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Ultrastructural Changes in Cells of Pea Root Cortical Explants Cultured *In Vitro*

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With 30 Figures

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Summary

Root cortical explants from seedlings of *Pisum sativum* L., cv. Little Marvel were cultured on a sterile nutrient medium in the presence of auxins or auxins and cytokinin. Explants were fixed (and subsequently processed for electron microscopic observation) at the outset and after 30, 60, and 72 hours of culture under the two hormonal conditions. In the presence of auxin alone, the cell walls of the cortical parenchyma showed distinctive structural changes involving the deposition of a new, diffusely fibrillar primary wall. A considerable increase of rough ER in the adjacent cytoplasm was associated with the new wall synthesis. These wall changes are interpreted as auxin-induced and prelude to cell enlargement and later cell separation. No dramatic changes occurred in other cytoplasmic organelles or in the nucleus. In the presence of cytokinin and auxin, the striking cytological events observed included marked nuclear changes and greater cytoplasmic density due to increased organelles associated with the onset of DNA synthesis, mitosis and cytokinesis. New cell walls formed from the developed phragmoplasts, cleaving the original parenchyma cells into smaller cellular compartments with no accompanying cell enlargement. No marked changes in the original primary cell walls were observed in cytokinin-auxin-treated explants. By 72 hours some cells already had completed two successive cell divisions. No ultrastructural evidence was obtained suggesting that these cells were committed to their known fate of differentiating into mature tracheary elements in the subsequent 2–4 days. At 72 hours each explant represented a population of actively dividing, still considerably vacuolated meristematic cells.

1. Introduction

The initiation of cell division and the induction of specific cyto-differentiation under hormonal control have been studied intensively in mature pea (*Pisum sativum* L.) root cortical cells grown in cultures as tissue explants (Libbenga and Torrey 1973, Phillips and Torrey 1973, 1974, Shininger and Torrey 1974). Earlier work (Matthysse and Torrey 1967 a, b, Torrey and Fosket

1970) with cultured segments excised from seedling pea roots centered on DNA synthesis and nuclear changes using light microscopy and autoradiography. Of particular interest has been the role played by specific hormones in activating cellular changes in mature parenchyma cells leading to their dedifferentiation and the formation of a population of meristematic cells (Gautheret 1966). After a phase of mitotic activity, the newly formed cells differentiate more or less directly into mature tracheary elements, forming specifically patterned, lignified secondary cell walls and thereafter undergoing autolysis and cell death.

Both auxin and cytokinin are required for tracheary element formation in this system; in the absence of cytokinin, the cortical cells enlarge but do not undergo DNA synthesis, mitosis, cell division or subsequent cyto-differentiation. Related studies on tracheary element formation in cultured tissue explants have been published (in *Coleus blumei* Benth. stem segments Fosket and Roberts 1964, Fosket 1968; in *Helianthus tuberosus* L. pith tissue Yeoman and Street 1973, Minocha and Halperin 1974; in *Nicotiana tabacum* L. pith tissue Das, Patau, and Skoog 1956, Bergmann 1964; in *Lactuca sativa* L. pith parenchyma Dalessandro and Roberts 1971, Dalessandro 1973; in *Glycine max* (L.) Merrill cotyledonary tissue Fosket and Torrey 1969, and others). For a general description of tracheary element differentiation in cultured cells and tissues and a comprehensive review of the literature one should refer to the monograph by Roberts (1976).

Despite extensive physiological, cultural, and cytological analyses at the light microscope level, there have been relatively few detailed accounts of the ultrastructural changes associated with cytoplasmic and nuclear events occurring during dedifferentiation and redirected cytodifferentiation in these experimental systems. The present paper concerns the events occurring during dedifferentiation of the pea root cortex into a meristematic state and examines whether there is, at this stage, any ultrastructural evidence as to which cells will subsequently redifferentiate into tracheary elements. The cytological changes evident in pea root cortical explants cultured in the absence of cytokinin are also described.

2. Materials and Methods

Cultural procedures: Cortical explants were prepared from seedling roots of the garden pea. *Pisum sativum* L. cv. "Little Marvel" according to the methods described by Phillips and Torrey (1973). Explants were cultured on the S 2 M medium described by Torrey and Fosket (1970) containing 0.8% Bacto-agar. The S 2 M medium contains the auxins, indoleacetic acid at 0.175 mg/l (10^{-6} M) and 2,4-dichlorophenoxyacetic acid at 1.1 mg/l (5×10^{-6} M). The auxin-containing medium was supplemented with cytokinin as kinetin (K) at 1 mg/l ($\sim 5 \times 10^{-6}$ M). Explants were fixed at time zero (*i.e.*, upon preparation) and thereafter at 30, 60, or 72 hours in culture on the S 2 M or S 2 M + K media. The timing of cellular events reported by Phillips and Torrey (1973) was directly comparable in explants examined in these studies.

Procedures for microscopy: Whole cortical explants were fixed in 3% glutaraldehyde in sodium cacodylate buffer at pH 7.2 at room temperature for 8 hours, then stored at 4 °C. Tissues were post-fixed in 1% osmic acid (buffered as above) for 2 hours, washed and dehydrated in an alcohol series and carried through propylene oxide to embedment in araldite with polymerization at 60 °C for 24 hours. Sections for light microscopy were cut 1–2 μm thick and observed with phase contrast either unstained or stained with toluidine blue. Ultrathin sections of the material were stained with uranyl acetate and lead citrate and examined by EM on an A.E.I. 6 B transmission electron microscope.

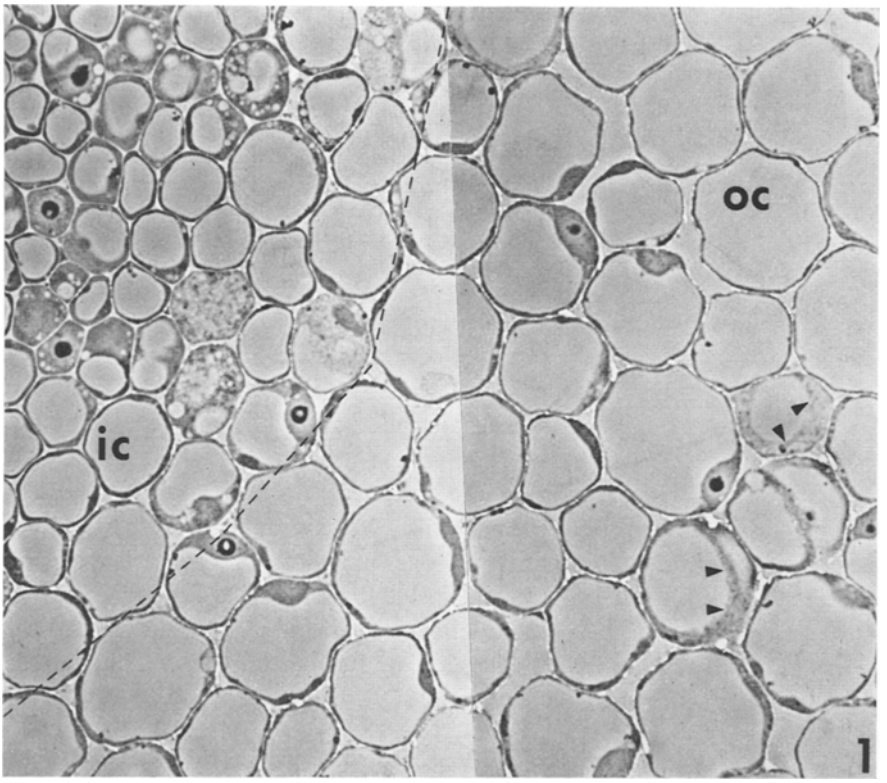
3. Observations

Pea root cortical explants at the beginning of culture period (day 0): The cell population comprising the pea root cortical explant is heterogeneous. The outermost cell layer of the hollow cylinder is the epidermis which is made up of small flattened cells with thickened suberized walls and an occasional rounded protuberance of early root hair formation (these cells are not involved in the general response of the tissue, *cf.*, TORREY and FOSKET 1970, although in some material cultured for 60–72 hours on a medium with both auxin and cytokinin, dense cytoplasm and prominent nuclei are visible in various root hairs). The inner cortical cell layers (Fig. 1, *ic*) are composed of small diameter cells and tend to be more densely cytoplasmic and less vacuolated than the larger-celled middle-outer cortex (*cf.*, TORREY and FOSKET 1970 and PHILLIPS and TORREY 1973).

The latter tissue is comprised of 10–12 concentric cell layers of relatively uniform and highly vacuolated cells ranging from about 20–50 μm in transverse diameter but elongated parallel to the long axis of the root and up to about 200 μm in length. Prominent intercellular spaces occur throughout the cortex. Occasionally larger tracts of cytoplasm (Fig. 1, arrows) are evident in some cells, probably corresponding to regions in which the section has grazed the cytoplasm adjacent to a transverse cell wall. The nuclei are typically flattened against the cell wall and in survey sections across an explant, only about 15% of cells show nuclei.

In subsequent discussions of the response of pea root cortical explants, attention will be focused on the behaviour of the middle and outer cortical cells (Fig. 1, *oc*) as typifying the tissue response. Assuming an explant cell number of 8,000 (PHILLIPS and TORREY 1973), these cells represent 55–60% of the initial population cell number and probably 80% of the explant cell volume.

Ultrastructure of middle-outer cortical cells at day 0: The most prominent feature of the cortical cells is the large vacuole surrounded by a narrow peripheral cytoplasmic layer containing the interphase nucleus and sparsely distributed organelles (Figs. 2–5). The tonoplast is generally retained intact but appears less densely staining than the plasmalemma. The primary cell wall is up to one μm thick and sometimes shows a layered texture (Figs. 3 and 6). The corners adjacent to intercellular spaces are usually thickened.

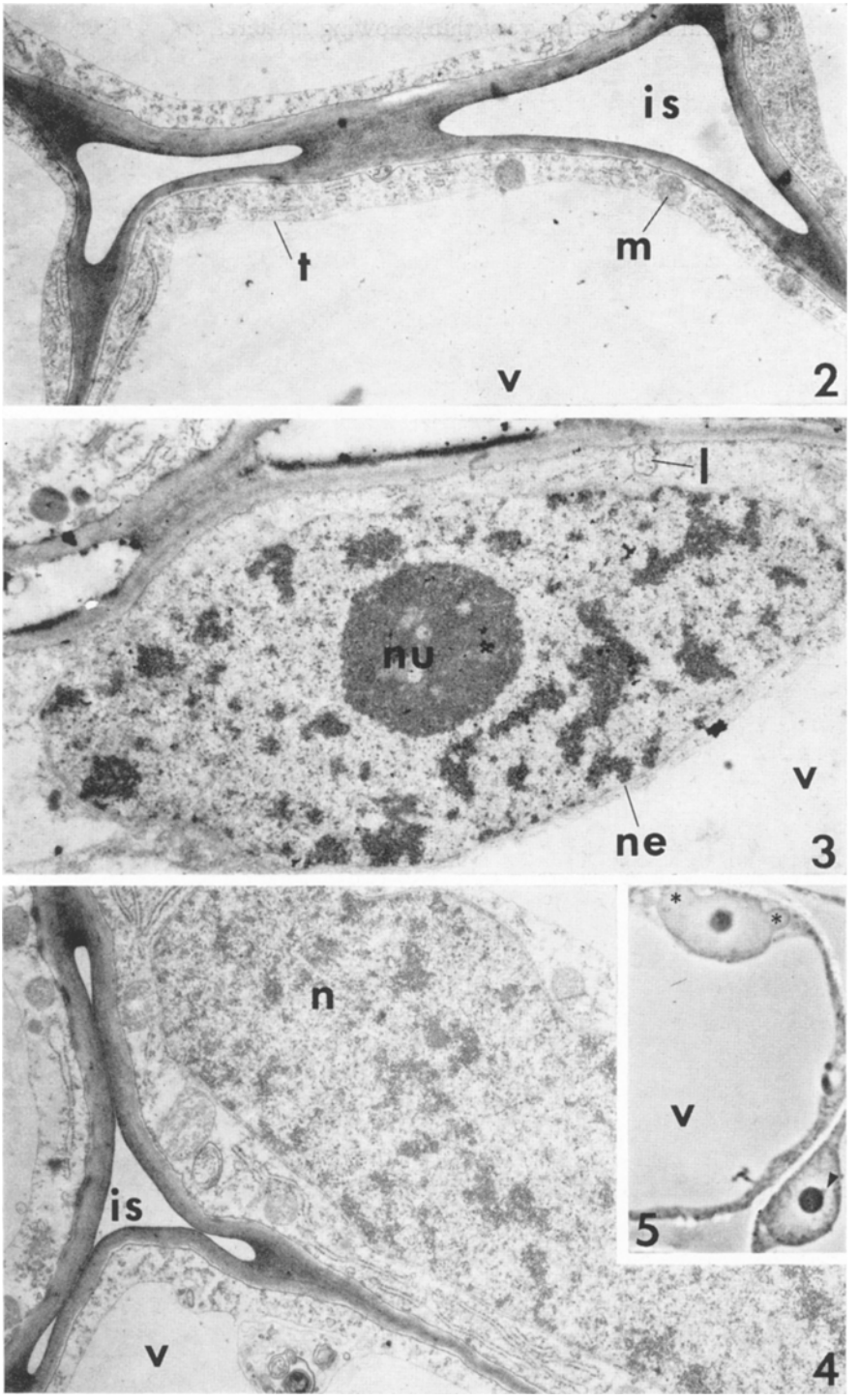


List of abbreviations in figures and text.
P.C. = phase contrast light microscopy, *T.E.M.* = transmission electron microscopy, *cp* = cell plate, *ic* = inner cortex, *is* = intercellular space, *l* = lomasome-like body, *m* = mitochondrion, *mt* = microtubule, *n* = nucleus, *ne* = nuclear envelope, *nu* = nucleolus, *nv* = nucleolor vacuole, *oc* = outer cortex, *p* = plasmalemma, *pcw* = primary cell wall, *pd* = plasmodesmata, *ps* = polysome, *rer* = rough endoplasmic reticulum, *sg* = starch grain, *t* = tonoplast, *v* = vacuole.

Fig. 1 (*P.C.* ×480). Survey transverse section of 0 hour explant. Dashed line shows approximate boundary between inner and outer cortex. Note nest of more densely cytoplasmic cells in inner cortex, whereas outer cortical cells are generally larger and more vacuolated but with some cells showing tracts of cytoplasm (arrows)

The plasmalemma is closely adherent to the cell wall except for occasional apparently empty spaces between them, presumably caused by osmotic shock during fixation. Lomasome-like bodies are also sometimes evident (Fig. 3). The surfaces of the intercellular spaces show smooth contours and the adjacent wall is more densely staining (Fig. 2).

Figs. 2–4 (*T.E.M.* ×10,000) and Fig. 5 (*P.C.* ×1,200). Transverse sections of 0 hour cortical explant: note prominent intercellular spaces, large central vacuoles and thin cytoplasm confined to narrow peripheral layer containing flattened nuclei. In Fig. 5 asterisks show lobes of nucleus and arrow indicates nucleolus with possible nucleolar organizer



Figs. 2–5

The cytoplasm is generally very thin, showing scattered rough ER paralleling the cell wall and tending to be swollen with vesicular endings (Figs. 2 and 4). Ribosomes are usually restricted to the surfaces of the ER. Mitochondria are relatively infrequent, spherical or oval in section (Figs. 2 and 4) and show poorly defined membranes. Golgi bodies are also not frequent. Occasional amyloplasts occur containing several starch grains. The nucleus of the cortical parenchyma cell is the most prominent cytoplasmic structure (Figs. 3–5). The interphase nucleus is generally oval or elliptical in transverse section although sometimes showing lobing (Fig. 5). The nucleus typically shows a single large, sometimes vacuolate, spherical nucleolus (Figs. 3 and 5) with scattered, densely staining chromatic material distributed throughout the nucleoplasm. Taken all together the cortical cells represent typical parenchyma with all the characteristics of a relatively inactive cytoplasm.

Cortical explants cultured for 3 days on S 2 M medium: In a medium containing auxins (2,4-D + IAA) but lacking cytokinin, cortical explants show a fresh weight increase at 3 days (Simpson 1975) and by 5 days, cell separation begins especially along the elongated cells of the inner cortex (Torrey and Fosket 1970). By 10 days the cortical tissue is quite friable (Phillips and Torrey 1973). According to Simpson (1975), the inner cortical cells particularly show a cell enlargement response which accounts for a large proportion of the fresh weight increase of the explants at 3 and 5 days.

Nuclear changes are not evident at day 3 on S 2 M medium middle-outer cortex (however, in one specimen a mitosis was noted in the *inner* cortical tissue). The most striking cytological change involves the deposition in some cortical cells, of a new wall layer upon the existing primary cell wall. The new cell wall is made of a diffuse, loose network of interconnected fibrils overlaid by the dense plasmalemma (Fig. 6). Extensive tracks of rough ER appear in the adjacent cytoplasm and lomasome-like invaginations of the plasmalemma, containing fibrillar material (Fig. 7), also occur. Golgi bodies are infrequent.

Fig. 8 shows an oblique section apparently cutting the old cell wall in an area of new wall synthesis. Microtubular arrays, vesicles, scattered rough ER and polysomes are evident, suggesting a greater metabolic activity at the cell wall cytoplasmic interface.

Cortical explants cultured for 3 days on S 2 M + K medium: When 1 ppm kinetin is added to the medium containing auxins, the cortical cells are

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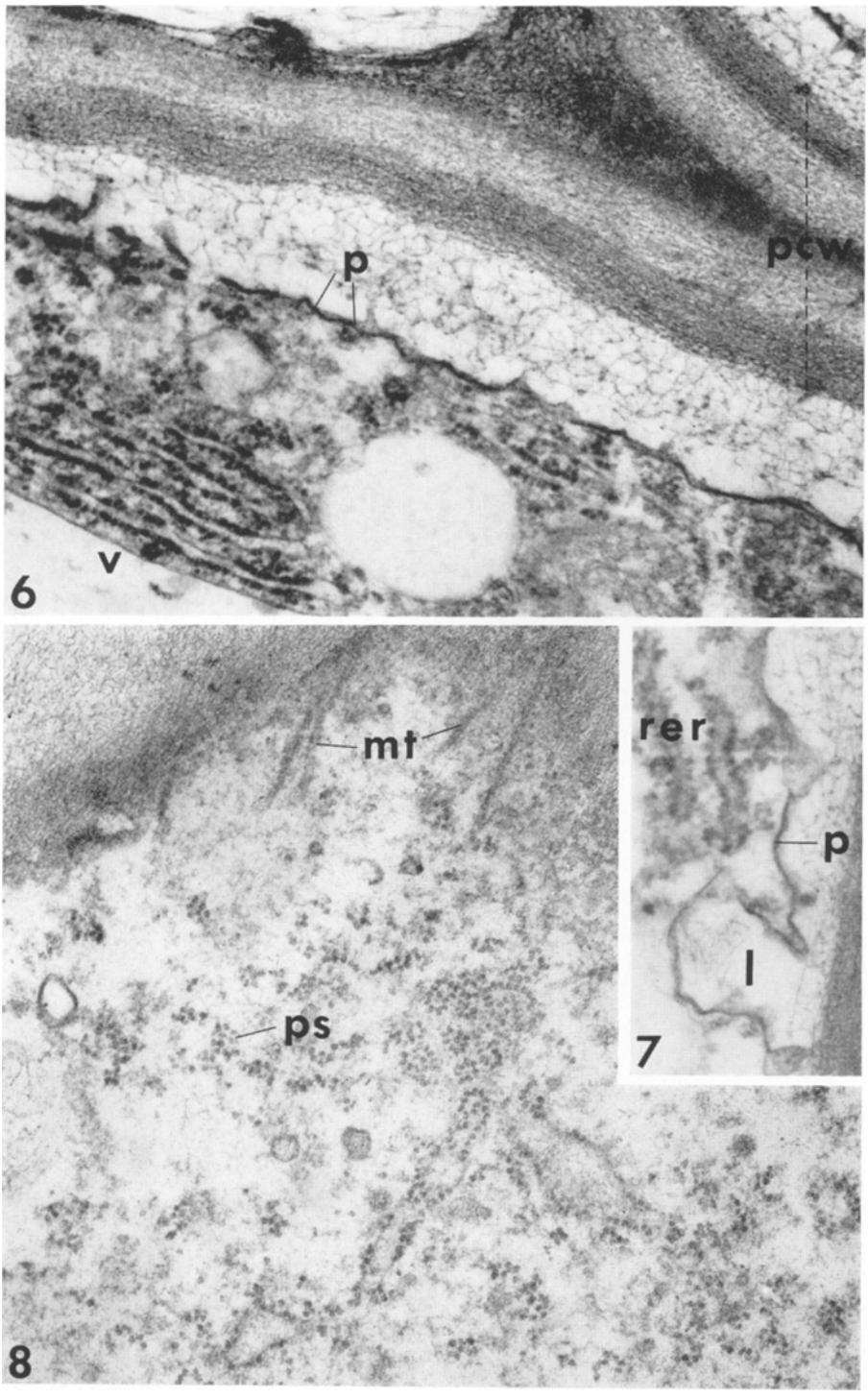
Figs. 6–8 (*T.E.M.* ×40,000). 72 hour cortical explants incubated on S 2 M medium. Note in transverse section (Figs. 6 and 7) thick layer of fibrillar wall material newly-deposited on primary cell wall and extensive tracts of rough ER in cytoplasm. Fig. 8 shows a glancing section of wall and cytoplasm (apparently in a region of new wall synthesis) with microtubules and polysomes prominent in cytoplasm



Figs. 6–8

activated to undergo dedifferentiation (Gautheret 1966, Yeoman and Street 1973) passing through DNA synthesis, mitosis, cell division, and finally redifferentiating to form a new cell type, the tracheary element. These changes have been documented at the light microscopic level (Torrey and Fosket 1970, Phillips and Torrey 1973).

Explants after 60 hours on S 2 M + K medium show a peak of mitotic activity, with however, the earliest cell divisions already apparent at 30 hours (Fig. 9). Divisions occur in the tangential or radial plane as well as in the transverse plane and the cortical cells become subdivided with little or no concomitant enlargement of the daughter cells. On occasion, tangential walls of cell divisions in adjacent cortical cells abut each other (Figs. 10 and 11), forming a row of aligned walls in an otherwise random distribution. Viewed in the longitudinal plane, a cell division is sometimes reminiscent of a dividing cambial initial (Fig. 14) as has been noted by Yeoman and Street (1973). At 60–72 hours some cortical cells have already divided once (Figs. 12, 14, 22, and 30) or are dividing a second time (Fig. 13) or occasionally have already undergone two sequences of divisions (Fig. 11). Thus the mitotic peak at 60 hours reported by Phillips and Torrey (1973) is complex, comprising mitoses of first and second divisions. All the cells still show extensive vacuolation with the enlarged interphase nuclei or mitotic figures frequently apparently suspended in the central vacuole by cytoplasmic strands (Fig. 29, arrows). Clearly, the cortical cells of the tissue explants show dramatic changes associated with activation into cell division. Over the sampling period of 60–72 hours on S 2 M + K medium one can observe a range of structures, including cells with nuclei in interphase before cell division, all stages of the first cell division, divided cells with nuclei in interphase and divided cells in stages of the second division. Differences in cell wall thickness assist one in deciding the sequence of events. Old mother cortical cell walls remain unchanged in thickness whilst new (daughter) cell walls are considerably thinner (Figs. 11, 15 *a*, and 30).

At the ultrastructural level, these changes are manifest in many cytological details. Mother cell walls of cortical cells cultured on S 2 M + K show none of the changes described for cells grown on S 2 M alone. However, new cell walls are formed in relation to the phragmoplast resulting from mitosis. Vesicular structures aligned on the cell plate are associated with microtubular

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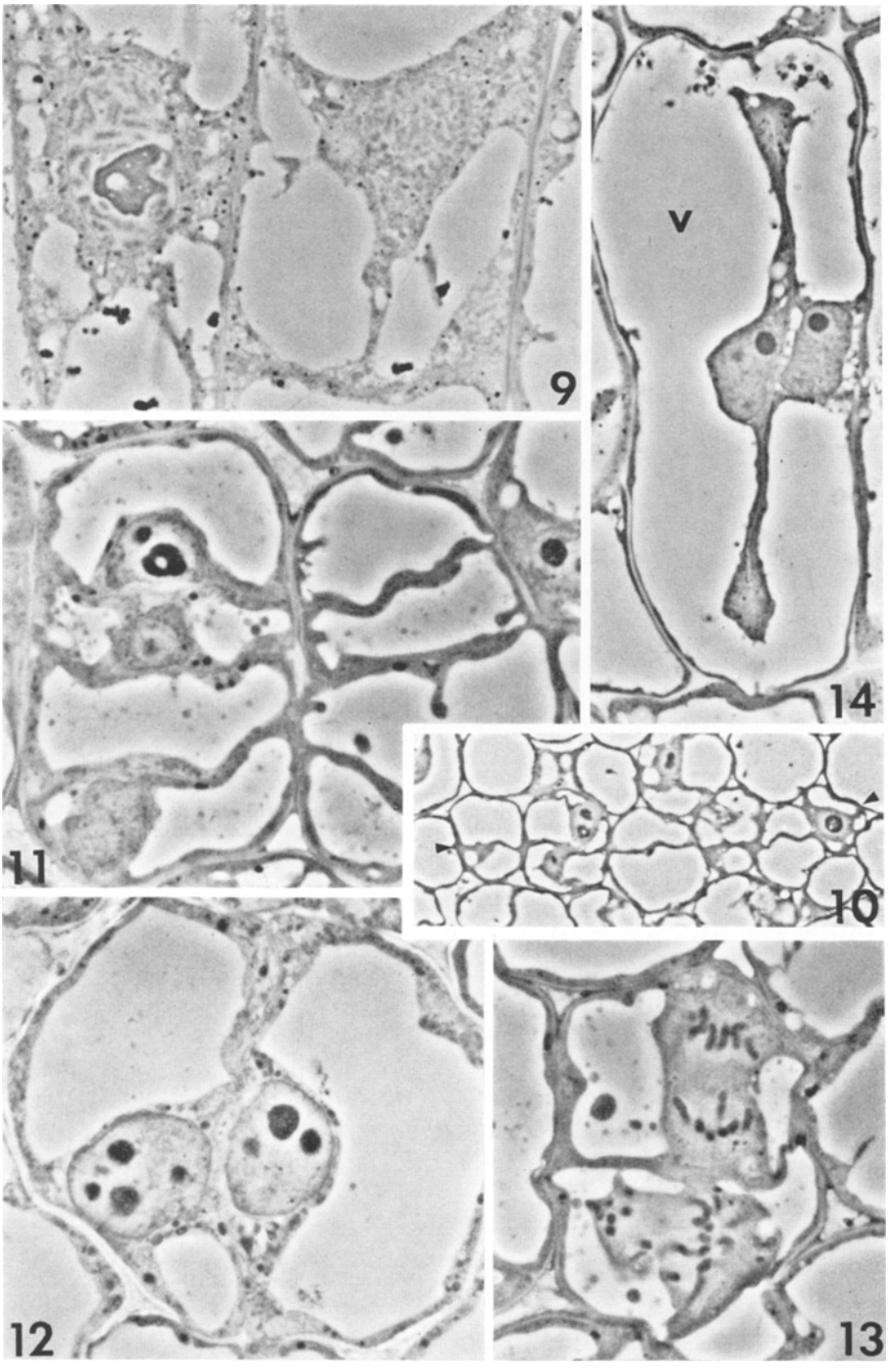
Figs. 9, 11 to 14 (*P.C.* ×1,200), and Fig. 10 (*P.C.* ×315). Explants cultured on S 2 M + K medium for 30 hours (Fig. 9), 60 hours (Figs. 10, 11, and 13), and 72 hours (Figs. 12 and 14). Note in Fig. 9 the suspended nuclei in prophase of the first mitoses in the two cortical cells and various stages in the cleavage of a mother cell into two (Figs. 12 and 14) and then four daughter cells (Figs. 11 and 13). Fig. 10 shows a group of cortical cells (delineated by arrows) in which the newly-formed daughter cell walls are aligned with each other



Figs. 9–14

arrays at right angles to the plate (Fig. 15 *a* and *b*). The middle lamella region is initially fibrillar (Fig. 15 *b*) and expanded ER, Golgi bodies and vesicles are found associated with this structure (Fig. 15 *a*). The extension of the cell plate generally occurs centrifugally (Fig. 15 *a*) eventually fusing with the much thicker primary (mother) cell wall. In the daughter cells the newly reconstituted nuclei frequently occur in proximity to the newly formed cell wall (Figs. 12 and 30). Invaginations of the mother cell wall may also occur (Figs. 16 and 17) but with no evidence of a centrifugally developing cell plate visible within the plane of section. Such invaginations resemble the in-growths occurring in *Marchantia polymorpha* L. in which Volker (1972) has described a centripetal development of the cell plate, although this has not been reported in higher plants. Clearly more detailed observations on the situation in pea may be of considerable interest.

The proportion of cytoplasm/cell in the now divided mother cells is markedly increased compared to the day 0 cells, although even after two sequences of divisions the former are still higher vacuolated (Fig. 11). Simpson (1975) reported a substantial increase in total protein nitrogen in explants cultured on S 2 M + K for 3 days. A general increase in cytoplasmic organelles is apparent at the ultrastructural level. Especially notable is the increase in rough ER and free ribosomes, especially in the region surrounding the nucleus and newly formed cell walls. In some cases, the cytoplasm is very dense, showing expanded ER and densely crowded free ribosomes. Numerous vesicles occur in the cytoplasm some of which are derived from the Golgi apparatus while others are probably formed from the expanded terminations of the ER.

In some sections Golgi are very frequent and usually intermingled with the rough ER. Mitochondria tend to be circular or oval in section but elongate and tubular structures are also present, apparently occurring with greater frequency than at day 0. Sometimes such mitochondria show constrictions (Fig. 19), perhaps indicating a division stage of this organelle. The membranous structure of the plastids is generally poorly defined although some contain numerous well-developed starch grains (Figs. 20 and 21). The still very prominent vacuoles frequently contain membranous or vesicular material which is sometimes attached to the tonoplast (Figs. 18 *a* and *b*). The vesicular structures sometime contain small particles (Fig. 18 *b*, asterisks), of a dimen-

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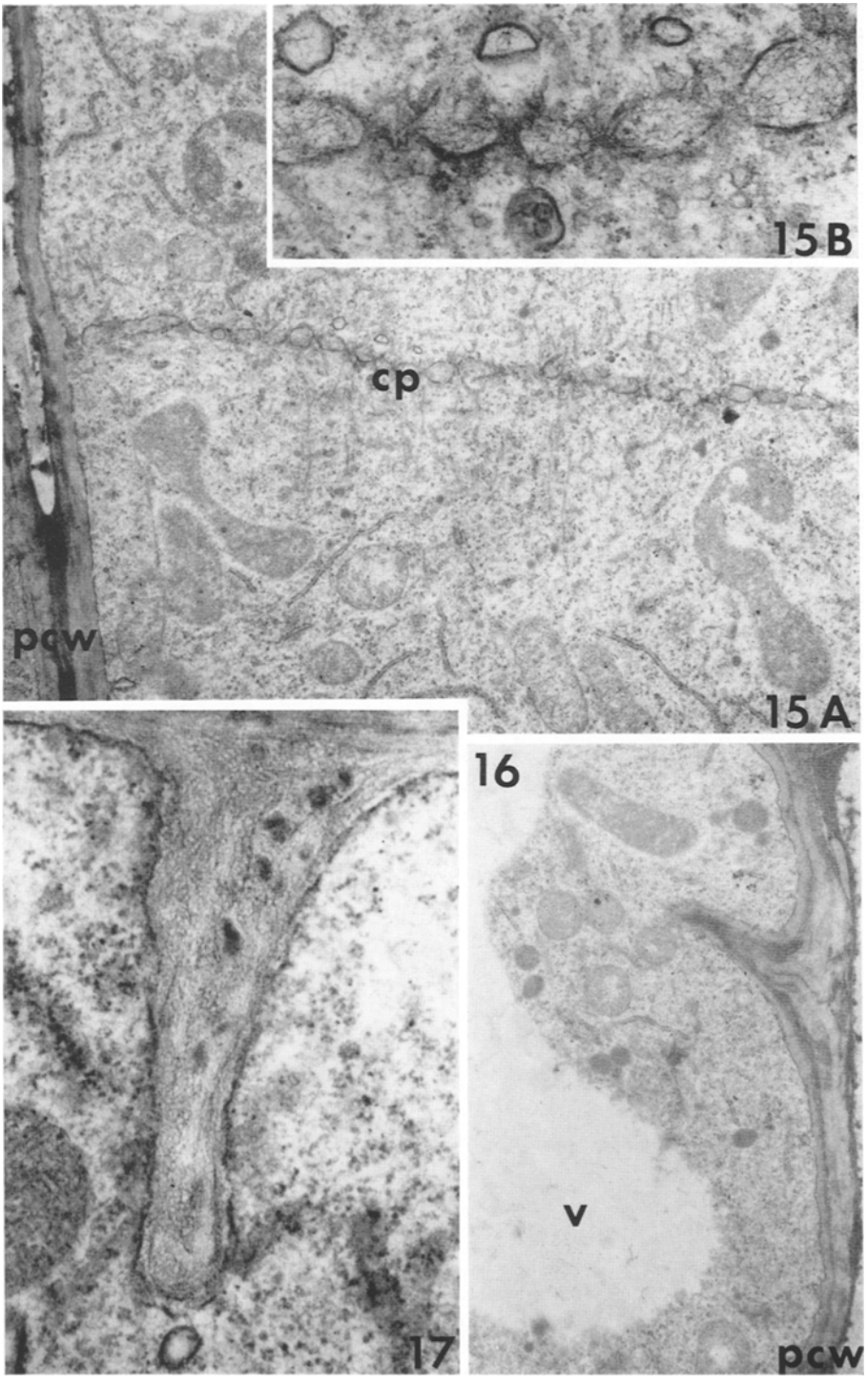
Figs. 15 *a* and 16 (*T.E.M.* ×10,000), and Fig. 15 *b* and 17 (*T.E.M.* ×40,000). Explants incubated on S 2 M + K medium for 60 hours. In Fig. 15 *a* the centrifugally developing vesicular cell plate has almost fused with the mother cell wall. Fig. 15 *b* shows detail of these cell plate vesicles with fibrillar material evident within them. Fig. 16 illustrates an invagination of the mother cell wall, apparently forming in the absence of a centrifugally developing cell plate. Fig. 17 shows details of a similar invagination



Figs. 15–17

sion similar to that of ribosomes, which are often embedded in a densely staining amorphous material.

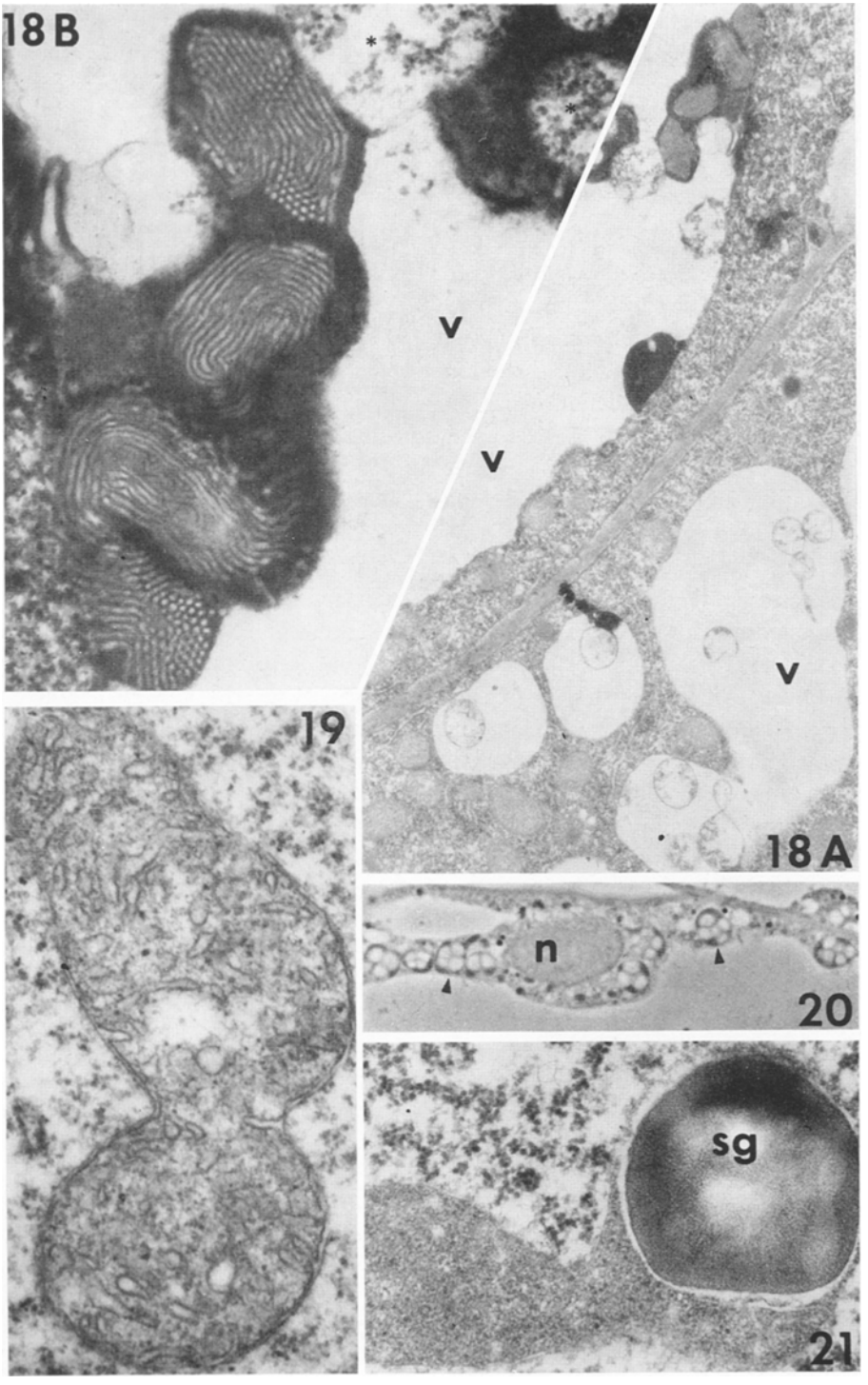
The nucleus shows dramatic changes, evident both at the light microscopic and electron microscopic level. As early as 30 hours in S 2 M + K medium, nuclear enlargement is evident in some cortical cells with prominent vacuolated and polymorphic nucleoli beginning to fade as condensing chromosomes of prophase appear (Fig. 9). By 60 hours, the first division has already occurred. Before the first mitosis the nucleus is often irregular in shape and sometimes shows lobing (Figs. 5, 23, and 25) and contains an enlarged nucleolus. The nucleoplasm is granular with diffusely distributed chromatin material and occasionally circular bodies occur in the nucleoplasm adjacent to the nucleolus (Figs. 24, arrow, and 28 *a* and *b*; see also day 0 nuclei in Figs. 1 and 5). These bodies possibly correspond to the nucleolar-organizing region of the nucleolar chromosome noted by Jordan and Chapman (1971) in excised discs of *Helianthus tuberosus* L. Similar structures termed karyosomes were observed by Hyde (1966) in root tips of *Plantago ovata* Forsk. and by Chaly and Setterfield (1975) in root tip cells of *Pisum sativum* L.

Fig. 29 shows metaphase of a first division with the chromosomes easily visualized at the EM level as highly condensed densely staining structures. In the area of chromosomes in the center of the cytoplasm, the organelles have been displaced and rough ER occurs generally in the periphery. Although the nuclear membrane is no longer intact, the nucleolus (asterisk) has apparently not fully disappeared.

Reconstituted nuclei after mitosis are well formed and large (Fig. 30), containing one to several enlarged nucleoli (Fig. 12), which are often vacuolate and polymorphic and whose edges are frequently fuzzy (Fig. 22). In some cells dense granular nucleolar-like material is distributed throughout the nucleoplasm (Fig. 26), extending in some cases to the nuclear envelope (Fig. 27). Plasmodesmata are evident in the thin, newly formed cell walls (Fig. 30).

Thus the daughter cells in these 3-day explants represent a population of actively dividing, still considerably vacuolated meristematic cells in which there is as yet no ultrastructural feature to hint at their future redifferentiation (by days 5–7) into tracheary elements.

Figs. 18 *a* (*T.E.M.* ×10,000), 18 *b*, 19, and 21 (*T.E.M.* ×40,000), and 20 (*P.C.* ×1,200). Explants incubated on S 2 M + K medium for 30 hours (Fig. 20), 60 hours (Figs. 18 and 19) or 72 hours (Fig. 21). Fig. 18 *a* shows a deposit in the vacuole adjacent to the tonoplast and Fig. 18 *b* illustrates this in greater detail (asterisks indicate particles in this deposit similar in size to cytoplasmic ribosomes). Fig. 19 illustrates a mitochondrion (note the well developed cristae) with a pronounced constriction. Figs. 20 and 21 show amyloplasts (indicated by arrows in Fig. 20)



Figs. 18–21

4. Discussion

The initiation of cell division in populations of mature parenchyma cells is a primary event in callus tissue formation from sterile tissue culture explants. Ultrastructural studies of the cellular events accompanying this activation from quiescence are relatively few. Surveys or general descriptions exist for carrot *Daucus carota* L. (Israel and Steward 1966); for tobacco *Nicotiana tabacum* L. Nitsch and Lance-Nougarède 1967, Nitsch 1968, Ross, Thorpe, and Costerton 1973); for Jerusalem artichoke *Helianthus tuberosus* L. (Fowke and Setterfield 1968, Yeoman and Street 1973); and for sweet pea *Lathyrus odorata* L. (Vasil 1971, 1973). Bowes (in the press) has also recently described the fine structure of callus formation in non-sterile root explants of dandelion *Taraxacum officinale* Weber whilst Jordan and Chapman (1971 and 1973) examined non-sterile discs of carrot and Jerusalem artichoke. EM observations on specific details of cellular changes in callus formation are scattered throughout the tissue culture and EM literature and are reviewed by Yeoman and Street (1973). The changes described above for pea root cortical explants have many features in common with the events described in these other explants but there is considerable variation in detail. Although all observations have been made in a single (cortical parenchyma) tissue, nevertheless there still exist problems in interpretation of the observations because of a certain degree of heterogeneity of the initial explant cell population, the lack of synchrony of the cellular responses and the difficulties in the fixation of initially highly vacuolated cells.

Cells of the pea root cortex show quite divergent responses when cultured as explants, depending on the hormones provided to them in the nutrient medium. On medium containing auxin, cell wall changes predominated during the first 3 days and associated cytoplasmic changes occurred which accompany the cell wall response. The explants increased in fresh weight and showed volume changes, especially in the inner cortical region (Simpson 1975), which are very probably attribuable to cells showing new wall synthesis as described in the present paper. Later, these same cells tended to round up and separate from each other accompanied by intercellular changes and dissolution of the middle lamellae. By 7–10 days both inner and outer cortical cells were

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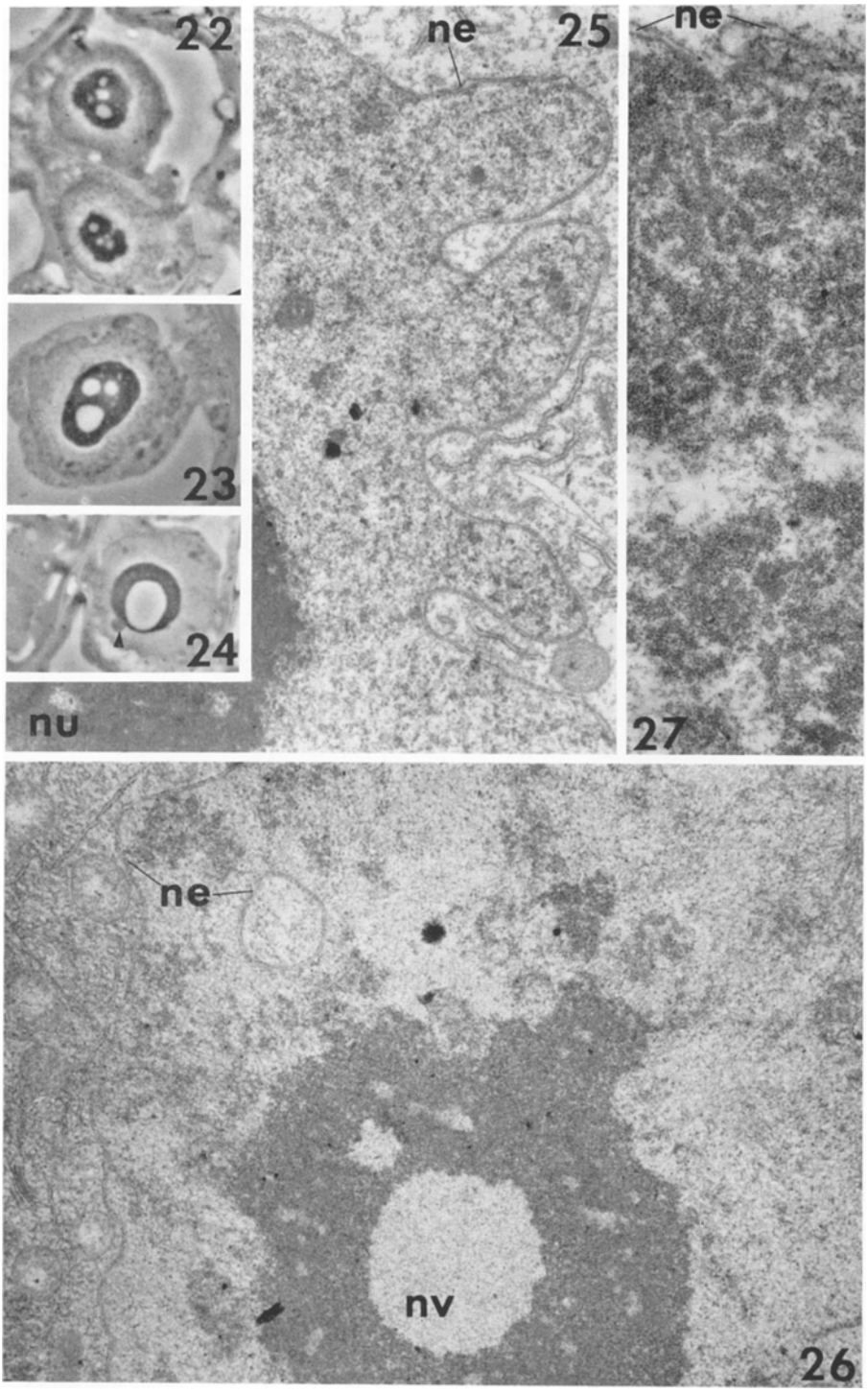
Figs. 22–24 (*P.C.* ×1,200), 25 and 26 (*T.E.M.* ×10,000), and 27 (*T.E.M.* ×20,000). Explants cultured on S 2 M + K medium for 60 hours (Fig. 26) or 72 hours (Figs. 22–25, and 27). Note the conspicuous lobing of the nucleus before the first mitosis (Figs. 23 and 25) and the possible presence of a nucleolar organizing region of the nucleolar chromosome (arrow) in Fig. 24. The reconstituted nuclei after mitosis show nucleoli with fuzzy margins (Figs. 22 and 26) and with granular nucleolar-like material distributed throughout the nucleoplasm (Fig. 26). Fig. 27 shows detail (from another nucleus) of this nucleolar-like material which extends as far as the nuclear envelope



Figs. 22–27

separating from each other (Simpson 1975). These changes are the classical response of storage tissues explanted and cultured in the presence of auxin.

New cell wall formation appeared to be associated with increased activity of the rough ER over the first 3 days in culture. The new diffusely fibrillar wall formation occurred as a layering on the inner surface of existing primary cell wall. Vesicles (presumably originating from the ER) occurred in the cytoplasm and these, together with microtubules, appeared to be associated with this new wall formation. The changes in the first 3 days may be presumed to be preliminary auxin-induced events leading to further cell enlargement and cell separation.

In the medium containing only auxin, nuclear changes were minimal. All nuclei were in interphase at the time of explanting and remained without DNA synthesis or other evident change with the exception of one mitosis noted in an inner cortical cell.

In marked contrast were the cytological changes occurring in cells of the pea root cortical explants cultured in the presence of auxin and cytokinin. When both hormones were present, wall changes in the mother cell walls seen in auxin-treated cells were not evident; rather, marked dedifferentiation (Gautheret 1966) changes affecting the nuclei and the cytoplasm were apparent. A whole new machinery developed in the cytoplasm, coupled with the onset of DNA synthesis and the subsequent nuclear changes associated with mitosis and cytokinesis (Phillips and Torrey 1973). These cellular changes occurred with almost no change in mother cell volume. Accompanying successive cell divisions, there was a progressive reduction in total vacuolation and an increase in total cytoplasm within the confines of the mother-cell wall; the latter phenomenon is evidently accompanied by synthesis of ER, ribosomes and other organelles. However, there is no real evidence in pea as to whether new (cytoplasmic) organelles are formed by replication (division or budding) from existing organelles or by *de novo* synthesis from the ground cytoplasm. Apart from the obvious difficulty of interpreting stages in such dynamic processes from static micrographs (coupled with only chance observations on single sections of a given organelle), it is also possible that there is a periodicity in cytoplasmic organelle formation which does not coincide with the sampling times (selected for peaks of mitotic activity) adopted in the current investigation. Thus Maruyama (1968) found that in developing

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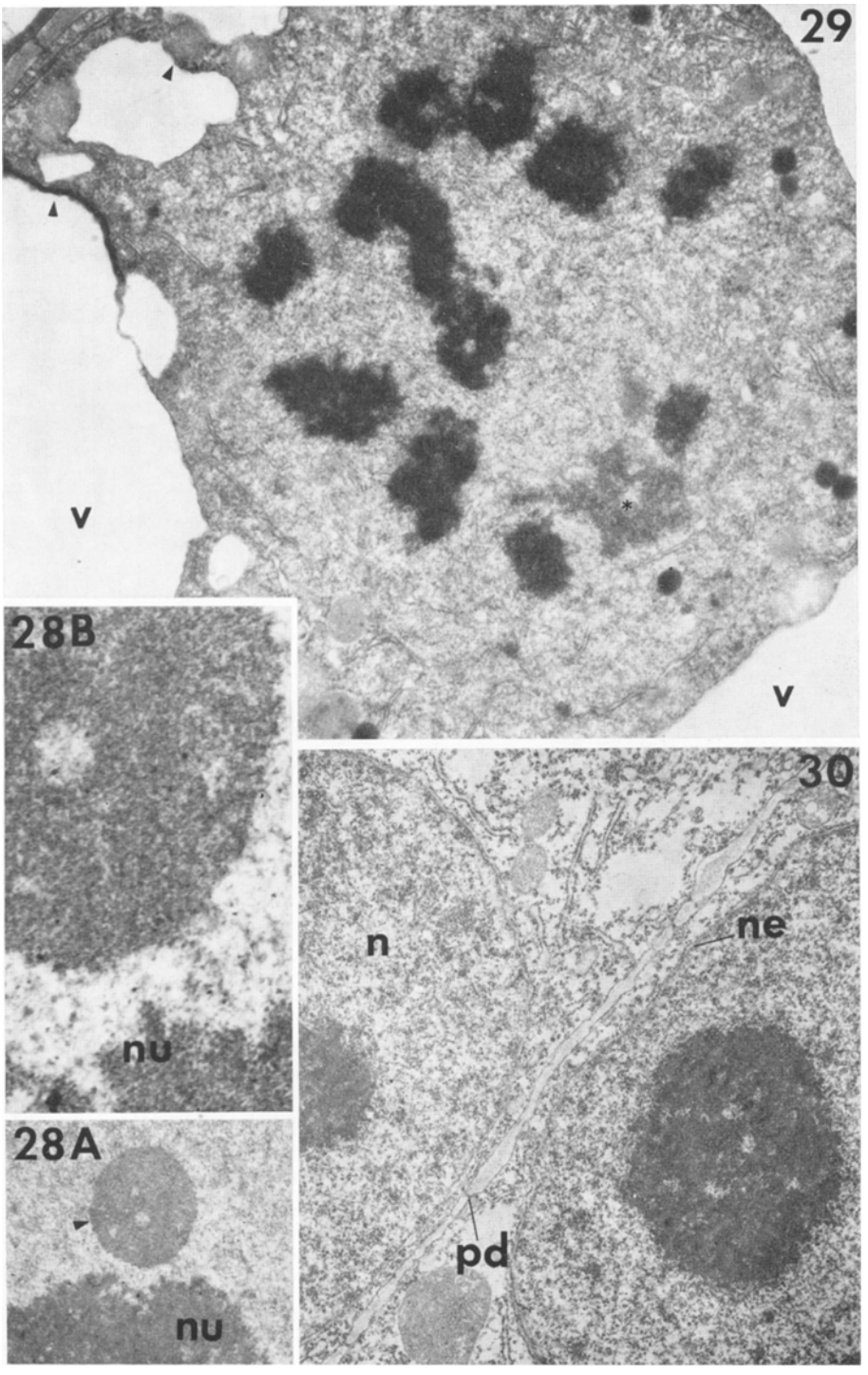
Figs. 28 *a*, 29, and 30 (*T.E.M.* ×10,000), and 28 *b* (*T.E.M.* ×40,000). 72 hour explants cultured on S 2 M + K medium. Fig. 28 *a* (arrow) shows a possible nucleolar organizer (see also Fig. 24) while Fig. 28 *b* shows details of the latter. Fig. 29 shows a metaphase nucleus, suspended by cytoplasmic strand (arrows) within the mother cell vacuole, with the remains of the nucleolus apparently still visible (asterisk). Fig. 30 shows two daughter cells with rounded nuclei adjacent to the thin dividing wall containing plasmodesmata



Figs. 28–30

microspores of *Tradescantia paludosa* divisions of mitochondria and plastids occurred at different times both from each other and nuclear divisions. The progressive decrease in cell size which accompanied activation of the pea root cortical cell is a concomitant of cellular dedifferentiation in other cultured explants (Nitsch and Lance-Nourgarède 1967, Yeoman and Street 1973) and has also been noted in adventive organogenesis of *in vivo* grown material (Crooks 1933, Bowes, in press).

The nucleolar changes visible at the light microscopic level in dedifferentiating root cortical parenchyma cells of pea include considerable enlargement, increased vacuolation, polymorphism, and formation of multiple nucleoli. Some or all of these changes have been noted in other dedifferentiating plant tissues (Fowke and Setterfield 1968, Gifford and Nitsch 1969, Jordan and Chapman 1971, 1973, Vasil 1971, 1973, Bowes, in press). At the electron microscopic level the activated pea nucleoli contain a large population of granular particles and in some nucleoli these form very diffuse, cloud-like arrays spreading throughout the nucleoplasm. Such nucleoli resemble greatly the nucleoli illustrated by Vasil (1973, Figs. 23 and 24) in callusing hypocotyls of sweet pea *Lathyrus odoratus* cultured *in vitro* on a 2,4-D medium. Vasil concluded that these granular particles are nucleolar ribosomes and suggested that the enlargement and diffusion of the nucleolus with the formation of multiple nucleoli, provide a more efficient system for the synthesis of rRNA and formation of ribosomal subunits which are presumably transported (by as yet unidentified means) to the cytoplasm. This proposed role for the diffused nucleolus would accord well with the situation in dedifferentiating pea cells where large scale and rapid synthesis of new cytoplasm is occurring. Thus the addition of a low molar concentration of cytokinin to the S 2 M medium completely switched the activities of the cell, suppressing further synthesis of the mother-cell wall and diverting the cellular energies into cell replication.

Cell divisions proceeded in rapid succession, cleaving the cytoplasm into packets of 2, 4, 6–8 daughter cells within 72 hours following explanting without apparent alteration in mother-cell volume, with newly formed thin walls separating the compartments within the thick wall of the original cortical cells. The ultrastructural changes in the first 3 days are events concerned with cell division and the reactivation of mature differentiated cortical parenchyma cells into a population of meristematic cells (Gautheret 1966).

The question arises—are the cells formed by these cell divisions already committed to the later formation by days 5–7 of tracheary elements? Is there evidence at the EM level presaging the cytodifferentiation events which are known to follow this hormonal treatment? The answer to the second question is no. If changes have occurred which involve the commitment of cortical cell derivatives to tracheary element formation, one cannot recognize them at the EM level in these newly formed cells. Evidence for the possibility of

tissue pattern formation is evident, for example, in the formation of arrays of cells with aligned cell walls (Figs. 10 and 11) or from the prior development of cell wall invaginations before cell plate formation (Figs. 16 and 17) but these are limited and random events leading to no organized structure. Cortical cell derivatives formed by 72 hours appear to be simply meristematic cells, uncommitted to any specific course of development. Only by biochemical means or measures other than microscopy is it likely to be possible to demonstrate whether or not the cells are already determined to a specific differentiation fate.

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