

## Activation of embryo during rape (*Brassica napus* L.) seed germination

### II. Transversal organisation of radicle apical meristem

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#### Abstract

Series of microtome cross sections of the root apical meristem were investigated in the mature embryo and young seedling of rape. The cell patterns are described in 3 layers of promeristem. Radial sectors of the root cap and protoderm, formed by common dermatocalyptrogen initials, and radial sectors of the cortex, produced by periblem initials were identified on all cross sections of the root. Between these sectors 4 segmentation boundaries of proembryo quadrants were distinguished, running across the whole root proper. The boundaries between the 4 sectors of connecting cells arising from the upper hypophysis derivative and the boundaries between the 4 sectors of the columella originating from the lower hypophysis derivative do not follow the same course and are not identical with the boundaries of the proembryo quadrants. Therefore during the whole embryogenesis, the central connecting cells, considered generally as cortex initials (iec), take no part in the development of the cortex but they form the quiescent centre of the radicle. Neither do the columella initial cells participate in the development of the lateral parts of the root cap.

#### INTRODUCTION

In the preceding paper (K u r a ś, 1978) the organisation of the apical meristem of the radicle in mature rape seeds was described on the basis of longitudinal sections. It was found that the meristematic root primordium is produced by the three-tier promeristem (Fig. 1) consisting of central cylinder initials (plerome), cortex initials (periblem), cells initiating the protoderm and the lateral parts of the root cap (dermatocalyptrogen), and of the initials of the central part of the root cap

(columella). These initials surround the intermediate layer of central connecting cells.

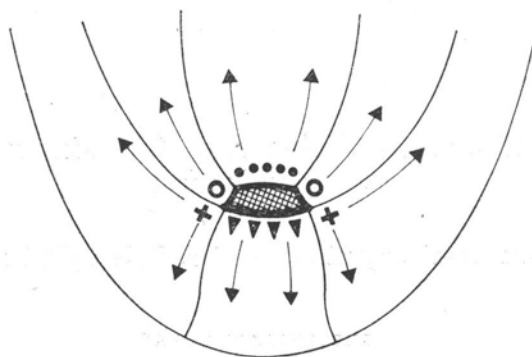


Fig. 1. Promeristem of rape radicle

Full circles — plerome initials; open circles — periblem initials; crosses — dermatocalyptrogen initials; triangles — columella initials; hatched — layer of central connecting cells

The central connecting cells are generally considered to be the initials of the root cortex. Tykarska (1979) indicates, however, unequivocally that these cells take no part in the development of the cortex in the primary stage of radicle development in rape. Analysis of the cell pattern on longitudinal sections of the mature embryo root suggests that these connecting cells do not either take part in the last stages of embryogenesis (Kuraš, 1978).

The cell arrangement visible at the boundary of the columella and lateral parts of the root cap on longitudinal sections through mature embryos seems to suggest that the lateral parts of the root cap adjacent to the columella might be of common origin with the peripheral cell files of the columella. If this were true the youngest parts of the cortex would also be of common origin with the peripheral binding cells since the growth of the promeristem cells is sympastic.

The origin of the lateral parts of the root cap or cortex could be ascertained on cross sections through the rape root apical meristem if the boundaries between the cell families forming the radial sectors could be distinguished.

The layer of central connecting cells and the columella arise in rape and other *Cruciferae* from the hypophysis after its periclinal division to two cells, whereas the plerome, periblem and dermatocalyptrogen initials develop in the quadrants of the proembryo (Souèges, 1916, 1919; Lebègue, 1952; Tykarska, 1976). Each of the two hypophysis derivatives is independently divided into 4 sectors which may eventually undergo further divisions. The boundaries of the sectors in

both these layers may, therefore, have a different course, and not coincide with those of the proembryo quadrants, running across the plerome, periblem, dermatogen and the lateral parts of the root cap.

It was found that these boundaries can be distinguished not only in the embryo but in the young seedlings. The cell pattern in the layer of central connecting cells and in the columella initial layer can be established. The initials and radial sectors of the cortex as well as those of the lateral parts of the root cap can also be recognized.

Owing to this, it was possible in the present study to demonstrate that in the course of embryogenesis the central connecting cells constitute the quiescent centre of the radicle and do not take part in the development of the cortex, and that the initial cells of the columella do not contribute to the development of the peripheral parts of the root cap.

#### MATERIAL AND METHODS

The radicles of embryos from winter rape seeds *Brassica napus* L. var. *oleifera*, cf. Górczańska) and roots of 2.5 cm seedlings were fixed in chromacetoformalin (CrAF-0.5-1-20) and after passing through xylene were embedded in paraffin. The serial cross sections 5  $\mu$ m thick were stained as described by Kuraś (1978).

Analysis of the cell pattern was performed on a continuous series of cross sections photographs of two roots.

At first the boundaries between the quadrants were distinguished and outlined in the plerome initials layer and then these boundaries were recognized and prolonged between the sectors of cortex and root cap. Then boundaries of the 4 sectors in the layers of central connecting cells and the columella initials were demarcated.

The radial cortex and root cap sectors started by the periblem initials and dermatocalyptrogen are easily identified at various levels. Much more difficult was the recognition of the plerome initials derivatives. It was, however, possible, owing to the preservation by the daughter cells of the same contacts with the neighbouring cells as those of the mother cells.

#### RESULTS

##### COLUMELLA AND DERMATOCALYPTROGEN

When distinguishing the derivatives of the columella initial cells on the basis of their position and mutual contacts on a series of cross sections through the cap, it is easy to note that the columella develops quite separately from other histogens, both in the radicle and in the root of young seedlings (Plates I (2-6), II (3), IV (3) and Fig. 2 (C)).

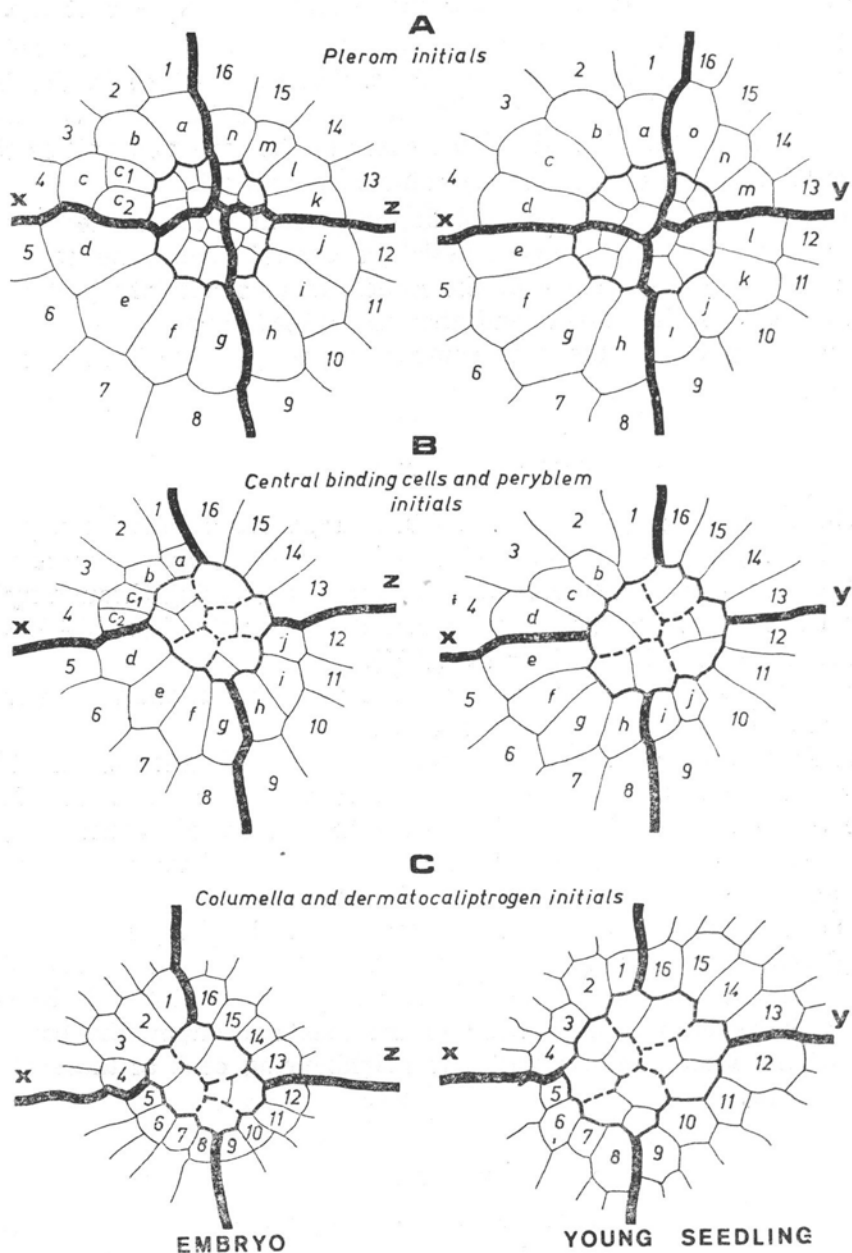


Fig. 2. Cross section through promeristem of embryo and young seedling  
1-16 — dermatogen sectors; a-o — periblem sectors



In the group of several initial cells of the columella (7 or 11) 4 sectors comprising cells of common origin can be distinguished. They may not be of equal size, however, the course of the boundaries between them indicates that the first division of the columella initial divided it into two almost equal parts, and the differentiation of the sizes of the 4 sectors occurred in both analysed roots in the second division. The complete lack of coincidence between the boundaries of the columella sectors and those of the dermatocalyptrogen quadrants and its lack between the boundaries of the outer initials of the columella and those the dermatocalyptrogen are evidence of the separate development of the lateral parts of the root cap and the columella.

Dermatogen and the outer layers of the lateral part of the root cap were formed (in both the roots examined in detail) by 16 dermatocalyptrogen initial cells denoted in Plates II (3), III (3) and in Fig. 2 (C) by Figs. from 1 to 16. On all cross sections containing the lateral parts of the root cap, 16 radial sectors of this cap started by these initials can be recognized. In the early period of embryogenesis, at the moment of formation of the external layers, particularly of the first layer of the root cap, the number of the dermatocalyptrogen initials was probably lower. Therefore not all the boundaries of the 16 dermatocalyptrogen sectors can be identified in the external layers. The boundaries of the quadrants, however, are quite distinct even in the first root cap layer (Plates II (3), and IV (3)).

#### CENTRAL CONNECTING CELLS

On the cross sections directly above the layer of columella and dermatocalyptrogen initials a plate of about 10 central connecting cells, surrounded by periblem initials is visible. Four sectors of the cells, separated by walls perpendicular to one another can be distinguished. The course of the boundaries of these sectors does not agree with that of the boundaries of columella sectors and with those of the quadrants of the root proper (Plates II (2), IV (2) and Fig. 2 (B)). It is therefore, obvious that the central connecting cells could not take any part in the development of the periblem either in the period of embryogenesis or in the first period of root growth in the seedling.

#### PERIBLEM

In both roots there were 15 periblem initials encircling the central group of connecting cells denoted in Fig. 2 (A, B) and in Plates II-VI by letters.

In the root of the young seedling to each of the 3 periblem quadrants correspond 4 radial periblem sectors, and to the smaller fourth quadrant

correspond 3 sectors (m, n, o, Plate IV (1) and Fig. 2 (A)). On the sections lying higher one of the sectors of this fourth periblem quadrant is divided in two by radial divisions of all its cells. The number of radial periblem sectors thus increases in the central part of the meristem to 16.

It increased similarly during embryogenesis of the examined radicle. In this there are 14 radial periblem sectors, one of which (C) ends in two sister initials (Plate II (1)). It is obvious that in the first period of development of this radicle there were 14 periblem initials in it (2 quadrants with 4 initials and 2 quadrants with 3 initials). In the end phase of embryogenesis division of the initial cell occurred in one of the smaller periblem quadrants, therefore in the higher lying parts of the apical meristem of this root there are 16 periblem sectors.

In the widening part of the radicle and in the basal part of the seedling root meristem, gradually further radial divisions of the cortex cells occur. These begin with the division of the proendodermis and the outer cortex layer cells.

The number of concentric cortex layers does not change within the root apical meristem after forming 4 cell layers by periclinal centripetal division of successive periblem segments.

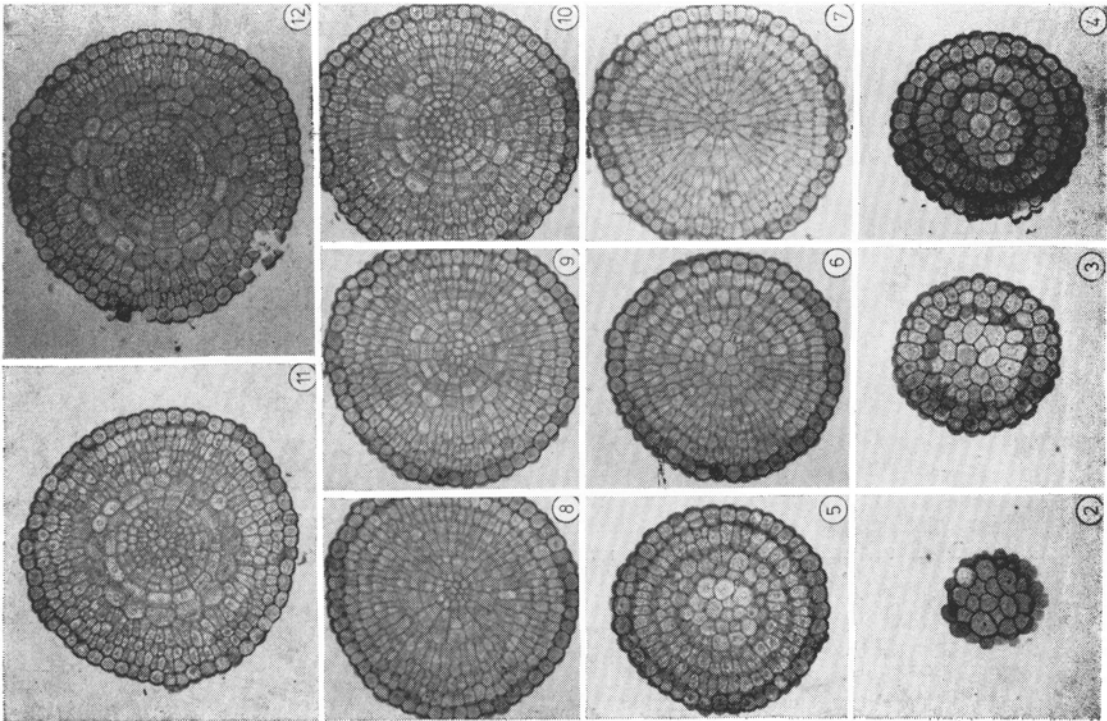
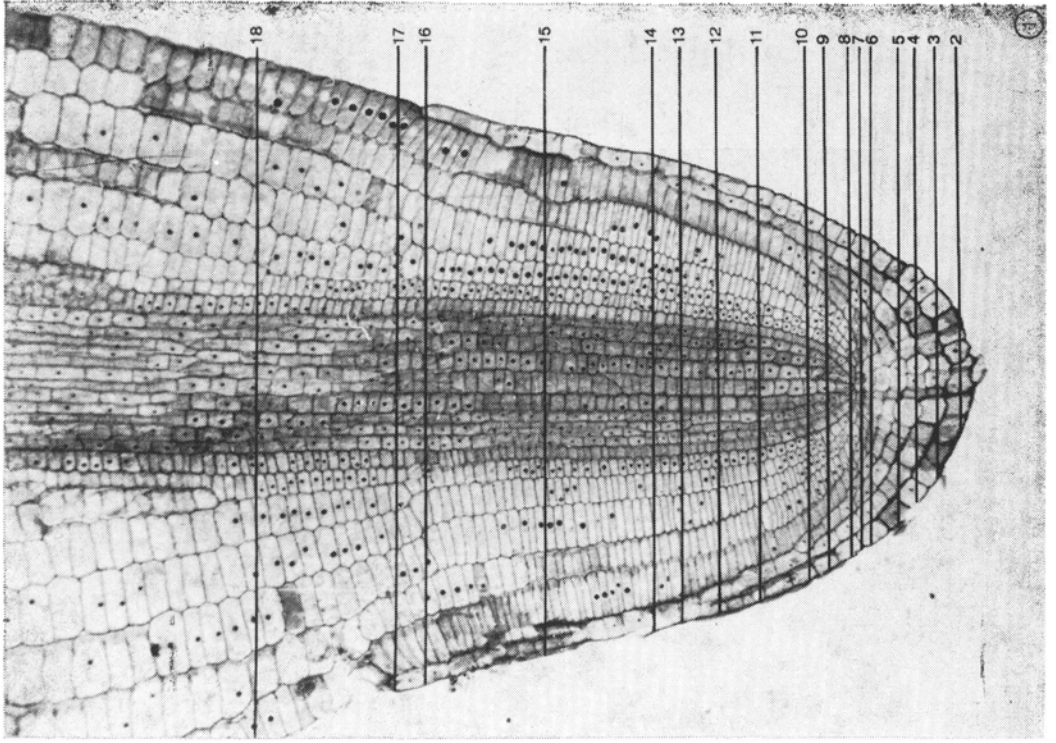
#### PLEROME

The layer of plerome initials consists of a number of cells (15 in the radicle and 19 in the seedling root examined) forming 4 quadrants which correspond to those of periblem and dermatocalyptrogen (Plates II (1), IV (1), Fig. 2 (A)).

In the serial cross sections the boundaries of these quadrants can be identified up to the level at which the sieve tubes of protophloem differentiate. In each quadrant pericycle and inner plerome initials are present, starting provascular tissue.

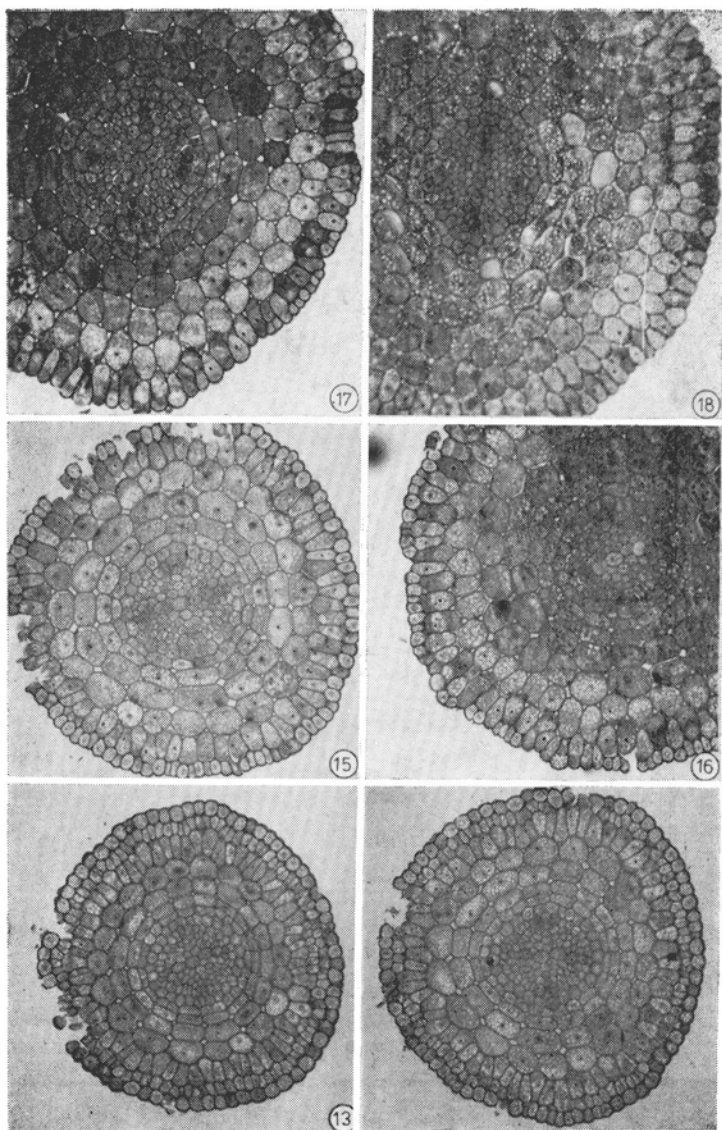
All the initials of the pericycle are usually distinguishable in the mature embryo. They are usually formed at the beginning of radicle development (Tykarska, 1979). Even in the young seedling root, however, sporadically single common pericycle and inner plerome initials can be found.

The course of xylem and phloem differentiation can be reconstructed by identifying on the successive cross sections the initial cells of the inner plerome and the cell complexes formed from them. It appears that, in the group of inner plerome initials of radicle an initial cell of the central metaxylem vessel may be present, denoted M (Plate II (1), Fig. 2 (A)), as was suggested by the analysis of the longitudinal sections (Kuraś, 1978).



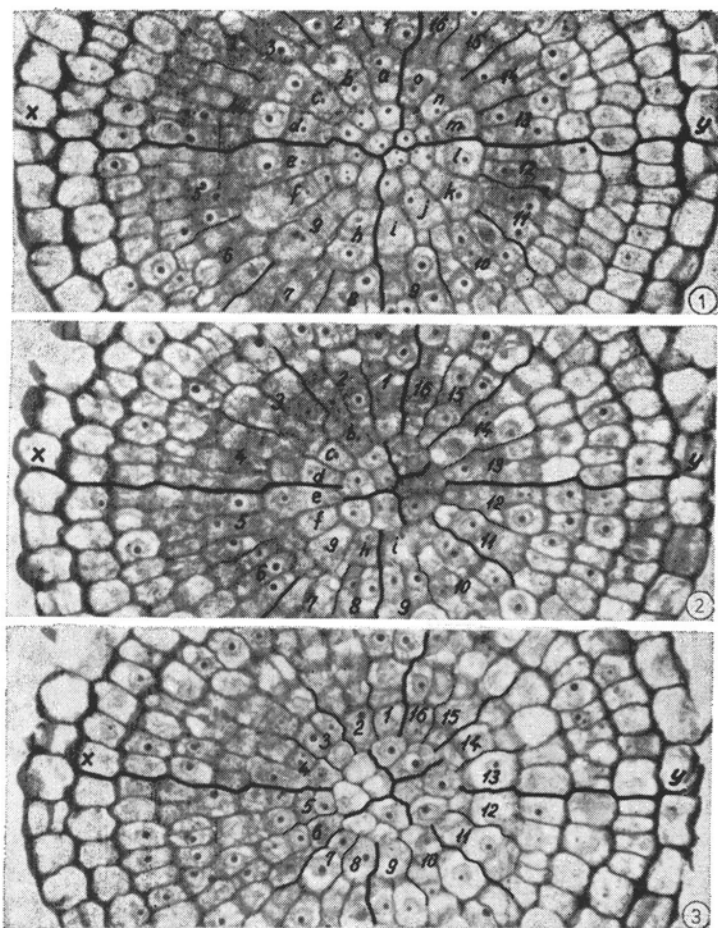
The explanations on the next page.

Plate I continued.



Series of cross sections through radicle of mature embryo (photos 2-18) at levels marked on central longitudinal section (photo 1 — 450 X, photos 2-18 — 350 X)

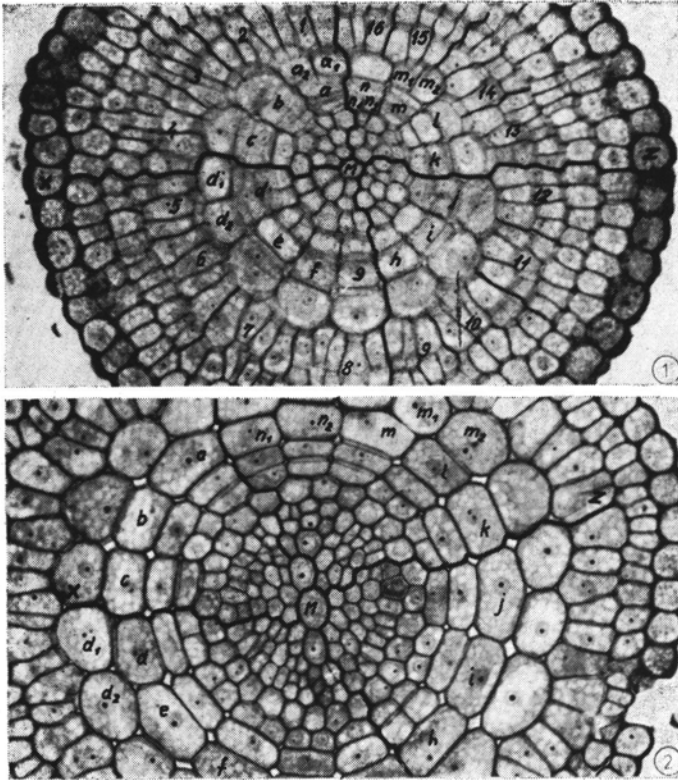
# Plate II



Boundaries of quadrants and radial sectors on cross sections through root meristem of maturing embryo

1 — layer of plerome initials; 2 — layer of central connecting cells and periblem initials; 3 — layer of columella and dermatocalyptrogen initials; a-n — radial periblem sectors; 1-16 — radial dermatocalyptrogen sectors. 600 X

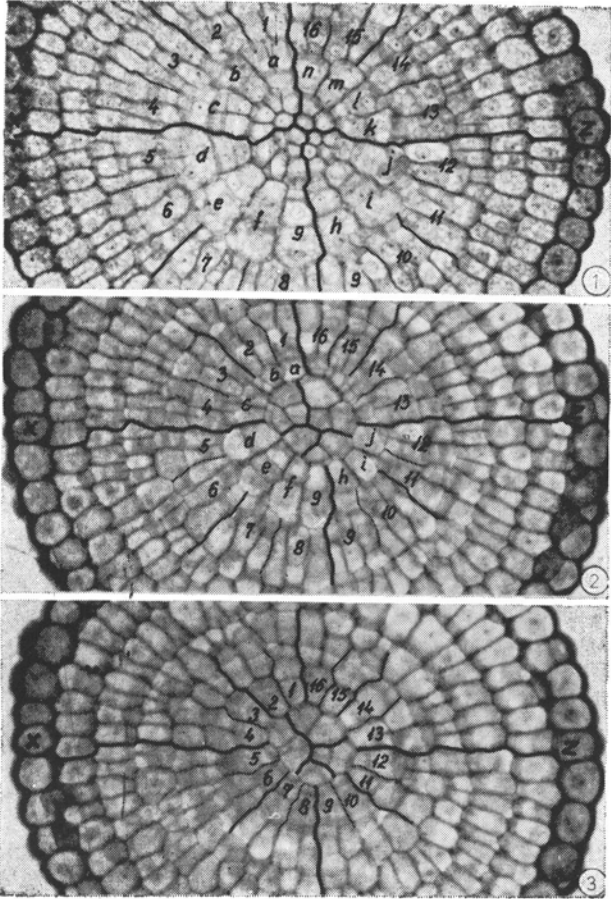
Plate III



Polar differentiation of plerome on cross sections through radicle of mature embryo.

M — central metaxylem vessel. Other notations as in Plate II. 600 ×

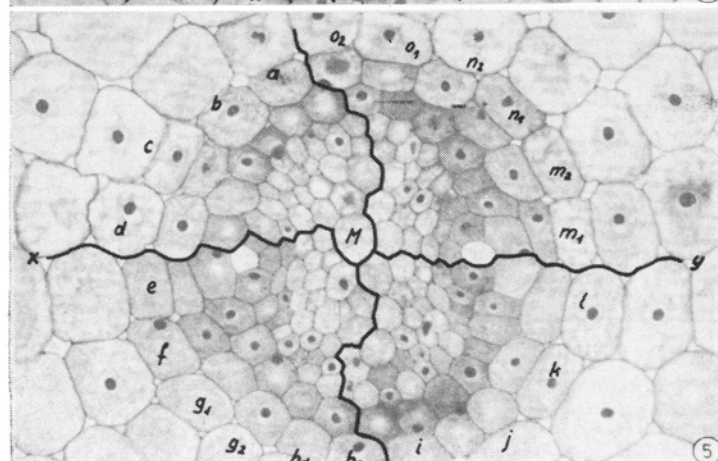
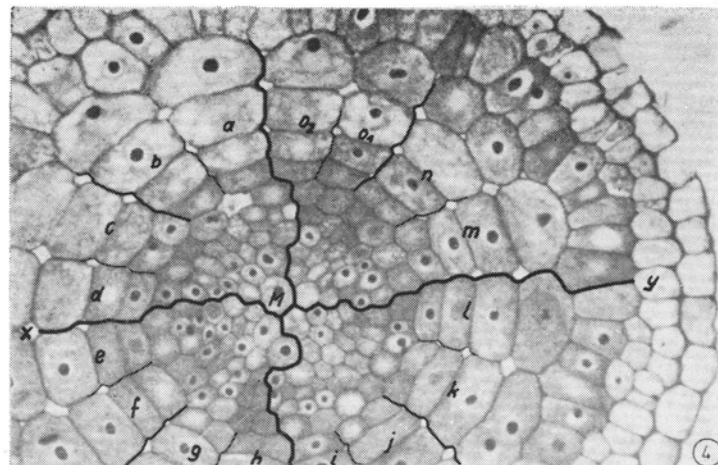
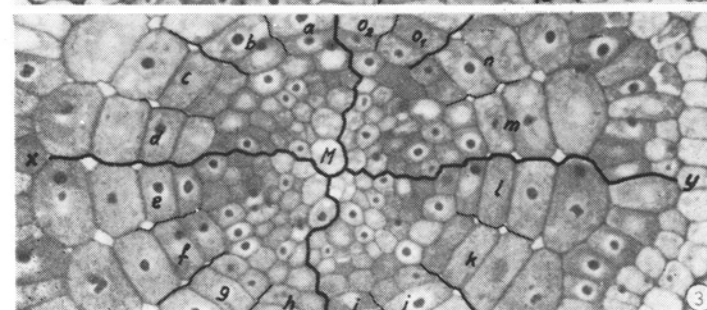
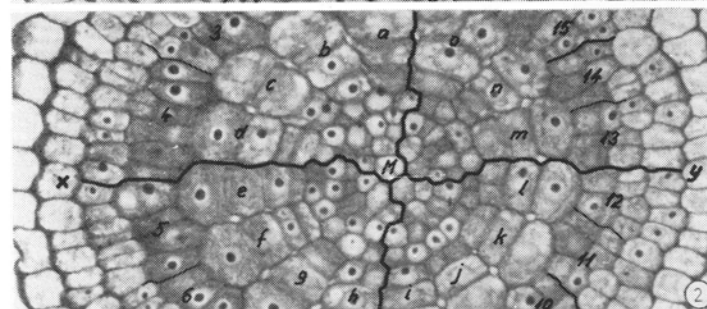
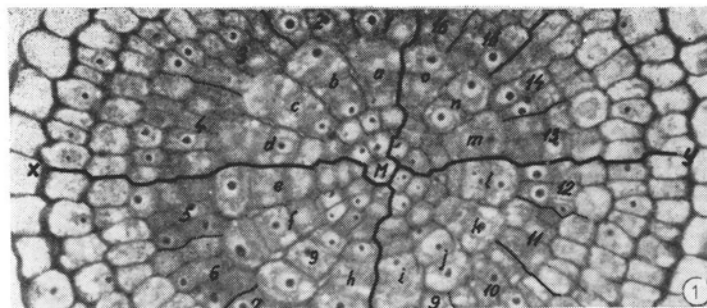
Plate IV



Boundaries of quadrants and radial sectors on cross sections through root promeristem of 2.5 cm long seedling.

Notations as in Plate II. 600 X





Polar differentiation of pterome on cross sections of 2.5 cm long seedling. Notations as in Plate II. 600 ×



In other radicles the initial of the central metaxylem vessel is one of two sister cells formed in the division of the derivative of an inner plerome initial. On the opposite sides of the central metaxylem vessel large initial cells of the next two metaxylem vessels differentiate.

Further elements of xylem develop centrifugally in two directions determined by the arrangement of the first metaxylem initials. These directions are concurrent with the boundary between the pairs of plerome quadrants in both examined roots (Plates III-VI). The first protoxylem cells are not yet definitively differentiated in the root of the mature embryo.

The two groups of phloem develop between the poles of the still undifferentiated protoxylem, around the boundaries of the plerome quadrants. The first sieve tubes of the protophloem begin to mature during embryogenesis in the basal part of the radicle (Plate I 14/17).

#### DISCUSSION

The cells patterns on a series of cross sections through the radicle and the young seedling root of rape were compared and the transverse structural organisation of the apical meristem of the radicle was described.

The initial cells of the three-tier promeristem, surrounding the intermediate layer of central connecting cells, and their derivatives were identified in the serial cross sections.

It was found that the root promeristem in the mature embryo and young seedling is of closed type since it is composed of permanent initial cells differentiated during embryogenesis.

Promeristem proper consists of about 60-80 initials of 4 histogens.

The central cylinder of the meristematic radicle is produced by plerome initials which are differentiated to initials of the pericycle and of the provascular tissue. There are about 12 initials of the pericycle and 4-8 of the provascular tissue (inner plerome). Among the latter there may be initials of 1 or 2 central metaxylem vessels.

It is found in both examined roots that the xylem plate develops along the boundary between the pairs of plerome quadrants.

This suggest that the polar differentiation of diarchic plerome might be determined by the direction of the first segmentation wall in the proembryo apical cell. If it is a so, the bilateral symmetry of the embryo and the position of the cotyledons must be determined in the same way.

The cortex of the radicle is formed from the ring of about 16 periblem initials of the inner and outer cortex layer (subdermatogen).

The radicle epidermis and the peripheral parts of the root cap are formed by common dermatocalyptrogen initials. In the mature embryo there are 16 radial sectors of dermatocalyptrogen and its derivatives.

The central part of the root cap (the columella) is formed by 8-12 initials.

It is demonstrated that the layer of central connecting cells situated between the plerome and columella initials and surrounded by a ring of periblem initials does not take part in histogenesis in the course of embryogenesis. These 8-12 cells form the quiescent centre of the radicle apical meristem. Cells of this layer are generally considered as the initials of the cortex (Lebègue, 1952; Guttentberg, 1969, 1964; Esau, 1965). We have no doubt, however, that they do not contribute to the root cortex development at any stage of embryogenesis in rape and probably in all other plants with a similar apical meristem organization. The whole cortex of the radicle is formed from the ring of primary periblem initials surrounding the central connecting cells. The same is observed in the root of young seedlings. It is possible, however, that in old roots the interrelated, symplastic growth in radial direction of the connecting cells and of the columella initials may lead to a reorganization of the meristem. In such a case the peripheral connecting cells or their derivatives would start to function as secondary periblem initials, and the peripheral initial cells of the columella as secondary dermatocalyptragen initials. The participation of these secondary initials in the growth of meristem would, however, be negligible in view of the gradual inactivation of the promeristem, connected with the development of the quiescent centre in the root (Clowes, 1958, 1960).

The present studies confirmed and supplemented the results obtained earlier on the basis of analysis of cell arrangement on longitudinal sections (Kuraś, 1978) and demonstrated that the apical meristem of the radicle in rape exhibits an organisation corresponding to Hanstein's (1868, 1870) histogen theory. This organisation can be described in the simplest and most accurate way by means of the presently unfashionable histogen terminology.

#### Acknowledgments

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### *Aktywacja zarodka podczas kiełkowania nasion rzepaku (Brassica napus L.)*

#### *II. Poprzeczna organizacja merystemu wierzchołkowego korzenia*

#### Streszczenie

Zbadano serie poprzecznych przekrojów mikrotomowych merystemu wierzchołkowego korzenia dojrzałego zarodka i młodej siewki rzepaku. Opisano i zilustrowano układy komórek w 3 piętrach promerystemu. Zidentyfikowano na różnych poziomach sektory czapeczki i protodermy wytworzone przez wspólne inicjały dermatokaliptrogeny oraz promieniste sektory kory wytworzone przez inicjały peryblemu. Rozpoznano między tymi sektorami 4 granice segmentacyjne kwadrantów prazarodka, przebiegające w poprzek całego właściwego korzenia. Stwierdzono, że granice między 4 sektorami komórek wiążących, powstałych z górnej pochodnej hypofizy, oraz granice między 4 sektorami kolumelli, powstałej z dolnej pochodnej hypofizy, przebiegają odmiennie i nie pokrywają się z granicami kwadrantów prazarodka. Wykazano w ten sposób, że w czasie całej embriogenezy centralne komórki wiążące, uważane powszechnie za komórki inicjalne kory (iec) nie biorą żadnego udziału w jej rozwoju, stanowiąc centrum spoczynkowe radikuli, oraz że komórki inicjalne kolumelli nie biorą udziału w rozwoju bocznych części czapeczki.