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DNA Synthesis, Cell Division and Specific Cytodifferentiation in Cultured Pea Root Cortical Explants[1]

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Pea root segments cut 10-11 mm behind the tip of germinating seedlings were prepared by removal of the central cylinders with a tissue punch. These cortical explants were cultured aseptically on nutrient medium containing auxin with and without added cytokinin. In the absence of kinetin, the cortical cells enlarged and separated but failed to show DNA synthesis, mitosis, cell division or subsequent cytodifferentiation. In the presence of 1 ppm kinetin, cortical nuclei showed ^{3}H-thymidine incorporation beginning between 24 and 32 hr; mitoses began about 48 hr, reaching a maximum of 6% at 60 hr. From an initial number of 8000 cells per segment, the cell count increased to 37,000 by day 7 and 140,000 by day 21. At the outset all mitoses were tetraploid; with time the proportion of tetraploid mitotic cells decreased and an octaploid population increased. A frequency of less than 10% diploid mitoses was observed after day 5. Only 25% of the cortical cells showed initial labeling. Beginning on day 7 tracheary elements differentiated from cortical derivatives. By day 14 about 25% and by day 21 about 35% of the total cell population had formed tracheary elements. As a system for analysis in biochemical and cytological terms, pea cortical explants represent an excellent system for the study of cytodifferentiation.

INTRODUCTION

Perhaps the most dramatic instance of cytodifferentiation in higher plants is the formation of tracheary elements, whose strikingly sculptured cell walls and lack of cytoplasmic contents render them distinctive and easy to identify. For this reason, and because cells of this type were observed to develop spontaneously in early tissue culture studies (e.g., Gautheret, 1950; Ball, 1950), the formation of tracheary elements in tissue culture has been regarded for some time as a model system for the study of cytodifferentiation (Torrey *et al.*, 1971).

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Earlier studies have concentrated on the chemical or hormonal control of xylogenesis; the importance of auxin was demonstrated by Jacobs (1952, 1954) by the analysis of xylem regeneration in wounded *Coleus* stems. Wetmore and Rier (1963) demonstrated in cultured callus tissue the importance of balanced levels of auxin and sugar for tracheary element formation, studies which were later confirmed by Jeffs and Northcote (1967). In addition, experimental systems have been reported in which cytokinins were the limiting factor for differentiation (Bergmann, 1964; Torrey, 1968; Torrey and Fosket, 1970). It seems likely that in fact auxins, cytokinins and perhaps other hormones and nutrients are required in balanced amounts for cytodifferentiation. These same substances are also required for cell division activity (Das *et al.*, 1956).

Another experimental approach is the careful analysis of changes in the cell population when an undifferentiated tissue is induced to form tracheary elements, the objective being to understand the history

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of the cells which ultimately differentiate, from the time that the chemical stimulus is given to the recognizable appearance of the differentiated state. Of interest in this context is the relationship between the cell cycle and cytodifferentiation and, in particular, the role of cell division. In animal systems, Stockdale and Topper (1966) concluded that prior mitosis was required before hormonally induced differentiation could proceed in mouse mammary gland epithelial cells, and they proposed that a division in the appropriate hormonal milieu was a critical step. In higher plant culture systems most reports refer to mitotic activity preceding xylogenesis (Cronshaw, 1967; Fosket and Torrey, 1969; Dalessandro and Roberts, 1971; Jeffs and Northcote, 1967). Fosket (1968) demonstrated that the inhibition of cell division by mitomycin C, fluorodeoxyuridine, or colchicine in each case also inhibits tracheary element formation in cultured *Coleus* segments.

If an experimental system is to be of use in the analysis of cellular changes preceding differentiation, several conditions should be fulfilled. First, the system should be highly reproducible, so that an accurate time-course of events can be constructed and used as a reliable guide for further experiments; second, a significant proportion of the cell population should differentiate; and third, it is preferable that the differentiative process be initiated by a single added substance. The studies of Torrey and Fosket (1970) showed that the culture of 1-mm discs cut from seedling pea roots approximated these conditions; in the presence of auxin and a cytokinin, cells in the cortex were stimulated to DNA synthesis and cell division, followed by differentiation to form tracheary elements. In this system, however, the cells of the central cylinder (i.e., within the endodermis) did not require kinetin to divide, their derivatives apparently did not differentiate, and in addition they divided as diploids while the cortical cells were largely polyploid. A study of cell population kinetics by Van't Hof and McMillan (1969) confirmed that cultured pea root segments exhibit properties of 2 cell populations, one associated with the cortex and the other with the central cylinder tissues, particularly the pericycle cells.

Consequently it was decided to culture pea root segments from which the central cylinder had been removed, i.e., cortical explants. It was hoped that such a system would simplify the analysis of cytological changes, increase the percentage yield of tracheary elements and be entirely dependent on exogenously supplied hormone for cell division as well as tracheary element formation. A method for culturing cortical explants of pea root segments was developed by Libbenga (1970), and was used by Libbenga and Torrey (1973) in a cytophotometric study of DNA changes in cortical cells treated with cytokinin and auxin.

MATERIALS AND METHODS

Culture techniques. Seeds of the garden pea, *Pisum sativum*, cv. Little Marvel, were surface-sterilized with 5% Pittchlor solution (a commercial preparation of sodium hypochlorite) for 15 min, rinsed in sterile distilled water, imbibed in sterile distilled water for 6–8 hr, and then germinated on 0.7% agar in distilled water in the dark at 20°C for 60–65 hr. Segments 1-mm thick were cut aseptically at 10–11 mm from the root tip, and the central cylinders were removed using the tissue punch described by Libbenga and Torrey (1973). The cortical explants were transferred aseptically to a strip of Whatman No. 40 (acid-extracted) filter paper moistened with liquid culture medium. The strips were held in slanted 25-mm diameter test tubes with the lower end dipping into 2.5 ml of medium. During the culture period the tubes were stored in darkness at 22°C,

except for brief periods of white light during samplings.

The basal medium, designated S2M, was that described by Torrey and Fosket (1970) except that agar was omitted. Comparisons were made between the response of cortical explants to this medium and to a similar medium containing 1.0 ppm kinetin designated S2M + K.

Cell counting. Cell and tracheary element counts were made by a modification of the acid-maceration procedure of Brown and Rickless (1949). Each cortical explant was placed in 0.5 ml of a solution of 5% chromium trioxide in 5% HCl and allowed to stand for 24 hr at 20°C. The sample was then drawn repeatedly into a glass syringe through a 20-gauge hypodermic needle, until the cells were well separated. The sample was then diluted with distilled water to a final volume, depending on the age of the explant in culture, to give a suspension of about 5000 cells per milliliter. Replicate 0.1 ml aliquots of the suspension were transferred to a cell-counting chamber made from microscope slides as described by Henshaw *et al.* (1966), and the total number of cells in this volume determined by counting at 60 × magnification. Calculations were made to determine the total cell and tracheary element numbers per segment. In Figs. 3–7 the vertical bars at each point represent two times the standard error of the mean value.

Histological procedures. For the preparation of sections, explants were fixed in 3% glutaraldehyde in 0.025 *M* phosphate buffer and embedded in glycol methacrylate according to the procedures of Feder and O'Brien (1968). Sections 2–3 μ thick were cut with a glass knife on a Porter-Blum microtome, mounted on glass slides, stained with 0.05% toluidine blue in benzoate buffer at pH 4.4, rinsed with water, air dried, and mounted.

For squash preparations, explants were fixed briefly (1 hr) in Carnoy's solution, rinsed in water, hydrolyzed for 12 min with 1 *N* HCl at 60°C, stained with Schiff's reagent for 30–60 min, rinsed with SO_2-water and tap water, and then squashed onto microscope slides coated with albumin. Cover slips were removed after freezing with Dry Ice (Conger and Fairchild, 1953) and the preparation was rinsed, air-dried, and mounted. In some cases it was found that better squashes could be obtained by treating the explants with 5% solution of pectinase (Rohm and Haas Pectinol R-10) in distilled water for 90 min at 20°C immediately prior to squashing.

Autoradiography. To label interphase nuclei, the cortical explants were transferred for 1 hr to paper strips moistened with S2M or S2M + K containing 1.0 μCi/ml tritiated thymidine at a specific activity of 19 Ci/mmole thymidine (New England Nuclear; NET, 027 ×). In earlier studies it had been found that radioactive label from tritiated thymidine incorporated into nuclei was removed by DNase. After the labeling period, explants were washed briefly in medium containing 10^{-4} *M* cold thymidine and fixed at once in Carnoy's solution. The explants were Feulgen-stained and squashed as above, and after air drying, were dipped in Kodak nuclear track emulsion type NTB3. On drying, the slides were stored in light-tight boxes at 2°C for 7 days, the emulsion was developed, and the slides were rinsed, air dried, and mounted.

RESULTS

Figure 1A shows in transverse section the appearance of a pea seedling root cut at 10 mm behind the tip: the ring of densely stained cells around the central region of the root segment indicates the position of the pericycle. A 1-mm thick segment cut at this level of the root contains about 20,000 cells. In Fig. 1B is shown a transection of a cortical explant prepared for culture; the punch has removed all the tissues of the central cylinder and one or two layers of inner cortical cells. Occasion-

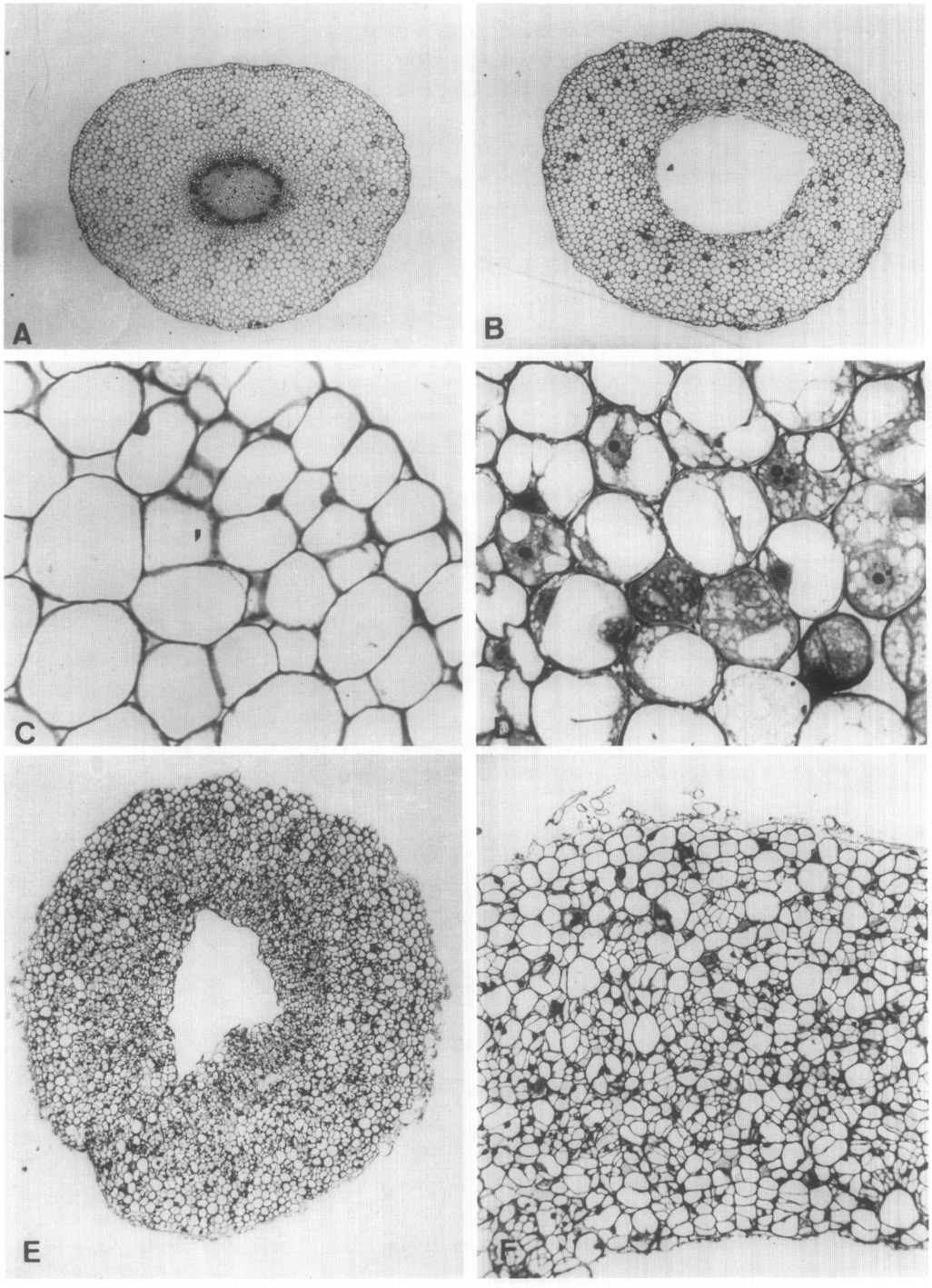

FIG. 1. Transverse sections of pea root segments. (A) Section of the whole root cut at 10 mm behind the root tip. Densely stained region is the pericycle. (B) Section of the cortical explant prepared for culture; central cylinder tissues have been punched out. (C) Enlarged view of section of cortical explant at beginning of culture. Note the scarcity of cytoplasm. Several small nuclei are seen in Section. (D) Section of cortical explant after culture for 3 days on S2M + K medium. Abundant cytoplasm, enlarged nuclei, and cytoplasmic strands are apparent. (E) Section of a whole cortical explant after culture on S2M + K for 7 days, showing extensive subdivision of cortical cells. Note the apparent disintegration of the epidermis at top. (F) Enlarged view of section shown in E. A, B, E, × 40; C, D, × 330; F, × 115.

ally some pericycle tissue may remain in explants that were not well centered in the segment holder. Such explants can usually be removed on inspection, and their frequency can be minimized by practice and selection of roots. Cortical explants contain 7000–8000 cells, of which 1500–2000 are epidermal and the rest cortical. Before culture, cortical cells contain very little cytoplasm and the nuclei are small and appressed to the walls (Fig. 1C). The epidermal cells are smaller and equally empty in appearance.

Morphological and Anatomical Changes in Explants Cultured on S2M

Cell division did not occur in explants on medium containing auxins but lacking cytokinin. However, cultured explants increased markedly in size concomitant with a 5- or 6-fold increase in fresh weight after two weeks of culture, resulting in a white, very loose and friable tissue mass. This change was due to expansion of individual cells coupled with a tendency for the cells to separate (Fig. 2A). These changes appear to be very similar to those described by Gautheret (1950) for Jerusalem artichoke explants cultured on medium containing high levels of auxin without added cytokinins.

Morphological and Anatomical Changes in Explants Cultured on S2M + K

During culture on medium containing auxins and 1 ppm kinetin, the explants increased in diameter up to 4–5 mm over a period of 3 weeks, but remained firm and compact in contrast to explants cultured without kinetin. A tendency for the explants to brown slightly was apparent after 12–14 days; up to this time they were white and slightly translucent.

Anatomical observation showed a considerable increase in cytoplasmic contents with enlargement of nuclei and nucleoli after 2–3 days (Fig. 1D); by the third day numerous mitoses were observed in sec-

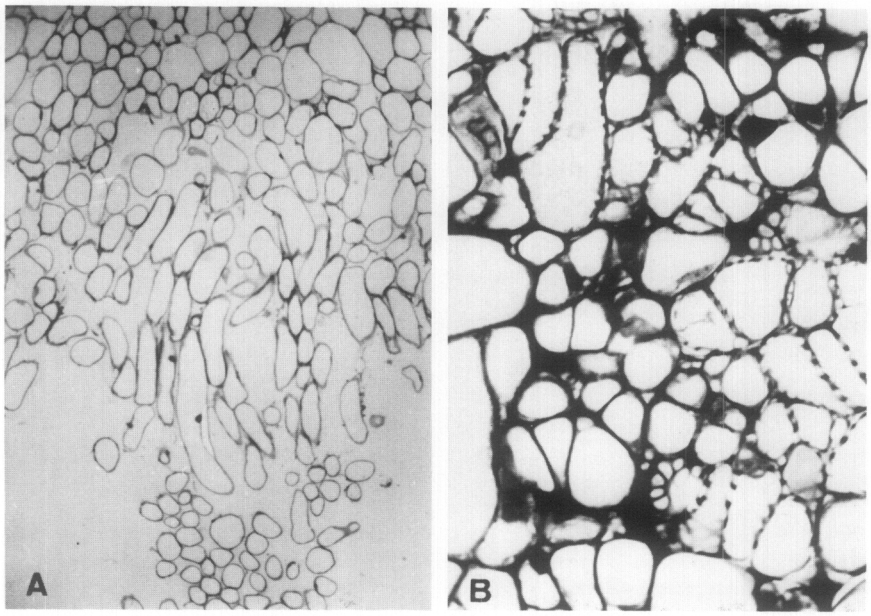

FIG. 2. Tissues and cells from cultured cortical explants. (A) Transverse section of part of a cortical explant after culture for 10 days on S2M. Note the expansion and separation of the cells. × 65. (B) Transverse section of the inner region of a cortical explant cultured for 10 days on S2M + K. The cut edges of thickened secondary cell walls of tracheary elements appear as broken lines. A, × 65; B, × 400.

tions and squashes. By day 4 some cortical cells were seen to be subdivided, and division continued until by day 7 the appearance was as shown in Fig. 1E and 1F. Note that Figs. 1B and 1E are at the same magnification. In most cases new cell walls were randomly oriented within the original cortical cells, and there was no evidence of organization in either transverse or longitudinal sections. However, in some transverse sections parts of the cortex adjacent to the inner surface of the explant divided by walls formed roughly parallel to the surface, giving the appearance of more regular rows of cells. A similar phenomenon was described by Gautheret (1957) for Jerusalem artichoke explants, occurring in tissue portions submerged in liquid medium containing auxin. Gautheret suggested that the orientation of such cell division was governed by the direction of penetration of auxin into the tissue; in the pea root cortical explant another possible factor is the release of nutrients in the region from cells damaged by the punch. There was no evidence from sections that these "cambial-like" derivatives were associated with higher rates of tracheary element differentiation than the regions of randomly oriented division.

Tracheary elements were observed in squashes and suspensions by day 7. Most of these elements contained nuclei and cytoplasmic contents which were lost apparently within 1 or 2 days thereafter, leaving empty, mature tracheary elements (Fig. 2B). By the tenth day of culture large areas in the central part of the cortex were seen in section to be composed almost entirely of masses of tracheary elements, with smaller groups of elements scattered randomly throughout the inner and outer edges of the explant.

Longitudinal sections of 10-day explants showed that elements were not localized with reference to the surface on the substrate but were randomly distributed throughout the 1-mm length of the explant.

The epidermal cells of explants cultured on S2M + K did not respond in the same way as cortical cells. Sections cut after 3 days of culture showed little if any increase in cytoplasmic contents, and after 5–7 days a general disintegration of the epidermis was apparent (Fig. 1F).

Cell Proliferation and Tracheary Element Differentiation

Figure 3 shows the increase with time in cell and tracheary element number in explants cultured on S2M + K. On medium without added kinetin (S2M) there was no significant increase in cell number above the original value of 8000 during a 21-day culture period. In the occasional segment that did show a small amount of proliferation, microscopic examination of cells separated by maceration indicated that this increase was due to divisions in remaining pericycle tissue. No tracheary elements were observed in segments cultured on S2M in any of a considerable number of experiments.

With medium containing 1 ppm kinetin (S2M + K), a small increase in cell number was detected by the third day, after which the number per explant increased at regular increments to 37,000 by day 7 and to 140,000 after 21 days of culture. It was not clear whether the steplike increases in cell number after day 9 were due to sampling errors or were real.

No tracheary elements were observed until day 7, at which time approximately 2500 per explant had differentiated. By day 14 the explants contained about 12,000 tracheary elements, and by the end of the 3-week culture period almost 50,000 elements had differentiated per explant.

The results shown in Fig. 3 were confirmed in a separate experiment of the same type, which gave values in close agreement. It is clear that the response of pea root cortical explants is similar to that described previously for the cortex of whole pea root segments (Torrey and Fos-

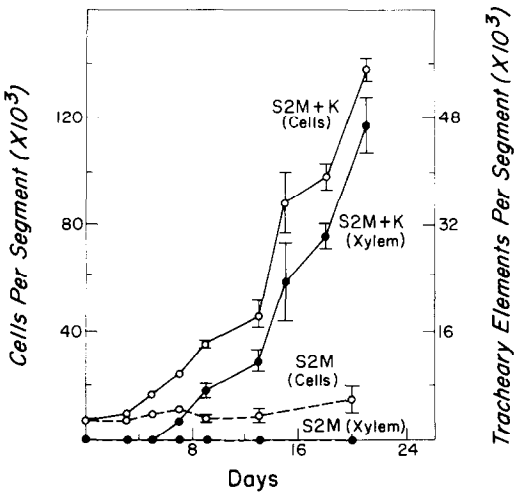

FIG. 3. Changes in cell and tracheary element numbers in cortical explants cultured on S2M and S2M + 1 ppm kinetin (S2M + K) as a function of time in days. The term cells refers to the total cell number per segment including tracheary elements. Vertical bars in this figure and subsequent figures represent two times the standard error of the mean value.

ket, 1970) and that the analysis of cellular events prior to differentiation can be greatly simplified by removal of the central cylinder.

The differentiation of tracheary elements in segments cultured on S2M + K continued at least up to 3 weeks. This finding excludes the possibility that such differentiation represents merely the maturation of a population of cells in the original explant that was already predestined to differentiate. Instead there was a continuing induction of differentiation that appears to be closely linked to cell-division activity (see Fig. 3).

The yield of tracheary elements expressed as a percentage of the total population in segments cultured on S2M + K is illustrated in Fig. 4. It is possible that this yield may be increased further by manipulation of medium components such as auxins and cytokinins (Dalessandro and Roberts, 1971), sugar levels and types (Jeffs and Northcote, 1967), or other nutritional or environmental factors. High yields of differentiated cells are likely to be necessary for many kinds of biochemical analysis which may be undertaken in the light of increased knowledge of cytological changes.

Mitotic Changes

An experiment was carried out in which segments were grown on S2M + K and fixed at 12-hr intervals. After staining and squashing, the percentage of cells in mitosis was determined as a function of time in culture (Fig. 5). Mitoses were first observed after 2 days, and a peak of more than 6% of the cells were in division after 60 hr. The mitotic frequency then declined to a minimum, and a second peak was evident after 6 days. Tracheary elements were observed in all squashes from day 7 onwards. In a separate experiment 2 peaks of mitotic frequency were again observed but on days 3 and 5; the discrepancy in timing was probably due to the lower frequency of sampling in the latter experiment. From day 8 until at least day 21 the mitotic frequency remained between 1% and 2% with no further peaks. Segments

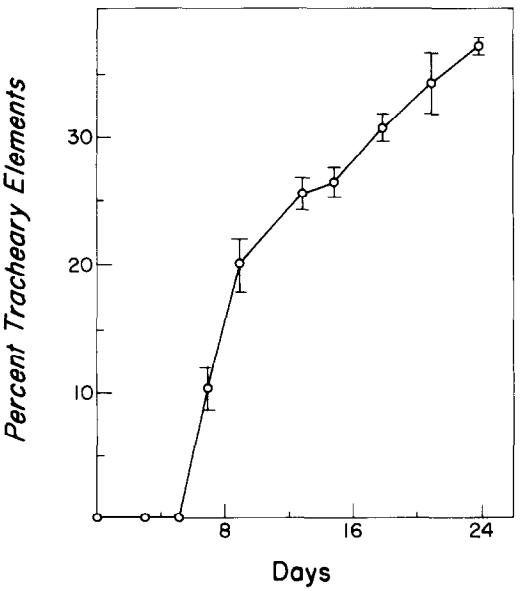

FIG. 4. Number of tracheary elements expressed as a percentage of the total cell population in cortical explants cultured on S2M + K for various periods of time.

cultured on S2M contained no mitotic figures, except for occasional segments with residual pericycle tissue.

From earlier cytological studies on whole pea root segments cultured on medium containing cytokinin (Matthysse and Torrey, 1967a,b) it was known that while diploid mitoses began within 24 hr of culture, a population of polyploid cells came into division after 72 hr. This observation was confirmed by Van't Hof and McMillan (1969) and from observations on histological sections of pea root segments. Torrey and Fosket (1970) concluded that the cells of the cortex divided as polyploids, while the cells of the central cylinder, particularly pericycle but also xylem parenchyma and procambium divided as diploids. It was interesting therefore to investigate the ploidy situation in cortical explants. Counts were made of metaphase figures in Feulgen-stained cytological squashes prepared after increasing culture periods on S2M + K. Three mitotic classes were scored viz., diploids with 14 chromosomes, tetraploids and octaploids or higher chromosome numbers. Initially all the divisions were tetraploid (Fig. 6), but the proportion of tetraploids declined throughout the 3-week period, while the percentage of the octaploid class showed a corresponding increase. Diploids constituted less than 10% of the dividing population from day 7 on, but a maximum of 35% was recorded on day 5. The significance of this peak is not known.

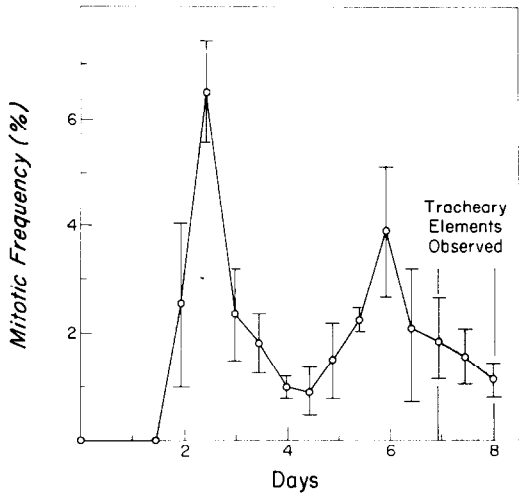

FIG. 5. Mitotic index of cortical explants cultured on S2M + K. Counts were made of the number of mitotic figures per 1000 nuclei in Feulgen-stained squash preparations. Segments cultured on medium lacking cytokinin (S2M) contained no mitotic figures, except in those occasional segments where incomplete removal of pericycle tissue was suspected. The shaded area indicates when tracheary elements were observed in the squashes.

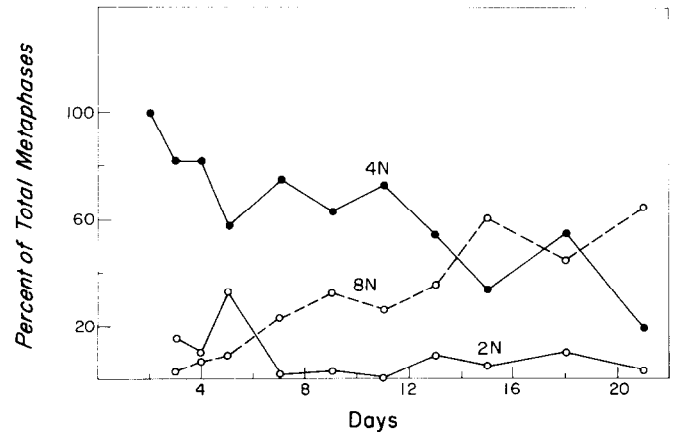

FIG. 6. Distribution of ploidy classes in metaphase mitoses from cortical explants cultured on S2M + K as a function of time. The total number of metaphases scored on days 2, 3, 4, 5, 7, 9, 11, 13, 15, 18 and 21 were, respectively, 7, 56, 30, 36, 110, 94, 11, 11, 44, 20, and 36. $2N$ = diploid, $4N$ = tetraploid, $8N$ = octaploid or higher chromosome numbers.

The origin of the tetraploid cells is a question of considerable importance. Two extreme conditions may be considered; either the cells in the 10–11 mm region were already tetraploid as a result of natural developmental processes or they were diploid prior to culture, in which case an endoreduplicative ploidy increase was stimulated by kinetin. Libbenga and Torrey (1973) have shown by fluorescent microspectrophotometric DNA measurements of Feulgen-stained cortical nuclei that at day 0 cells with 2c and 4c levels of DNA are present, the 2c predominating. The 2c cells must be diploids in the G_1 phase of the cell cycle; the 4c may be either diploids in G_2 or tetraploids in G_1. After culture on S2M + K for 3 days the DNA levels observed were 4c, 8c, and 16c, with no 2c nuclei and with 8c cells predominating (8c mitotic cells would be tetraploid or 4*N*). This result, which is in agreement with results in this study (Fig. 6), supports the theory that cortical nuclei at this level in the root prior to culture are diploid and that endoreduplication of DNA is stimulated by kinetin in culture.

^{3}H-Thymidine Incorporation During the Early Stages of Cortical Explant Culture

An important goal in the analysis of cellular events preceding tracheary element differentiation is an understanding of the timing of DNA synthesis in relation to cell division and differentiation. This is particularly so since polyploidy is involved in this system, and it is not known at present whether polyploidy is an essential prerequisite to differentiation or merely a separate but accompanying phenomenon.

A preliminary experiment was performed to investigate DNA synthesis during the first 4 days of culture by following ^{3}H-thymidine incorporation. Cortical explants were cultured on S2M + K and at 8 hr intervals, were transferred to the same medium containing tritiated thymidine for 60 min, then fixed and squashed, and autoradiographs were prepared. The nuclei of explants cultured on S2M did not show any significant incorporation of labeled thymidine (Fig. 7A). This finding contrasts with those reported by Das *et al.* (1958) and Simard (1971), who found that tobacco pith nuclei incorporated thymidine when maintained on a medium devoid of hormones, and at a higher rate when the medium contained an auxin. In the pea root cortical explant wounding alone does not stimulate DNA synthesis, even in the presence of auxins. In the presence of 1 ppm kinetin, thymidine was not incorpo-

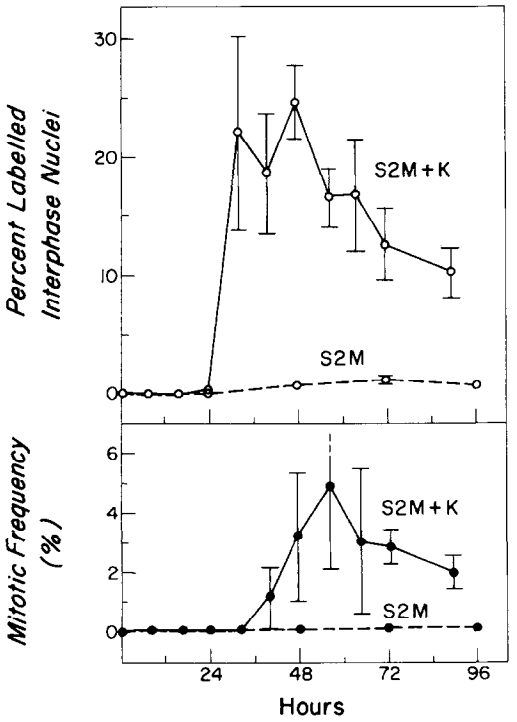

FIG. 7A (above). Cortical explants grown on S2M or S2M + K were transferred at 8-hr intervals to corresponding medium containing 1.0 μCi tritiated thymidine per milliliter for 1-hr periods. At the end of the labeling period, segments were immediately fixed and squashed; the percentage interphase nuclei containing radioactivity was determined after autoradiography.

FIG. 7B (below). The mitotic frequency was determined by counting all mitoses in the same preparations as in Fig. 7A.

rated at 0, 8, 16, or 24 hr of culture, but significant incorporation was observed from 32 hr on. A maximum of 25% of the nuclei were labeled during the period 32–33 hr and, while the variation between segments was rather wide (owing perhaps to differences in the timing of the onset of DNA synthesis), there was an indication of a possible second peak at 48–49 hr. While this observation requires stringent checking, it is of interest in view of the possible involvement of endoreduplicative cycles of DNA synthesis leading to tetraploid mitoses, i.e., cells going from 2c to 8c before dividing. In Fig. 7B are plotted the mitotic frequencies of segments shown in Fig. 7A. These values agree well with those shown in Fig. 5 with a peak at about 60 hr in segments cultured on S2M + K. In order to check the possibility that the 1-hr labeling period was failing to detect nuclei synthesizing DNA between samplings, segments were cultured continuously on S2M or S2M + K containing labeled thymidine, and were fixed and squashed after 54 hr. Under these conditions 26.7% of the nuclei in segments cultured on S2M + K, and 2.9% on S2M showed label. These values indicate a high degree of synchrony within at least some segments after 24 and 40 hr of culture (Fig. 7A), and in addition confirm that only a minority of the cell population, around 25–30% are sensitive to kinetin during the early stages of culture.

DISCUSSION AND CONCLUSIONS

Cells in cortical explants respond in essentially the same way to hormonal stimuli as do cortical cells in excised segments as described by Torrey and Fosket (1970). There is evidence that the isolated cortical explant is more sensitive to exogenous cytokinin, showing an observable response to 0.01 ppm kinetin (Libbenga and Torrey, 1973). Furthermore, there is an experimental advantage in removing all the central cylinder cells since the latter cells divide as diploids and complicate the analysis of cytodifferentiation from the cortical cells themselves. The cortical explant tissue system is ideally suited to further biochemical and cytological analysis since one begins experimentation with an almost pure population to which the epidermal cells appear to make little or no contribution.

Yet the cortical cells at day 0 are a mixture, expecially with respect to their DNA content. Of the total cortical population, only about 25% show DNA synthesis (as measured by ^{3}H-thymidine incorporation) in response to auxin-cytokinin treatment. Many of these cells may be presumed to begin at the 2c level of DNA. However, the first cells to divide do so at about 48 hr, even though one sees no ^{3}H-thymidine incorporation until after 24 hr (Fig. 7A). Since the cells observed to divide at the outset were tetraploid, it seems reasonable to assume that these first dividing cells were at the 4c level at day 0, underwent one round of DNA synthesis and then divided. Later, some cortical cells which had undergone two rounds of DNA synthesis divided as tetraploids. Either of these two cell types might show diplochromosomes. Still later in culture, cells with nuclear DNA at the 16c level (three rounds from the 2c level or two rounds from the 4c level) came into division in increasing proportion, driven apparently by the hormonal stimuli provided in culture.

A number of problems remain unsolved. First is the problem of why only 25% of the cortical cells respond to hormones. Can the percentage of responsive cells be increased so that all cortical cells divide? A second question is which of the various cellular derivatives of the cortical cells that do divide go on to differentiate as tracheary elements. By selective labeling it should be possible to determine specifically whether cytodifferentiation in this system is dependent upon endoreduplication of DNA and, if so, what this implies about the process of cell determination.

In the intact seedling root, the mature parenchyma cells that comprise the cortex show no further differentiation, that is, they remain as living fully differentiated cells. Never in nature do they show the type of reactivation and novel differentiation elicited in the excised cultured system described here. The only case in which these cells normally might divide again is in the initiation of root nodules under the hormonal stimulation produced by the infective bacterium *Rhizobium* (Phillips and Torrey, 1972). A further fundamental question thus concerns the nature of this reactivation, which involves the reprogramming of the cellular fates and an imposition of a new differentiation state. Can yet other fates be elicited by different hormones or combinations of hormones? The cortical explant system seems to present an ideal situation in which to study these fundamental questions.

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