells in the center of this twelve-celled sub-unit of the root cap then undergo periclinal divisions to become eight cells in two layers (four in each layer), resulting in a sixteen-celled sub-unit of the root cap (Figs. 3f, 3n). Pal and Pal (1962) stated that this periclinal division occurs in the sixteen-celled stage which they studied from material obtained in

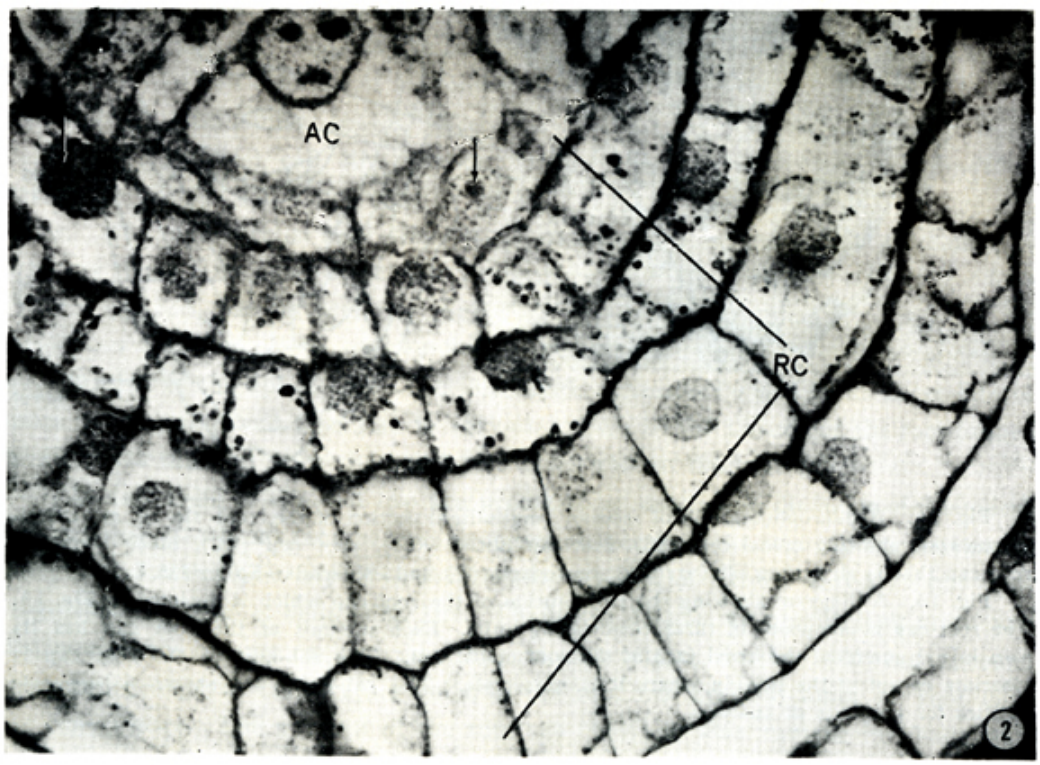

Fig. 2. A median longitudinal section of root tip through apical cell and root cap, showing the apical cell (AC) and successive layers of cap cells (RC), ×1,700. Note the homogeneously stained nuclei in older cap cells and identifiable nucleoli in young cap cells (arrows).

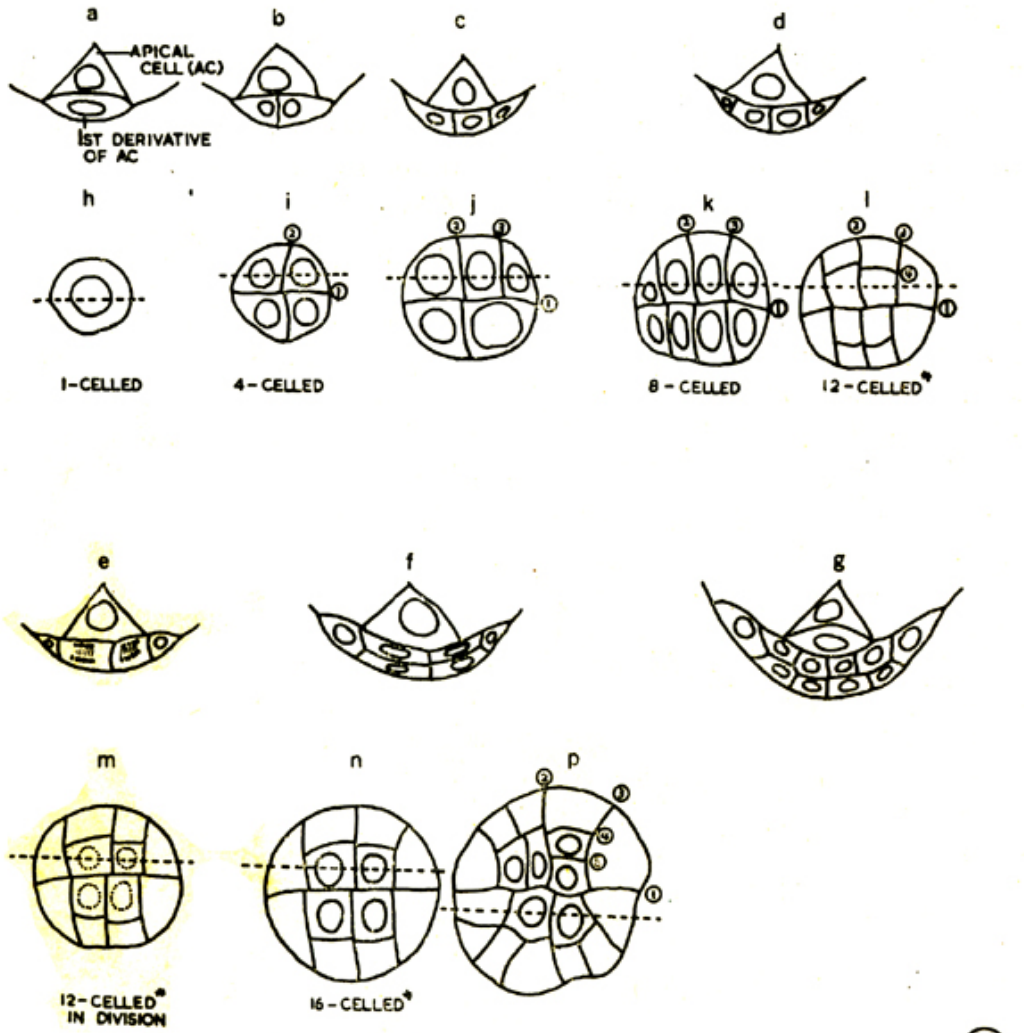

Fig. 3. Successive stages in the development of the root cap, all ×260. Figs. a-g. Longitudinal sections of the root cap through the apical cell and the root cap. Figs. h-p. Semi-diagramatic or diagramatic (*) illustrations of successive stages in the development of the root cap as seen in transverse sections through the root cap. Broken lines indicate where the longitudinal sections in Figs. a-g were made. The Arabic numbers in the circles indicate the sequence of wall formation. The nuclei in Figs. n-p indicate that the cells are in two layers. The lenticular root cap initial is seen in Figs. a, g and h.

India. The subsequent divisions of this group of sixteen cells occur only anticlinally, *i.e.*, no periclinal division occurs again in this sub-unit of the root cap (Figs. 3g, 3k). The root cap development in lateral roots is essentially similar to that of the main root.

The submicroscopic structure of root cap cells of rapidly growing roots

measuring about 5 cm in length was investigated with the electron microscope. The magnification used for this investigation ranged from 4,000 to 25,000 times.

The young root cap cells which are located close to the apical meristem (especially the apical cell) contain more cytoplasmic organelles. As can be seen in Fig. 4, a large nucleus occupies the central part of the young cap cell. The nucleus has a two-layered envelope with numerous pores. The outer membrane of the nuclear envelope has some external extensions. Both envelope and its extensions resemble endoplasmic reticulum in having two layered membrane which are approximately 30 mμ apart. No extension of the inner membrane of the nuclear envelope toward the inside of the nucleus was observed. The nucleoplasm of the interphase nucleus (the mitotic figure has not been observed in the root cap under electron microscope during this investigation) is structurally homogeneous with many fine granules which are uniformly distributed. The nucleus of a young cap cell contains two or more nucleoli which are reticulate and densely stained and are not delimited by any membrane (Figs. 4, 5). The nucleolus of the young cap cell can be easily identified even in the material prepared by the paraffin method (Fig. 2). On the contrary, the nucleus appears to be homogeneous in the old root cap cells (Fig. 2) and an identifiable nucleolus can not be found. Though the cap cells increase considerably in size as they develop (Figs. 1A, 2), there is no conspicuous change in the nuclear size (Table 1). The nuclei of older root cap cells are slightly larger than those of younger root cap cells. The nucleus is still present even in the old cap cells which are in the process of sloughing off (Fig. 1A).

Table 1. Diameter of nucleus in root cap cell (in μ)

	Young sub-unit (12-16 celled stage)	Mature sub-unit	Old sub-unit
Average*	11.26	11.52	12.73

* Average of 5-9 cells from each of seven roots.

Like the nucleus the plastid is delimited by a double layered membrane (Figs. 4, 5, 6, 7). The grana are not well developed, having only one to three layered lamellae. Intergrana or frets are short. Starch granules are found in the stroma in some plastids. Statoliths were not found in the sections examined in this investigation. The plastids containing starch granules seem more abundant in the root cap cells than in root proper tissue. The disintegration of the plastids is the most conspicuous degenerating process among all of the cytoplasmic organelles during the development of root cap cells (Fig. 7). Most of the cytoplasmic organelles, such as nucleus, endoplasmic reticuulm, mitochondrion and spherosome-like body maintain rather complete figures even in the cap cells which are in the process of sloughing off (Fig. 6). The most significant phenomenon occurring in

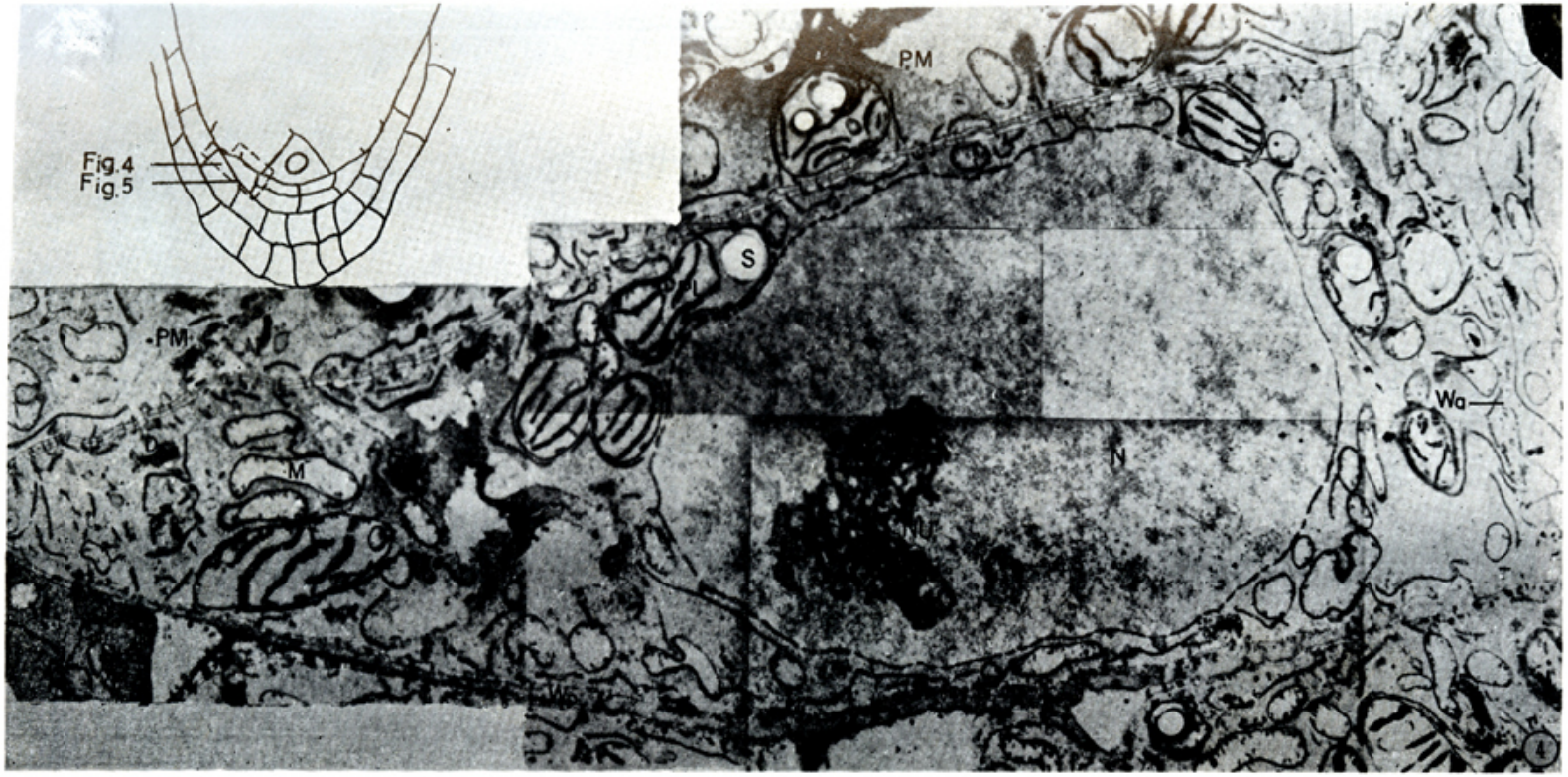

Fig. 4. Electron micrograph of longitudinal section of a young root cap cell, ×7,000. Insert: A drawing indicating the region where Figs. 5 & 6 were made. Key to labeling for electron micrographs Figs 4-7: AC, apical cell; D, dictyosome; ER, endoplasmic reticulum; G, granum; M, mitochondrion; N, nucleus; NE, nuclear envelope; NU, nucleolus; PD, plasmodesma; PL, plastid; (PL), disintegrating plastid; PM, protodermal cell; RC, root cap; S, starch granule; SP, spherosome-like body; V, vacuole; Wa, anticlinal wall in cap cells; Wp, periclinal wall in cap cells.

Fig. 5. Electron micrograph of longitudinal section of young root cap cell (partial enlargement of Fig. 4), ×18,000. See Fig. 4 for labeling.

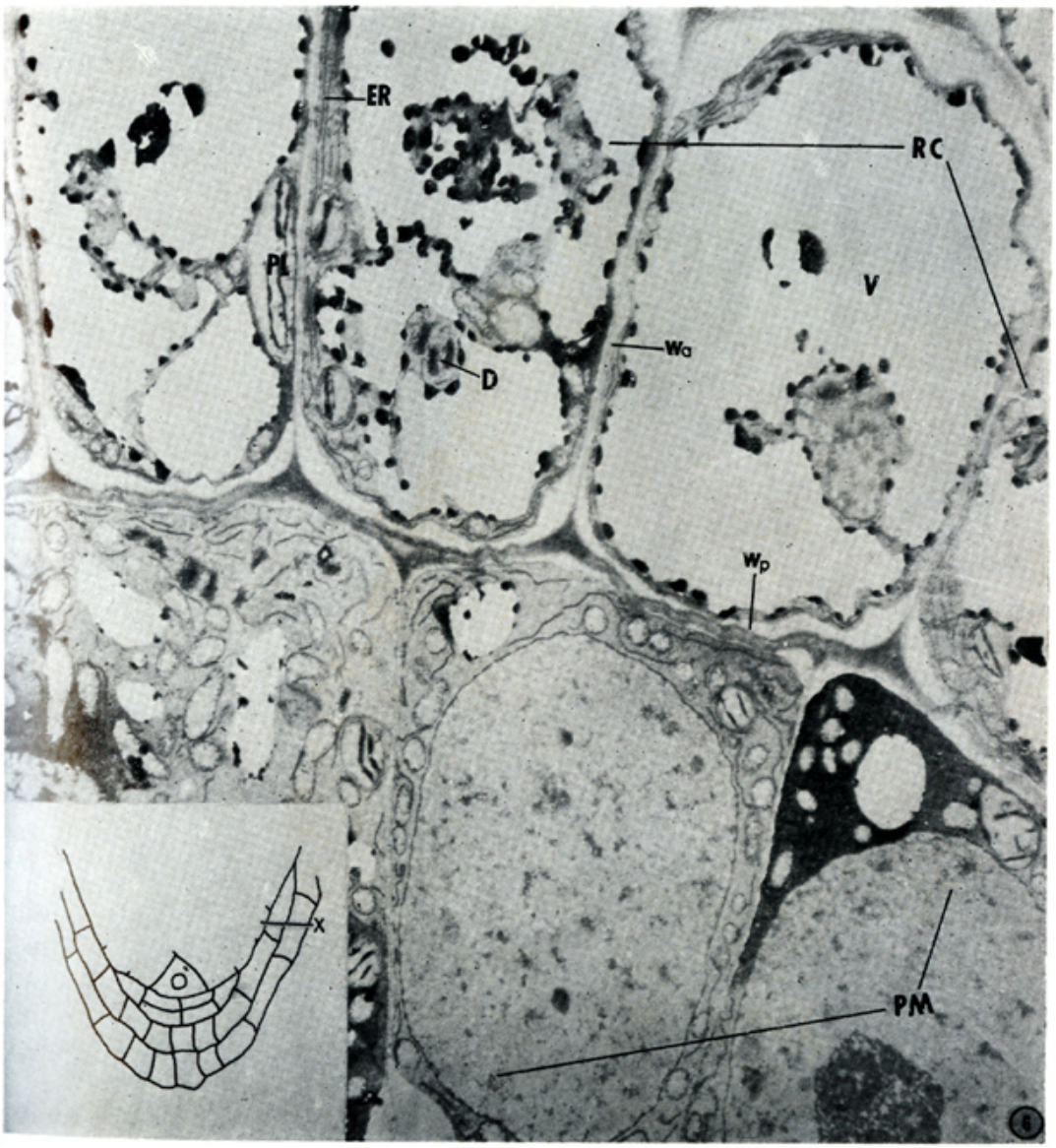

Fig. 6. Electron micrograph of transverse section through protoderm and mature root cap cells, ×5,200. Insert: A drawing indicating the region (×) where Figs. 6 & 7 were made. See Fig. 4 for labeling.

Fig. 7. Electron micrograph of transverse section of root cap cells, ×28,000. Note the disintegrating plastids, thicker periclinal wall; and large vacuoles. See Fig. 4 for labeling.

the developing root cap cells is the enlargement of vacuole which is delimited by a single membrane or tonoplast.

The periclinal wall in the root cap cell is always considerably thicker than the anticlinal wall (Figs. 4, 5, 6, 7). Numerous plasmodesmata are seen in the walls located between the apical cell as well as its close derivatives in the root proper and young root cap cells (Fig. 4). The number of plasmodesmata becomes fewer as the wall grows thicker (Figs. 6, 7).

DISCUSSION

As stated in earlier report (Chiang, 1967), all of the root tissues of *Ceratopteris thalictroides* including the root cap cells have a common origin or a single tetrahedral apical cell which has four faces, three lateral and one basal face. The segment formed from the basal face, the root cap initial, gives rise to the root cap, whereas the segments from the lateral faces give rise to various initials of the tissues of the root proper. These four faces of the apical cell do not divide with the same frequency. Typically every tenth division of the apical cell occurs at its basal face, resulting in a root cap initial (Chaing, 1967). The succession of three segments cut off from the lateral faces of the apical cell and then one from the basal face is strictly maintained in *Marsilea quadrifolia* (Clowes, 1961), *Schizaea rupestris* (Bartoo, 1929), and *S. pusilla* (Bartoo, 1930). The root cap initials of these ferns divide only by anticlinal divisions and so each layer of the older root cap cells remains only one cell in thickness. This comes about because cap initials are formed from the apical cell quite frequently in these species, *i.e.*, by every fourth division in the apical cell, and no further periclinal divisions are needed in order to maintain a definite number of layers of the root cap. In *C. thalictroides* (Kny, 1875; Lachmann, 1907; Pal and Pal, 1962), *Equisetum palustre* (Wagner, 1939), and *Azolla filiculoides* (Clowes, 1961), however, the number of segments produced from the side faces of the apical cell is considerably greater than that from the base. Therefore, in these species the number of cell layers in the root cap is increased by means of periclinal divisions in the cap initials. These divisions keep pace with the growth of the root proper. In these ferns, each layer of the older root cap in the central part is two layers in thickness. In *Pteris cretica* the cap initials divide only by anticlinal divisions, but in *Adiantum emerginatum* and *Polypodium falcatum* there are also periclinal divisions (Campbell, 1895). In *Dennstaedtia punctilobula*, the cap initial gives rise to a single layer of root cap cells, but periclinal walls were occasionally seen in three to five or six of the median cells, making the segment two layered at that point (Conard, 1908). Although Campbell and Conard did not discuss the behaviour of the root apical cell in these plants, there might be a general relationship between the sequence of apical cell division and that of cell division in root cap initials.

The function of the root cap of *C. thalictroides* has not been studied in the present work, and indeed has received little or no consideration from other authors. As mentioned earlier, no sign of statolith bodies has been seen in any part of the root cap, and the plastids in the root cap cells contain more starch granules than those in cells of any other part of the root. There is no information about whether the root cap has the ability to perceive gravity as does that of maize (Juniper, Groves, Schachar and Audus, 1966). Since the root cap is present without exception on the surface on the root apical meristem, the only region on the root where no epidermis is present, or differentiated, it may be considered that the root cap is an acting or substitute multiseriate epidermis which is capable of undergoing division and elongation in order to keep pace with the growth of the apical meristem. Its multiseriate and compact nature and its ability to be sloughed off and renewed are especially adapted to perform a function as a modified epidermis fitting over the naked active meristem of the root. The high thickness of periclinal wall may cause the old cap to separate easier from the adjacent layers of younger cap layer by layer (Fig. 1A). Besides, the sloughy root cap cells show the similar cell wall pattern as that in the annulus of fern sporangium (Fig. 1A, arrows). The uneven thickening of the cell wall causes the sloughing process after the contraction of cap cells. And the decrease of the number of plasmodesma may assist in cutting the movement of cellular materials from the root proper. These morphological characteristics of cap cells play an important role in the constant sloughing off of the root cap in this plant.

LITERATURE CITED

(1) BARTOO, D. R., 1929. Origin and development of tissues in root of *Schizaea rupestris*. Bot. Gaz. 87: 642-652.

(2) ———, 1930. Origin of tissues of *Schizaea pusilla*. Bot. Gaz. 89: 137-153.

(3) CAMPBELL, D. H., 1895. The Structure and Development of the Mosses and Ferns. Macmillan & Co., New York.

(4) CHIANG, S. H. T., 1967. Histological studies of the root of *Ceratopteris thalictroides* (L.) Brongn. Ph. D. thesis, Univ. of California, Davis.

(5) CLOWES, F. A. L., 1961. Apical meristems. Blackwell Scientific Pub. Oxford.

(6) CONARD, H. S., 1908. The structure and life-history of the Hay-Scented Fern. Carnegie Inst. of Washington Pub. no. 94. Washington D. C.

(7) ESAU, K., 1961. Anatomy of Seed Plants. John Wiley & Sons, Inc., New York & London.

(8) JOHANSEN, D. A., 1940. Plant Microtechnique. McGraw-Hill Book Co. Inc., New York.

(9) JUNIPER, B. E., GROVES, S., SCHACHAR, A. L. and AUDTS, L. J., 1966. Root cap and the perception of gravity. Nature; London. 209: 93-94.

(10) KNY, L., 1875. Die Entwickelung der Parkeriaceen dargestellt an *Ceratopteris thalictroides* (L.) Brongn. Abhandl. K. Leop.-Carol. Deutsch. Acad. Nat. 37: 1-80.

(11) LACHMANN, P., 1907. Origine et developpement des racines et des radicelles du *Ceratopteris thalictroides*. Rev. Gen. Bot. 19:523-556.

(12) PAL, N. and PAL, S., 1962. Studies on morphology and affinity of the Parkeriaceae. I. Morphological observations of *Ceratopteris thalictroides*. Bot. Gaz. 124: 132-143.

(13) PEASE, D. C., 1964. Histological Technique for Electron Microscopy. 2nd ed. Academic Press. New York.

(14) 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.

(15) RICHARDSON, S. D., 1955. The influence of rooting medium on the structure and development of the root-cap in seedlings of *Acer saccharinum* L. New Phytol. 54: 336-337.

(16) WAGNER, N., 1939. Uber die Entwicklungsmechanik der Wurzelhaube und der Wurzelrippenmeristems. Planta 30: 21-66.