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Ultrastructure of cells in an initiating lateral root primordium of *Raphanus sativus*

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Abstract

Lateral root primordia in *Raphanus sativus* had developed 10 hours after main root decapitation. The primordia consisted of three cell layers — basal layer continuous with the pericycle. The primordia were initiated by activated groups of pericycle cells.

Inactive pericycle cells with a thin layer of parietal cytoplasme large central vacuole and well developed leucoplasts with starch grains were transformed into meristematic cells. During transformation the amount of cytoplasm and number of cytoplasmic organelles greatly increased, the central vacuole disappeared, and an ER system continuous in many places with the nuclear envelope evolved. The lamellar structure of plastids underwent almost complete reduction; the dictyosomes became active. The newly formed meristem differed apparently from the apical root meristem only in the lack or scarcity of lipid bodies and starch.

INTRODUCTION

The initiation of a lateral root is connected with the deep reorganization of a group of cells in the pericycle layer (Bell and McCully 1970; Gramberg 1971). This process is closely dependent on RNA and protein metabolism (Jalouzot 1971). Differentiated pericycle cells undergo dedifferentiation, become actively dividing and eventually forming a primordium of the root apical meristem.

The processes of dedifferentiation occur regularly in normal development and were observed under experimental conditions. Extensive studies of this problem were made by Buvat (1944), who under the light microscope investigated the dedifferentiation in cultured carrot tissues and in the formation of bulbils during the normal development of Brimeura leaves. Cytological changes during dedifferentation consisted of an increase of nuclear volume, transformation of chromoplasts in carrot tissues and chloroplasts in leaf cells into leucoplasts, modification

of mitochondria and disappearance of starch grains. All these changes were related to the regaining of mitotic activity.

Quiescent carrot cells isolated from a root begin to grow and divide rapidly in an vitro culture. Activation of resting cells is associated with events that occur in ground cytoplasm and all the cytoplasmic inclusions. A great wealth of ribosomes appear, both free and attached to membranes a ramifying system of endoplasmic reticulum canals becomes more prominent, dictyosomes and mitochondria are more numerous and their structure is developed in relation to greater metabolic activity. After growth induction typical green chloroplasts develop in cells grown in the light, in dark-grown cells larger, structurless plastids occur (Israel and Steward 1966).

The egg is considered to be a quiescent differentiated cell with a large vacuole. After fertilization it undergoes several changes preceding the division. There is a notable increase in the number of active dictyosomes, the large vacuole decreases, and free ribosomes form polyribosomes. There is some increase of starch granules in plastids and lipid bodies in cytoplasm (Schulz and Jensen 1968; Diboll 1968; Deschamps 1969; Vazart 1969).

Somewhat similar processes are displayed when dormant meristematic cells of the embryo radicle in a dessicated seed resume growth during germination. In a soaked embryo, cisternae in mitochondria are better defined, and it is supposed that dictyosomes develop from prodictyosomes present in a dormant embryo. The most obvious change taking place is an extensive and rapid development of ER. Protein bodies with reserve proteins are transformed into vacuoles. Plastids and nuclei remain unaltered, typical for meristematic cells (Yoo 1970).

Dedifferentiation of chloroplasts occurs during formation of a reproductive meristem in the vegetative shoot apex (Lance-Nougarède 1960).

Each of these processes of activation and dedifferentiation displayed some distinction in scope and range in the discribed cytoplasmic changes. It may be assumed, that these differences in some measure depend upon the character of the cells undergoing activation and the direction of the process.

MATERIAL AND METHODS

The apical meristems in the 4 cm roots of *Raphanus sativus* grown in water were cut off, then the seedlings were again put in water culture for 10 hours. Upper parts of the roots were then fixed in glutaraldehyde and postfixed in osmium at pH 6,5 or in $KMnO_4$. The material

was embedded in Westopal -W. Ultrathin sections were stained with uranyl acetate and lead citrate (according to R e y n o l d s 1963). Preparations were examined in a Philips 100 electron microscope.

RESULTS

Removal of the apical meristem in the main root stimulates the development of lateral roots. Ten hours after decapitation small primordia are already bulging from the pericycle into the cortex. They arise in the region approximately three cm above the decapitation surface. The root primordia taken for the electron microscopic observations were at first examined under the light microscope. Histological structure of these root primordia resembles closely that of the lateral root primordia which develop in normal older roots.

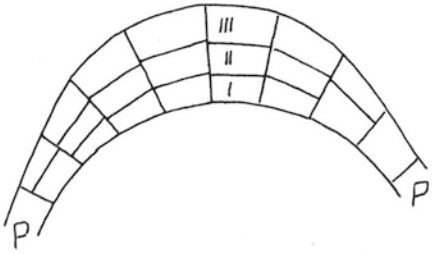


Fig. 1. Diagram of a lateral root primordium arising from the pericycle (*p—p*).

The primordium consisted of several cells arranged in a typical way (Figs. 1,2). The basal layer was formed by dividing pericycle cells, laterally continuous with nondividing cells of the pericycle. In the central part of the primordium the pericycle cells give rise to the two layers of derivative cells; in lateral sides one cell division took place in the pericycle. Thus the observed primordium was built of three cell layers in the centre and of two layers in the lateral parts.

A differentiated pericycle in *Raphanus* is formed by one layer of cells similar in internal structure to the parenchymatous cells. They have a large central vacuole, a thin layer of cytoplasm with sparsely scattered mitochondria, dictyosomes, plastids and ER cisterns. Dictyosomes are in the form of closely packed stacks of narrow cisterns with occasional adjacent vesicles; the internal lammellar structure of leucoplasts is well developed and they often contain starch grains (Fig. 3); ER occurs mostly as short cisterns.

An early stage of transformation of inactive pericycle cells into active ones is visible in the cells adjacent to the basal layer of the lateral root

Plate I

2. Part of a lateral root primordium and central cylinder in *Raphanus sativus;* pericycle cells (*p*—*p*), protoxylem cell — *pr.* (approximately × 3000).
3. Leucoplasts in pericycle cells. Lamellae and starch grain are visible. $KMnO_4$, × 25000.

Plate II

4. Dedifferentiating pericycle cell (*p*) filled with cytoplasm; vacuole — *v;* sieve tube — ST, companion cell — C, floem parenchyma cell — FP, inactive dictyosom — D, in the bottom of the picture a leucoplast with starch grains. $KMnO_4$.

Plate III

5. Pericycle cell in course of dedifferentiation. Plastid (Pl) with reduced lamellae and small optically empty space; developed system of ER continuous with nuclear envelope; nucleus — N, $KMnO_4$, × 18000.
6. Dense granular cytoplasm from the meristematic cell of lateral root primordium. Glutaraldehyhe and osmium.

Plate IV

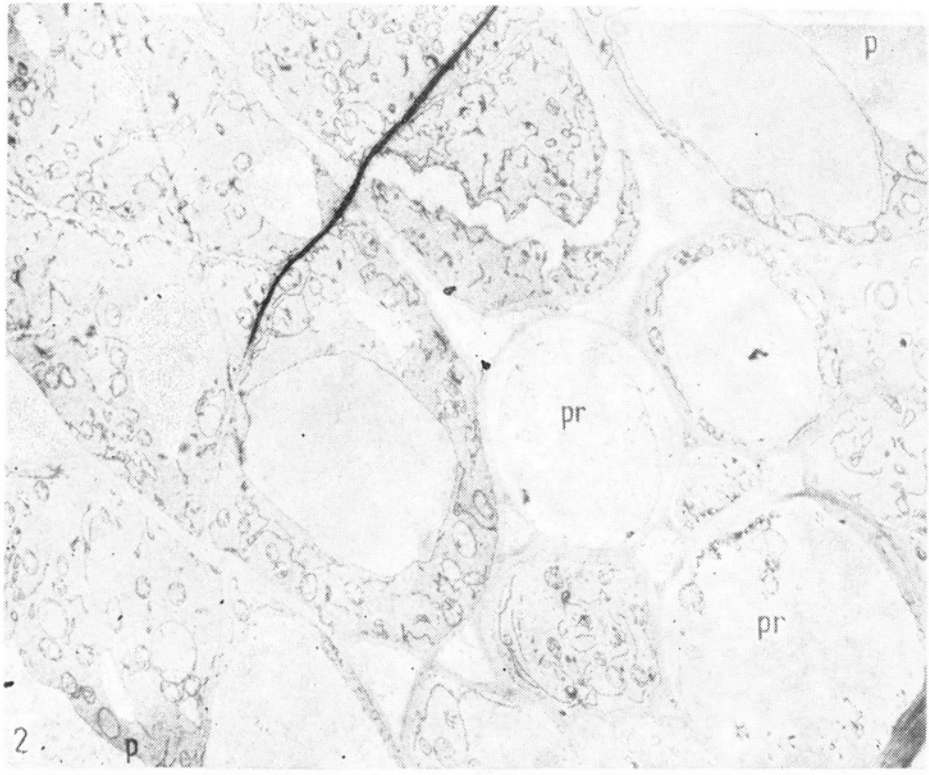
7. Meristematic cell from the central part of the three layered lateral root primordium. Constricted mitochondria — M, small vacuole — V, cisterns of the ER, one with terminal dilation — ER. $KMnO_4$.
8. Dense granular cytoplasm from the meristematic cell of lateral root primordium; constricted plastid — Pl. Glutaraldehyde and osmium, × 18000.

Plate V

9. Meristematic cell from the second layer of the three layered lateral root primordium. Nuclear envelope continuous with ER cisterns; nucleus — N, proplastids — P, some mitochondria with subterminal constrictions — M. $KMnO_4$, × 12000.
10, 11, 12. Circular and irregular arrangements of dictyosome cisterns. In picture unidentyfied body near a single cistern. $KMnO_4$, × 30000.

Plate VI

13. Cells from the third (outer) and second layer of lateral root primordium. Active dictyosomes — D, some mitochondria of elongated profiles. $KMnO_4$, × 12000.
14. Cells from the third (outer) layer of lateral root primordium Elongated mitochondria, dumb-bell plastids and plastids with double constrictions. $KMnO_4$, × 12000.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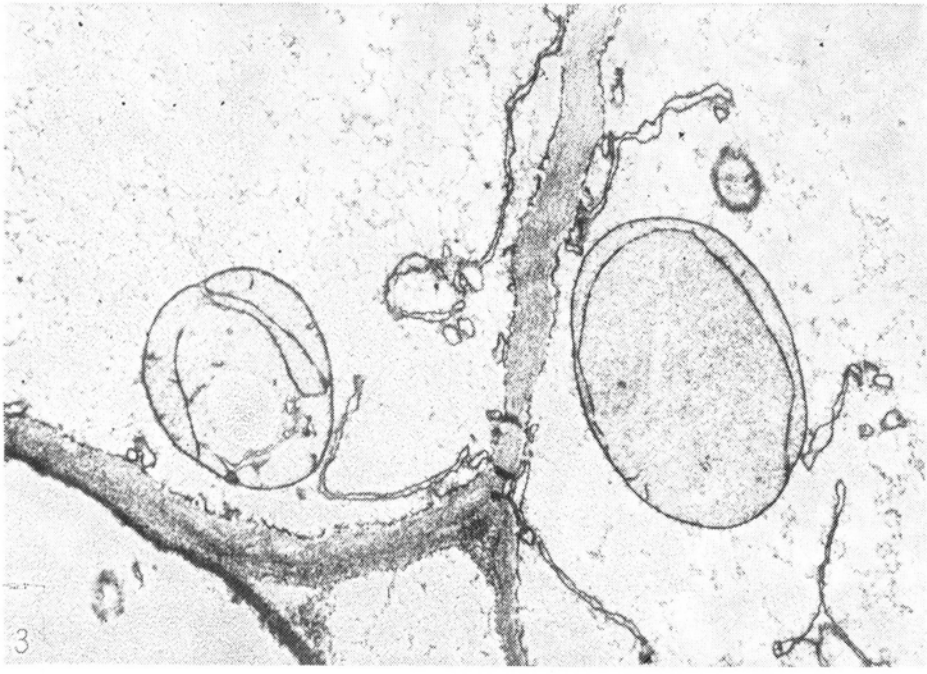
pr
pr
2
p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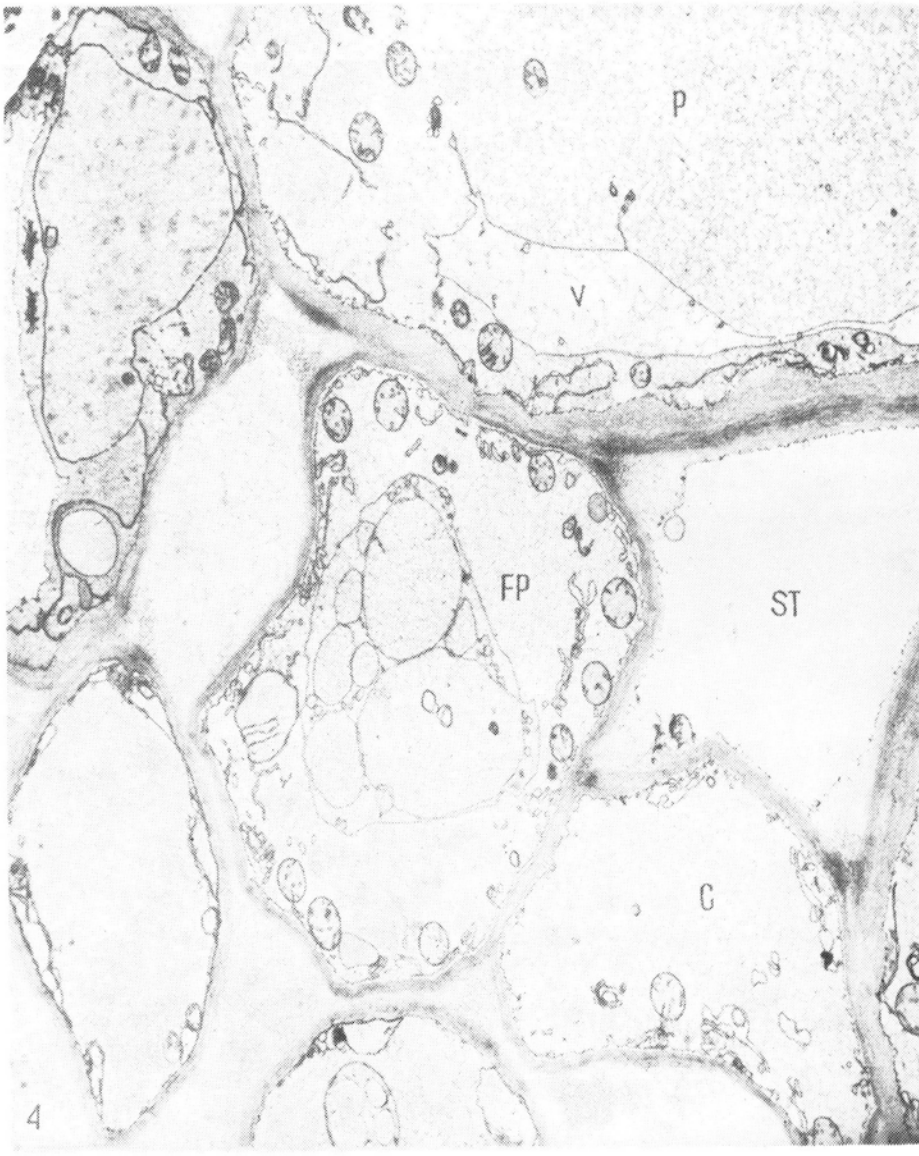
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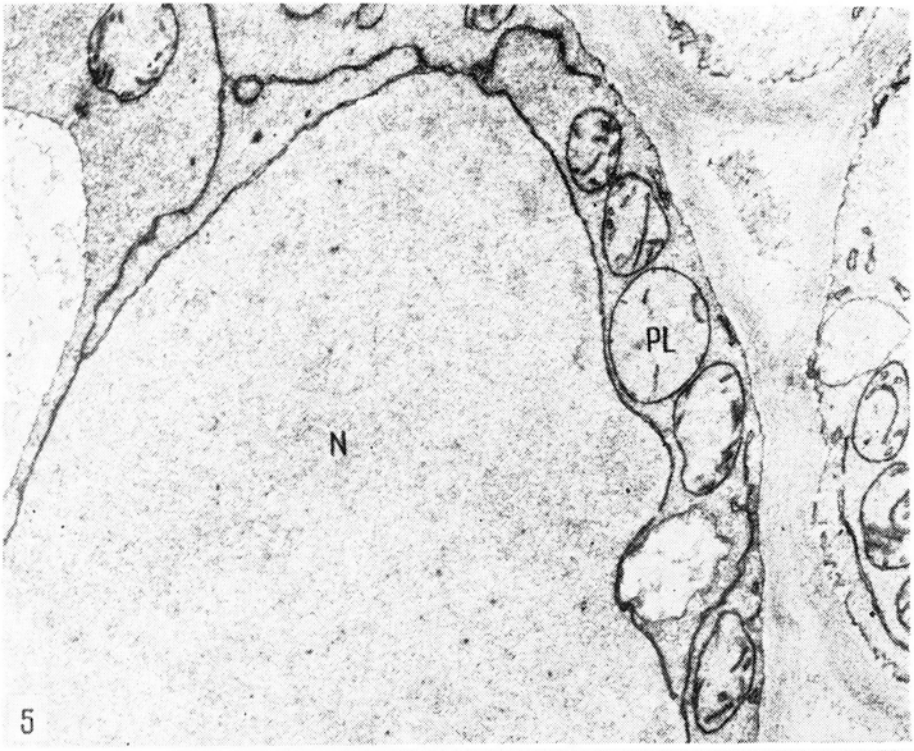

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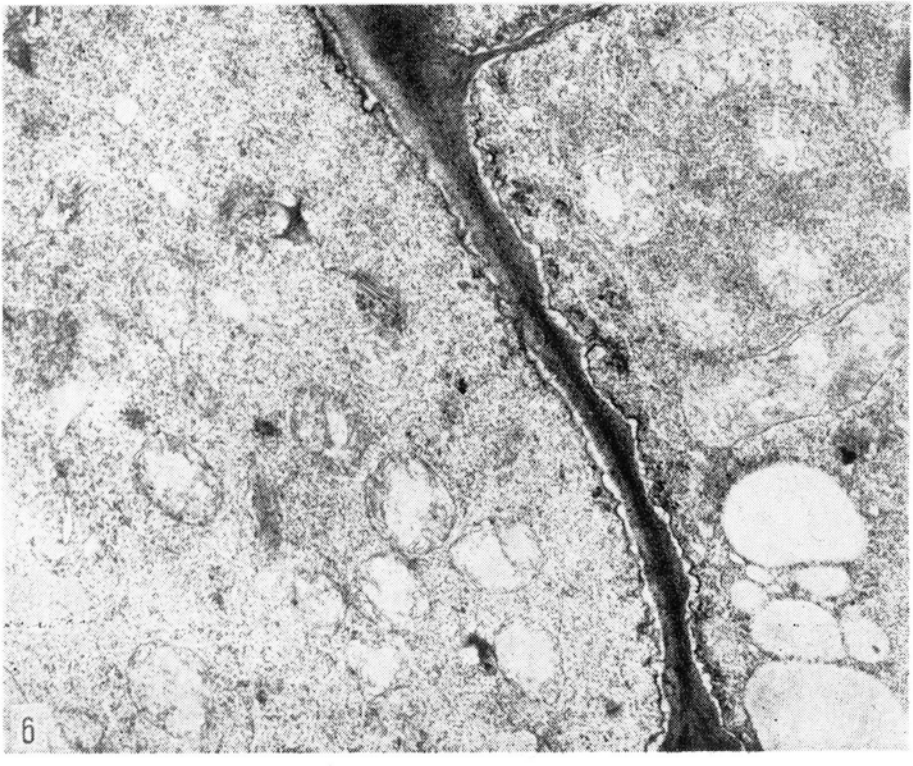

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Plate IV

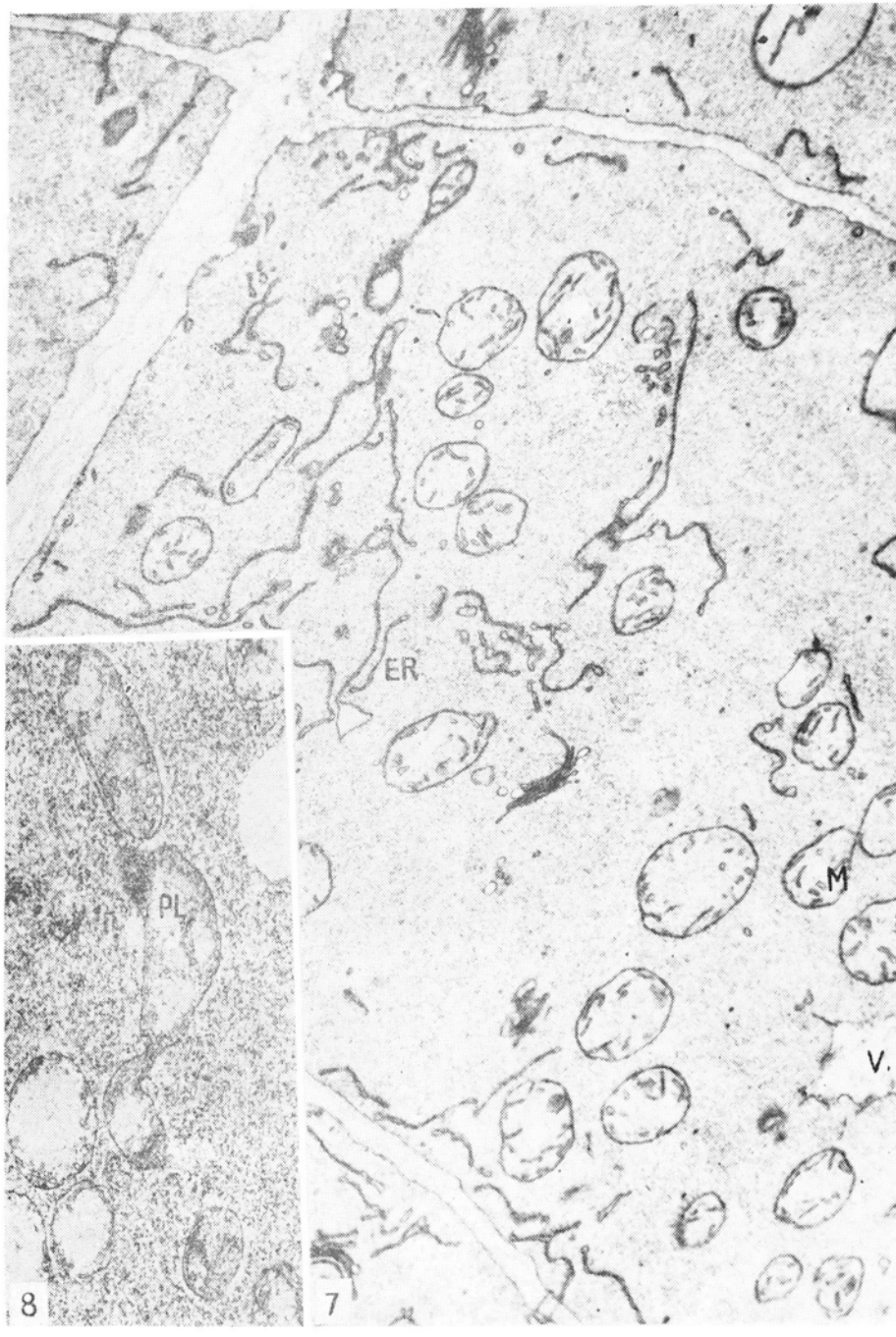
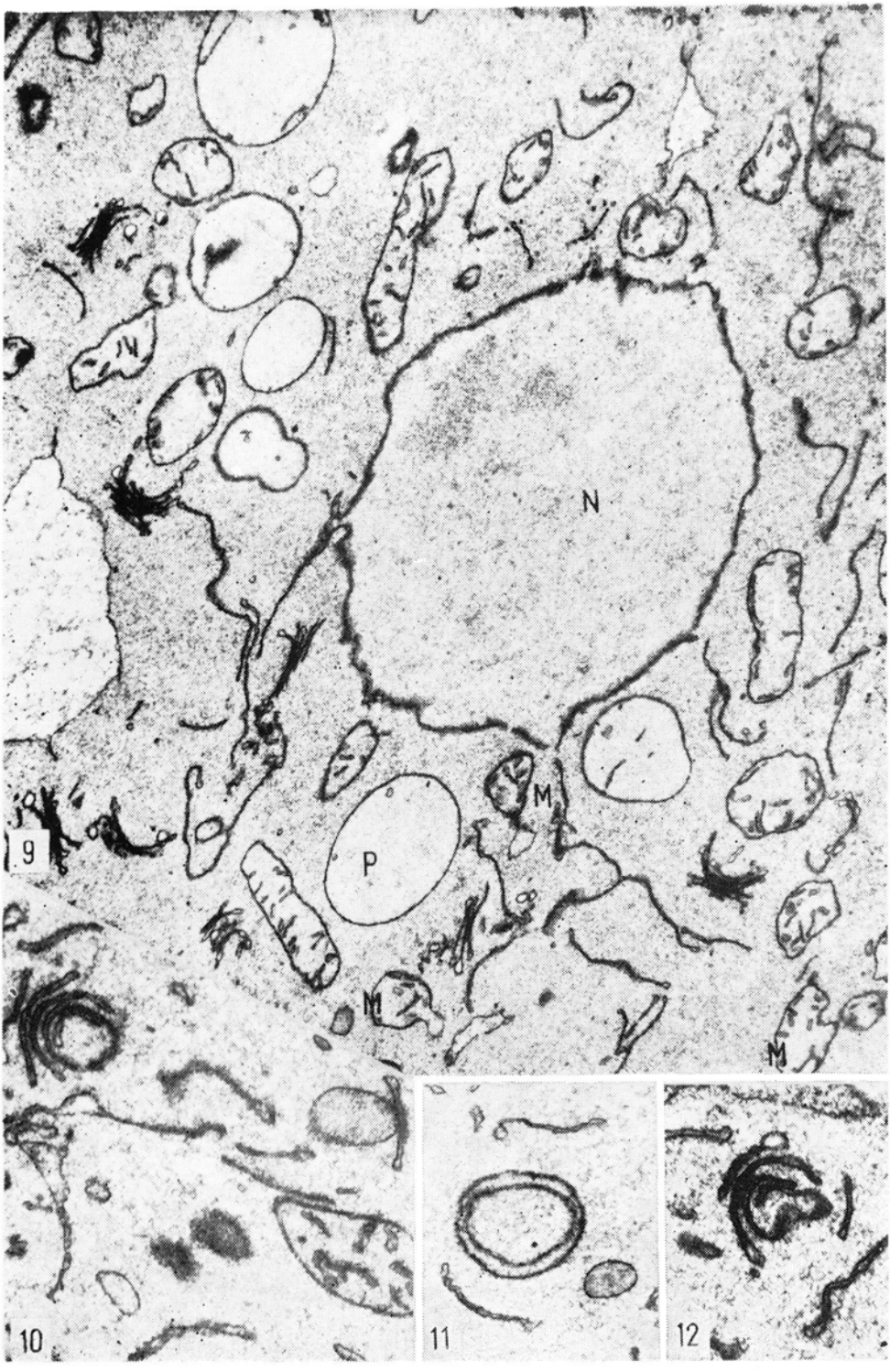
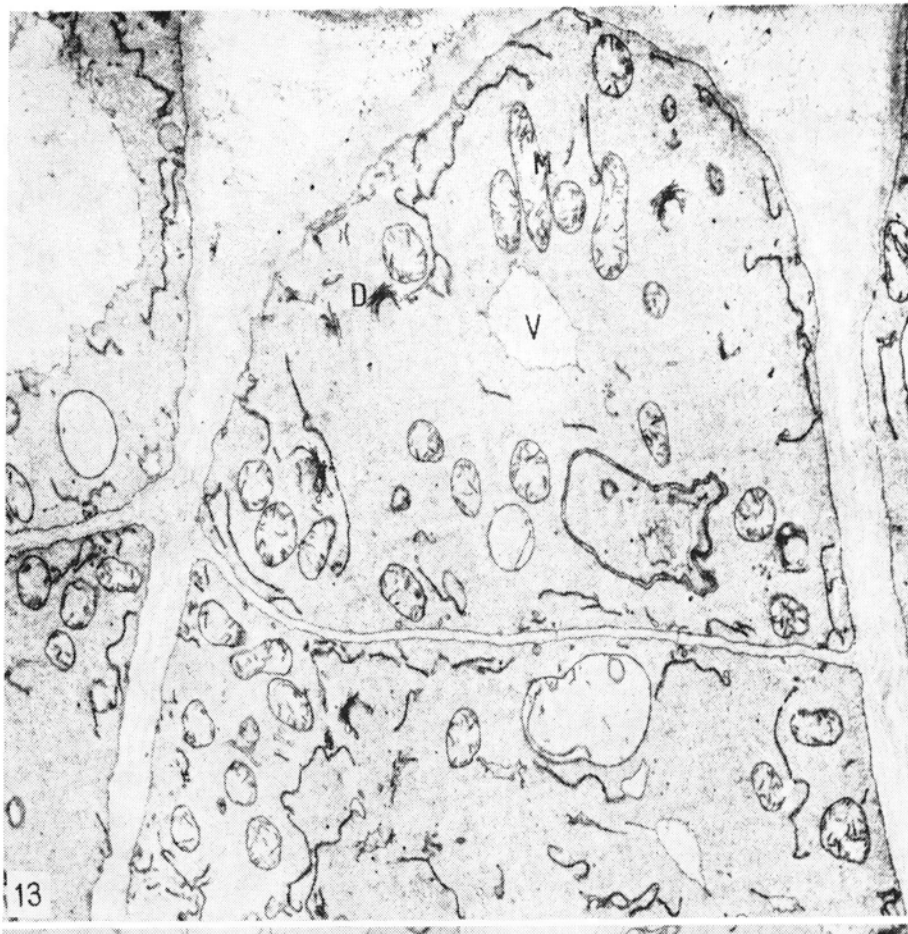


Plate V

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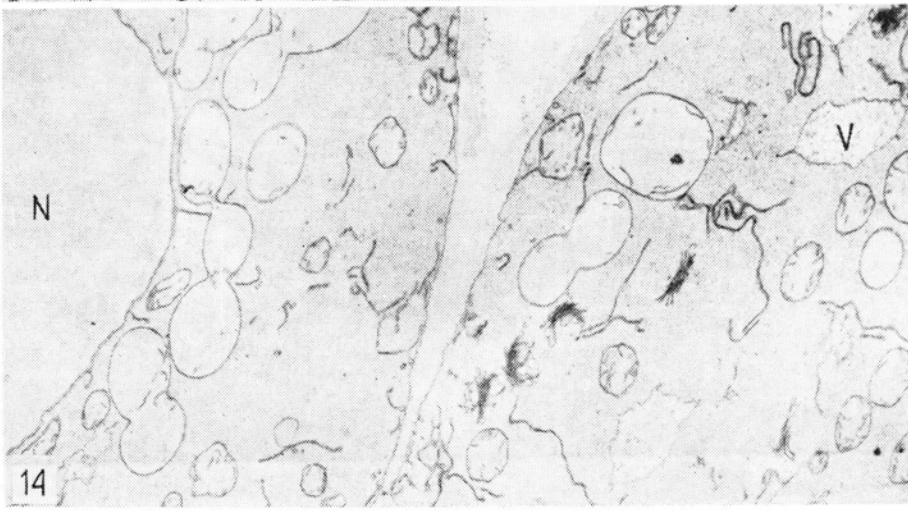
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primordium. Such a cell is shown in the picture (Fig. 4). Almost the entire cell is filled with cytoplasm, large areas of which are devoid of any organelles. A flattened vacuole of irregular profile is situated near the cell wall. Very narrow vacuolar canals go deep into the cytoplasm from the main vacuolar space. Oval mitochondria, dictyosomes — each with adjacent vesicles, and cisterns of ER are scattered in partietal and perinuclear cytoplasm. Reduced lamellar structures and starch are present in the plastids as shown in the other picture of the similar cell (Fig. 5).

The central cylinder cells contiguous to the activated pericycle cells seem to remain unaltered in comparison with a control root without lateral root primordia. In the picture (Fig. 4) there are central cylinder parenchyma cells and a phloem unit consisting of sieve cell, companion cell and phloem parenchyma cell. Dictyosomes in the parenchyma cell are flattened and devoided of vesicles; the leucoplast contains starch grains, and large central vacuoles are present.

In the pericycle cell situated in the lateral part of root primordium (Fig. 5) still further changes in the cytoplasm are noticeable. The leucoplast lamellae are reduced and become very short or vesicular, and an optically empty space is much smaller than in the leucoplast in the previously described cell. The cisterns of ER are developed in a netlike system continuous in many places with the nuclear envelope. The cells in the middle part of the basal (first) layer of root primordium and the cells of the derivative layers are almost completely filled by cytoplasm with a nucleus in the centre (Fig. 9). There are only small vacuoles scattered in the cytoplasm, often with short narrow protrusions. These vacuoles after $KMnO_4$ fixation show very little contents, whereas similar vacuoles in apical meristems of older roots display denser contents.

The density of the cytoplasm is greatly increased as compared with the lateral cells in the basal layer and adjacent pericycle cells. The density of the cytoplasm is particularly well seen in the preparations after glutaraldehyde fixative (Fig. 6). Numerous mitochondria are scattered throughout the whole cytoplasm. In the cells of the first and second layers their profiles are mostly round or oval, but in the cells of the third (outer) layer many mitochondrial profiles are of elongated shape (Fig. 13). There are some rare dumb-bell and more often rodlike mitochondria with subterminal constrictions which give the impression of budding (Figs. 7, 9).

The internal lamellae of plastids are almost completely reduced, and the plastids contain no starch grains. In the third layer of the root primordium there are often plastids with middle constrictions — biscuitlike or even with two constrictions, which gives to the plastid the appearance of a short chain composed of three elements (Figs. 14, 8).

Dictyosomes in the primordium are formed of 3—5 cisterns always

accompanied by small vesicles. Often dictyosomes occur in bizarre shapes — circular, wavy, whorled but these changes in shape are said to be $KMnO_4$ fixation artefacts (Figs. 10—12). Sometimes solitary short cisterns are near unidentified small bodies with optically dense contents, enclosed in a single membrane. Osmiophilic lipid bodies typical for meristems in root apices were conspicuously absent in the observed cells of the primordium.

DISCUSSION

The lateral root primordia composed of three cell layers are developed in ten hours after removal of the main root apical meristem. The lateral root primordium originates from the pericycle, where some cells are induced to dedifferentiation and then undergo divisions. The structure of original inactive pericycle cells is similar to the structure of parenchymatous cells. This structure after a relatively short time (several hours following the stimulus) is changed in some pericycle cells and replaced by a structure typical for a primary meristem of a root apex. Apical meristem structure was minutely described by many authors (ref. Mesquita 1970; Clowes and Juniper 1968).

The meristematic cells in young lateral root primordium appear to differ in one aspect from the meristematic cells of the main root, namely they seems lacking in typical osmiophilic lipid bodies and starch grains.

Comparing the structure of the cells in successive layers of the lateral root primordium, and the adjacent cells of the pericycle with the inactive pericycle cells, it may be possible to reconstruct some morphological processes associated with dedifferentiation and transformation of quiescent cells in the pericycle into active meristematic cells. During dedifferentiation a large amount of cytoplasm rich in ribosomes appears. Large areas of the new cytoplasm are devoid of any organelles. This cytoplasm invades and gradually squeezes out the central vacuole, which decreases, becomes irregular and splits into small vacuoles. The most obvious changes are noticeable in plastids, ER and dictyosomes. The internal lamellae of plastids are being reduced, starch grains disappear and almost structurless plastids remain in the meristematic cells. At the same time a system of ER canals is built up, and in many places cisterns come into direct conjunction with the nuclear envelope. All dictyosomes become active and produce vesicles. In the later stage there is a great increase in number of mitochondria, plastids and dictyosomes.

Although the increase in number of organelles takes place in a few houres it is impossible to assume any precise way of their multiplication. Attention may be drawn to atypical arrangements of dictyosome cisterns, constricted, or dumb-bell mitochondria and plastids. Such morphological

features in meristematic cells were repeatedly described by several authors.

Dedifferentiation processes in activated pericycle cells lead to the formation of typical root apex meristem cells. In the early primordium they differ only by the apparent lack or at least by a much smaller amount of ergastic substances (lipids, starch, vacuolar contents).

The activation processes in various plant cells described by authors referred to differ in some points from the presented data. This variability may stem from the differences in the structure of the cells being transformed and the type of cells which they were transformed into. The most common phenomena of activation and dedifferentiation were: increase in the amount of ground cytoplasm, formation of polyribosomes and increase in number of cytoplasm organelles.

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Ultrastruktura komórek zawiązków korzeni bocznych Raphanus sativus

Streszczenie

Zawiązki bocznych korzeni u *Raphanus sativus* rozwijały się w 10 godzin po dekapitacji korzenia głównego. Zawiązki składały się z trzech warstw komórek powstałych skutkiem podziałów grupy komórek perycyklu. Nieaktywne komórki perycyklu charakteryzujące się cienką warstwą przyściennej cytoplazmy, dużą wakuolą i dobrze rozwiniętymi leukoplastami ze skrobią ulegały przekształceniu w komórki merystematyczne.

W czasie przekształcania komórek powiększała się ilość podstawowej cytoplazmy i liczba organoidów, znikała centralna wakuola i wytwarzał się system ER połączony w wielu miejscach z błoną jądrową. Lamelarna struktura plastydów ulegała prawie kompletnej redukcji; diktiosomy stawały się aktywne. Nowe komórki merystematyczne różniły się widocznie od komórek wierzchołkowego merystemu korzenia tylko brakiem lub rzadkim występowaniem ciał tłuszczowych i skrobi.