

# Deoxyribonucleic Acid Synthesis in Root Cap Cells of Cultured Roots of *Convolvulus*<sup>1</sup>

Received for publication March 5, 1971

HARRY L. PHILLIPS, JR., AND JOHN G. TORREY  
*Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138*

## ABSTRACT

Isolated cultured roots of *Convolvulus arvensis* L. were incubated in 0.2 microcurie per milliliter methyl-<sup>3</sup>H-thymidine for 14 hours, for 64 hours, or for 14 hours followed by transfer to fresh nutrient medium without tritiated thymidine. Autoradiographs of serial, longitudinal sections of roots which were continuously incubated with tritiated thymidine showed that cells of the root cap columella did not undergo DNA synthesis after their formation from the root cap initials. In roots pulse-labeled with tritiated thymidine, the movement of labeled cells through the root cap columella was followed. Labeled cells were displaced at a constant rate of 72 microns per day over a period of 6 to 9 days before they were sloughed off from the root cap. The specialized role of the root cap cells in relation to their distinctive metabolism and longevity is discussed.

In experiments designed to determine the presence of a quiescent center in the apical meristem of roots by utilizing radioactively labeled precursors and autoradiography, investigators have noted that significant portions of the root cap remained unlabeled. While the initial cells of the root cap and the cells of the root cap periphery show active incorporation of DNA precursors, thereby delimiting the distal boundary of the quiescent center of the root proper, cells derived from the root cap initials and displaced subsequently through the columella show little or no incorporation. Clowes (3) reported that the mature, distal root cap cells of *Zea mays* did not incorporate radioactively labeled phosphate or adenine. Later, Clowes (5) noted that after supplying labeled sulfate or L-leucine, the nonmeristematic cells of the root caps of *Vicia faba*, *Zea mays*, *Allium ascalonium*, and *Sinapis alba* showed a lower density of silver grains after autoradiography than did the meristematic cells of the root cap and of the root proper. Clowes (4) observed in developing lateral roots that the first cells to lose the capacity to incorporate labeled adenine into DNA were the distal cap cells. Rabideau and Mericle (18) analyzed the distribution of labeled compounds in the shoot and root apices of intact corn plants after allowing the assimilation of <sup>14</sup>CO<sub>2</sub> into the leaves for 24 hr. Although the authors failed to comment on the distribution of label in the various regions of the root apex, their autoradiographs showed

that the cells of the central root cap were unlabeled, whereas the cells of the root cap initials and the cells at the surface of the root cap did show labeling.

Clowes (6) reported from absorption microdensitometric measurements of Feulgen-stained sections that, although the root cap initials rapidly synthesized DNA and were at the 4C level of DNA, the immediate derivatives of the root cap initials were at the 2C level and did not synthesize DNA. Although DNA synthesis resumed in some of the cells of the root cap located 100 to 200  $\mu$  from the root cap junction, these cells were not regularly arranged in the root cap. Only a few of the most distal cells were at the 8C level of DNA. Barlow (2) found that nearly all the central cap cells in *Zea mays* have a 2C DNA value, while cells in the root cap periphery have a 4C value, and cells in the root cap initials show intermediate values between 2C and 4C, as would be expected for actively dividing cells.

It was the purpose of the present work to study in detail the pattern of incorporation of radioactively labeled precursors of DNA into the nuclei of the root cap initial cells and the lack thereof in derivative cells of the root cap columella. The lack of DNA synthesis in maturing root cap cells of the columella allowed us to label specific cells of the root cap initials with a pulse treatment and then to follow the incorporated label during a subsequent period, thereby determining the age and longevity of the root cap cells.

## MATERIALS AND METHODS

Isolated cultured roots of *Convolvulus arvensis* L., obtained from a clone of roots maintained in culture over a period of 18 years by subculturing root segments and tips, were used in these experiments. Roots were cultured in 125-ml Erlenmeyer flasks containing 50 ml of liquid Bonner-Devirian medium (20) in the dark at 23 C. Cultures were agitated continuously on a horizontal rotary shaker at 80 rpm. Prior to use root tips were transferred to fresh medium for 3 to 4 days to determine the approximate rate of elongation. Root tips elongating at a rate of 15 to 30 mm per day were used in these experiments. Root tips were transferred aseptically to fresh medium containing methyl-<sup>3</sup>H-thymidine (specific radioactivity 6.0 c/mm) at a final concentration of 0.2  $\mu$ C/ml for either 14 or 64 hr. The root tips supplied with tritiated thymidine for 14 hr were either fixed at the end of the treatment or were transferred to fresh medium without tritiated thymidine for 24, 48, 120, 168, 288, and 336 hr before fixation. The root tips supplied with tritiated thymidine for 64 hr were fixed immediately after the treatment. Roots were fixed in 3% glutaraldehyde in 25 mM phosphate buffer, pH 6.8, for 2 to 2.5 hr at room temperature and subsequently stored in 50 mM phosphate buffer, pH 6.8, in the refrigerator until dehydration. Procedures for dehydration and embedding in Epon-Araldite were

<sup>1</sup> This research was supported in part by research Grant GM-08145 and fellowship Grant GM 41021 to H.L.P. from the National Institutes of Health.

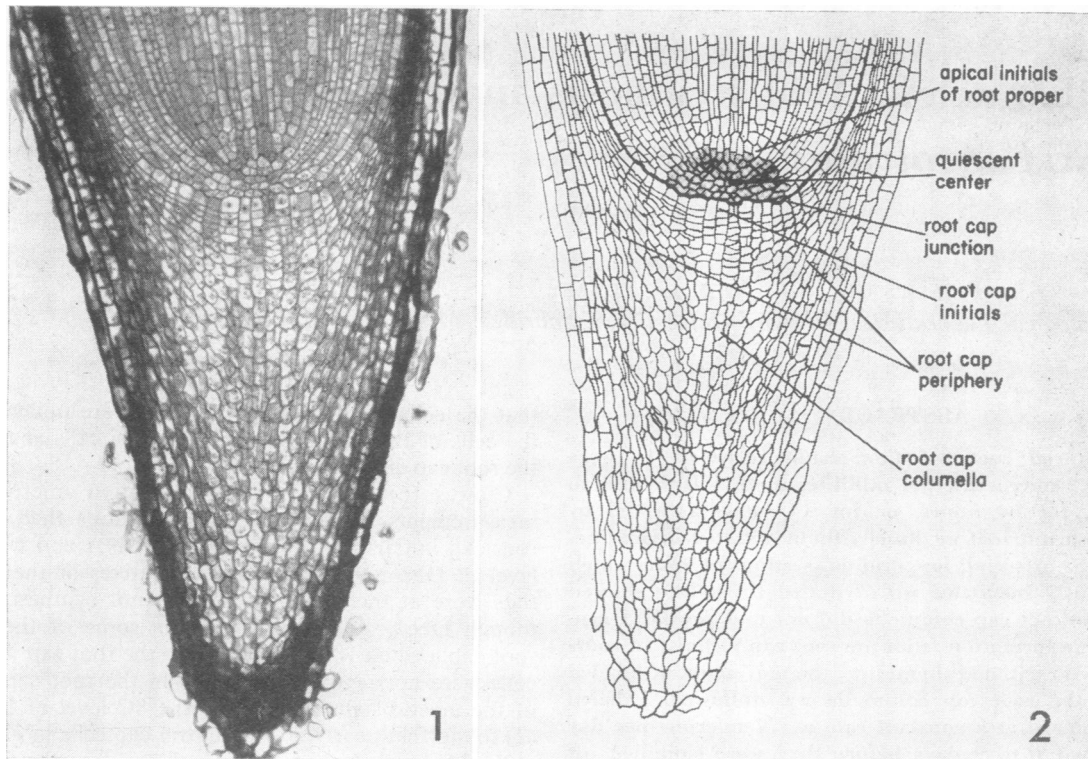


FIG. 1. Median longitudinal section of a root apex of *Convolvulus arvensis* L.  $\times 140$ .

FIG. 2. Diagrammatic representation of Figure 1, showing the regions of the root apex discussed in the text.

the same as those previously reported (17). Serial longitudinal sections  $5 \mu$  thick were obtained with a dry knife with a Huxley Ultramicrotome and mounted on slides which were then coated with Kodak nuclear track emulsion NTB3, diluted 1:1 with distilled water, in darkness. The dried slides were stored in slide boxes in the presence of anhydrous calcium chloride in complete darkness. The slides were developed with chilled Amidol in darkness with only the use of a Kodak adjustable safelight lamp with a Kodak Wratten series 2 filter after exposure periods ranging from 6 to 8 weeks. The sections were stained for 2 min with 0.05% toluidine blue in phosphate buffer, pH 6.8, to which 1% sodium borate was added. Measurements were made with the use of a calibrated ocular micrometer.

## RESULTS

A median longitudinal section of a cultured root tip which received no treatment at all is shown in Figure 1. Figure 2 is a schematic diagram based on a tracing of Figure 1. The root cap initial cells are separated from the cells of the root proper by a cell wall layer designated the root cap junction, which stains more intensely than walls between cells in the root proper. In addition, the root cap initial cells are morphologically distinct from cells of the root proper. The root cap initial cells referred to in this paper are those actively dividing cells which give rise to the root cap columella. Additional initial cells of the root cap produce cells of the root cap periphery. In the initial cells of the columella, cell divisions occurred in the first four tiers distal to the root cap junction; about 77% of the cell division figures observed in stained serial sections were found in the first of these tiers of cells immediately distal to the root cap junction. No cell division figures were observed in cells of the root cap columella or in cells of the quiescent center in the root proper.

Autoradiographs of median longitudinal sections of root tips supplied with tritiated thymidine for 14 hr showed that the nuclei of cells of the root cap initials, the root cap periphery, and the meristematic cells of the root proper were heavily labeled, whereas cells located in the quiescent center in the root proper and in the columella of the root cap showed little or no labeling. The root cap initial cells which give rise to the columella were labeled uniformly so that these cells formed a band extending across the columella. Immediately proximal to the root cap initial cells are the cells of the quiescent center (Fig. 2). After a 14-hr exposure to tritiated thymidine, the first two to four cells in each longitudinal tier of the root cap initials giving rise to the columella were heavily labeled (Fig. 3). The band of heavily labeled nuclei extended  $60 \pm 17 \mu$  from the root cap junction.

The variability in the number of labeled nuclei scored for each tier from three serial median sections indicated that some of the nuclei of the root cap initial cells were not synthesizing DNA during the 14-hr treatment period and thus did not incorporate tritiated thymidine. Examination of serial sections was important because in any given section the nuclei of some cells were not in the plane of the section, so that the cells appeared unlabeled. The standard deviation reported for root measurements reflects not only small differences in the accuracy of measuring, but also the fact that not all nuclei are found in the same plane of any given section.

In roots supplied with a pulse of tritiated thymidine for 14 hr and subsequently transferred to fresh medium without tritiated thymidine for different intervals of time before fixation, the cells which were initially labeled were progressively displaced through the root cap by continuing cell divisions of the root cap initials. In roots supplied with tritiated thymidine for 14 hr followed by a period of 24 hr in control medium before fixation, the cells of the root cap initials and their immediate derivatives were heavily labeled. The band of heavily

labeled nuclei extended  $102 \pm 30 \mu$  from the root cap junction and consisted of 2 to 6 labeled cells in each longitudinal tier. The increase in the number of labeled cells constituting the band of heavily labeled nuclei indicated that pools of tritiated thymidine built up during the 14-hr treatment were still available and were being utilized by the dividing root cap initials during the 24-hr period after removal of the tritiated thymidine.

In roots treated with tritiated thymidine for 14 hr, followed by 48 hr in fresh medium, the proximal and distal boundaries of the band of heavily labeled nuclei were  $45 \pm 17 \mu$  and  $175 \pm 33 \mu$  respectively from the root cap junction (Fig. 4). The band of heavily labeled nuclei consisted of 2 to 5 labeled cells. Since the number of cells constituting the band of heavily labeled nuclei remained unchanged between 24 and 48 hr, the pools of tritiated thymidine had been utilized by the dividing root cap initials during the first 24 hr after removal of the tritiated thymidine. During the next 24 hr the tritiated thymidine already incorporated into the nuclei of root cap initial cells began to be diluted out by continued DNA synthesis using unlabeled precursors and by subsequent cell division. The nuclear autoradiographs after 48 hr in medium without tritiated thymidine showed far fewer silver grains per nucleus in root cap initial cells than in the heavily labeled cells displaced into the columella.

Roots supplied a pulse of tritiated thymidine for 14 hr, followed by transfer to medium without tritiated thymidine for 48 hr, were in culture for a total of 62 hr before fixation. These roots may be compared to roots supplied with tritiated thymidine continuously for 64 hr and then immediately fixed. In the latter case the distal boundary of the band of heavily labeled nuclei was  $165 \pm 32 \mu$  from the root cap junction (Fig. 6). This distance is equal to the distance which labeled cells had been displaced in the pulse-labeled roots grown for 62 hr. This result indicates that the labeled cells in the continuously labeled roots were derivatives of the actively dividing root cap initials and that the rate of movement of cells through the root cap was constant. Furthermore, the nuclei of the more distal cells in the columella had not incorporated any tritiated thymidine during the 64-hr period in which the labeled precursor was available. Thus, there was no DNA synthesis in the columella cells once they were formed from the root cap initials.

After 120 hr the proximal and distal boundaries of the band of heavily labeled nuclei were  $188 \pm 29 \mu$  and  $427 \pm 60 \mu$  respectively from the root cap junction (Fig. 5). The remaining tritiated thymidine in the DNA of the nuclei of the root cap initials had been diluted still further, as was shown by the small number of silver grains over nuclei immediately distal to the root cap initials. After 168 hr the proximal and distal boundaries of the band of heavily labeled nuclei were  $280 \pm 51 \mu$  and  $489 \pm 38 \mu$  respectively from the root cap junction. By 288 hr the labeled columella cells were sloughed off from the root cap.

The distribution of labeled nuclei in cells of the root cap columella of a number of experimental roots is shown graphically in Figure 7. The total length of each bar represents the length of the root cap as measured from the root cap junction. The darkest band represents the position of heavily labeled nuclei in cells of the columella as the cells were progressively displaced through the root cap. The lighter band represents the region of lightly labeled nuclei in cells of the columella where the label had been diluted out of the nuclei of the root cap initials after the pulse. Comparison of roots with different root cap lengths, subjected to the same treatment, shows that the position and width of the band of heavily labeled nuclei in cells of the columella is independent of the length of

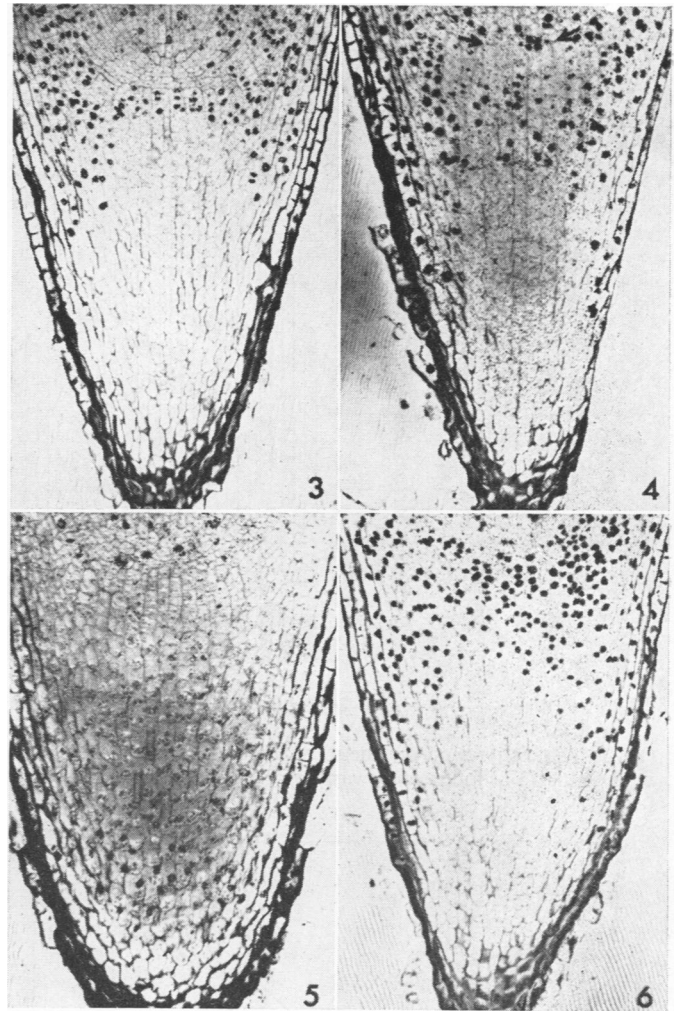


FIG. 3. Autoradiograph of a median longitudinal section of a root apex provided  $^3\text{H}$ -thymidine for 14 hr, showing silver grains associated with incorporation into nuclei of cells of the stele, cortex, root cap initials, and root cap periphery. Note the lack of label in cells of the quiescent center and in cells of the root cap columella.  $\times 100$ .

FIG. 4. Autoradiograph of a median longitudinal section of a root apex supplied with  $^3\text{H}$ -thymidine for 14 hr followed by transfer to fresh medium for 48 hr. Cells initially labeled in the root cap initials have been displaced into the root cap columella by further cell divisions in the root cap initials. The nuclei of the root cap initial cells are now only lightly labeled. The two arrows indicate the location of the root cap junction. Note the sloughing of labeled cells of the root cap periphery.  $\times 110$ .

FIG. 5. Autoradiograph of a median longitudinal section of a root apex supplied with  $^3\text{H}$ -thymidine for 14 hr followed by transfer to fresh medium for 120 hr. The heavily labeled cells have been displaced through the root cap columella toward the root tip. All cells of the root cap periphery labeled during the 14-hr treatment period have been sloughed off the root cap. Note the starch grains evident in the cells of the central columella.  $\times 130$ .

FIG. 6. Autoradiograph of a median longitudinal section of a root apex supplied  $^3\text{H}$ -thymidine continuously for 64 hr. Root cap initials, derivatives of the initials displaced into the root cap columella, and cells of the root cap periphery are heavily labeled. Note the complete absence of label in cells of the root cap columella.  $\times 90$ .

the root cap. This finding also indicates that the rate of movement of cells through the columella of the root cap is constant.

In Figure 8 the proximal and distal boundaries of the band of heavily labeled nuclei in the columella of the root cap as a

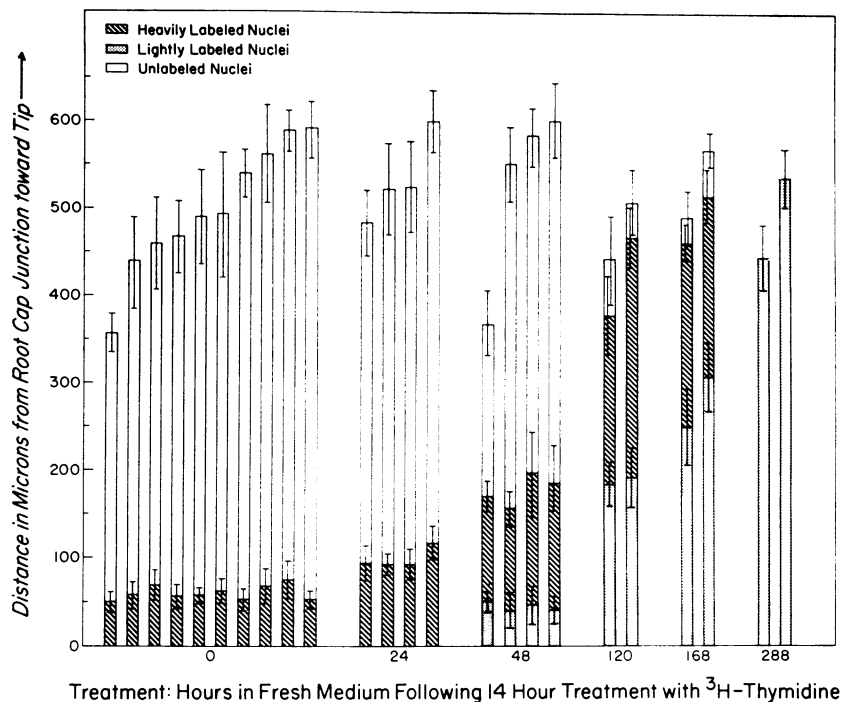


FIG. 7. The distribution of labeled nuclei in the cells of the root cap columella seen in autoradiographs of individual roots treated with  $^3\text{H}$ -thymidine for 14 hr followed by various periods in fresh medium lacking  $^3\text{H}$ -thymidine.

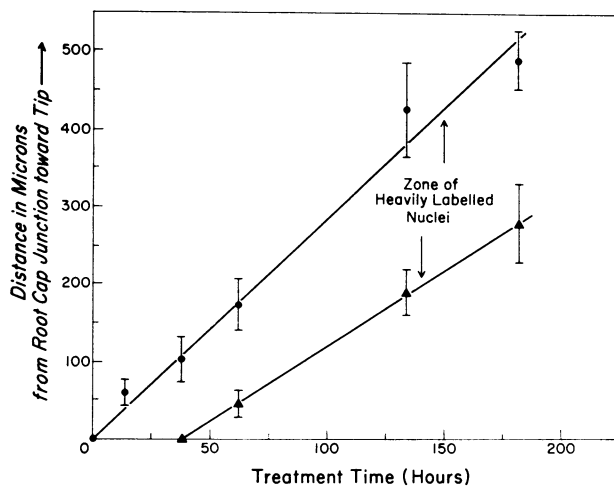


FIG. 8. The progression with time of the zone of heavily labeled nuclei summarized from measurements of the autoradiographs of *Convolvulus* root sections presented in Fig. 7. Two times the standard deviations are presented for each point. ●: Distal boundary; ▲: proximal boundary.

function of total culture time are plotted. The increase in the width of the band of heavily labeled nuclei with time is caused by two factors, namely, the difficulty in measuring the boundaries exactly with increasing time and the increase in size of the cells of the columella as they are progressively displaced toward the root cap tip. From the position of the distal boundary the rate of displacement of a cell through the root cap, after being formed from a root cap initial cell, can be estimated. The rate is approximately  $3 \mu/\text{hr}$  or  $72 \mu/\text{day}$ . This rate also includes the amount of displacement which occurs as the result of cell enlargement as the cell approaches the tip of the root

cap. From the rate, the lifetime of a cell in the columella of the root cap can be estimated to range between 6 and 9 days, depending upon the length of the root cap.

## DISCUSSION

The experimental evidence presented in this paper indicates that the cells of the root cap columella are quite different from the cells of the root cap initials, the root cap periphery, and the quiescent center with respect to the utilization of the precursor of DNA synthesis, tritiated thymidine. In contrast to the cells of the root cap initials and the root cap periphery which increase their level of DNA without any subsequent division or in preparation for cell division, the cells of the root cap columella completely terminate DNA synthesis after their formation from the root cap initials. Such behavior is also unlike that of the cells of the quiescent center which are characterized by a low rate of DNA synthesis or no DNA synthesis at all.

The cells in these four regions also differ in the use of labeled precursors of nuclear and nucleolar RNA synthesis. Himes (10) reported that in roots of *Zea mays* which were pulse-labeled with tritiated cytidine and uridine, the cells of the root cap initials and older root cap cells were characterized by a high rate of nucleolar RNA synthesis. Furthermore, Barlow (2) showed that nucleolar RNA synthesis occurred at a greater rate in cells of the root cap columella than in cells of the quiescent center in roots of *Zea mays*, although this rate was less than that observed in the initial cells of the root cap or of the root proper.

These regions of the root differ also with respect to their utilization of glucose. Thomas (19) showed that cells of the quiescent center did not incorporate radioactively labeled glucose into cellular components in roots of tomato. Dauwalder *et al.* (8) followed the incorporation of D-glucose-1- $^3\text{H}$  by autoradiography for 0.5, 1, and 4 hr in the root cap of *Zea*

*mays*. After 1 hr the youngest mid-cap cells and the inner portions of the mid-cap showed the most active labeling of starch. The starch grains of mid-cap cells further displaced from the root cap initials showed less labeling. After 4 hr the starch grains of the outer cap cells were still unlabeled. In addition, Northcote and Pickett-Heaps (16), using electron microscope autoradiography, found that plastids located in the inner cells of the root cap of *Triticum vulgare* were labeled after incubation with D-glucose-6-<sup>3</sup>H, whereas the plastids located in the outer root cap cells were unlabeled. In the outer root cap cells the labeled glucose was utilized almost exclusively via Golgi bodies to produce wall material, while, in the cells located internally in the root cap, both starch and small amounts of wall material were formed from the radioactively labeled glucose.

Ultrastructural studies indicate that cytodifferentiation occurs rapidly in cells of the root cap columella. Clowes and Juniper (7), from ultrastructural studies of *Zea mays*, noted that at a distance of 60 to 90  $\mu$  from the root cap junction, the density of the cell wall changed abruptly. This change was related to the point at which cell division ceased. A large increase in the amount of endoplasmic reticulum occurred in the root cap cells over the first 150  $\mu$  such that the endoplasmic reticulum was well developed in the expanding cells of the root cap. They also noted that the maturation of plastids from immature proplastids in the root cap initials to mature amyloplasts in the mature cap cells occurred over a distance of 250  $\mu$ . Juniper and Barlow (12) found that the actively dividing root cap initials had a higher number of plasmodesmata per unit area on the transverse and longitudinal walls than did the walls of cells located in the central region of the root cap in roots of *Zea mays*. The number of plasmodesmata per unit cell volume progressively decreased moving from the region of the root cap initials to the central cap (200–300  $\mu$  from the root cap junction) to the cap periphery. Juniper and Barlow believed that drastic change in the total number and the distribution of plasmodesmata in the three different regions was correlated with the control of differentiation, as shown by the rapid changes in the biochemical properties of these different populations of cells. Juniper and French (13) reported that an as yet unidentified substance is deposited in the walls of cells displaced into the root cap columella in roots of *Zea mays*. The appearance of this encrusting substance in the cell wall coincided with the accumulation of starch in amyloplasts, and the disappearance of this material from the cell walls coincided with the loss of starch from amyloplasts as these cells reached the periphery of the root cap just prior to being sloughed off.

Thus, all the experimental evidence indicates that rapid cellular differentiation occurs in the cells of the root cap columella after their formation from the root cap initials and one of the earliest events is the cessation of DNA synthesis. Many of these rapid changes in the cells are probably directly related to the ability of these cells to detect geotropic stimuli. Griffiths and Audus (9) reported for roots of *Vicia faba* that the only bodies which showed marked sedimentation to one site within cells in response to a geotropic stimulus were the amyloplasts. Reviews by Wilkins (21) and Audus (1) summarize the strong body of evidence in support of the statolith theory. In addition, Iversen (11) showed for *Lepidium sativum* L. that the starch-containing, mobile amyloplasts in the cells of the central columella of the root cap were responsible for the geotropic response. Amyloplasts located in the older columella cells and in cells surrounding the columella were immobile. Prior to horizontal stimulation, roots which were treated hormonally to remove the starch from the amyloplasts showed no subse-

quent curvature. The geotropic response was restored once the hormone-treated roots formed starch after illumination.

Juniper *et al.* (14) reported for roots of *Zea mays* that the geotropic response was dependent upon the presence of a root cap. In their experiments, removal of the root cap prevented the perception of the gravitational stimulus, as shown by the fact that the roots grew horizontally for about 15 to 30 hr without showing any curvature. Once a major proportion of the root cap regenerated, the geotropic response returned. The excision of the root cap did not affect the growth in length of the whole root. Juniper *et al.* noted that, although the root cap is unlikely to be the site for production of root growth regulators, the root cap must influence the production of growth regulators or the movement of such regulators in the meristem in response to the perception of gravity. Konings (15) reported for roots of *Pisum sativum* that the removal of that portion of the root cap which included the cells of the columella deprived the roots of their ability to respond to gravity.

The experimental evidence presented in this paper indicates that the lifetime of an average cell in the root cap columella ranges between 6 and 9 days. Within this period of time, a cell formed by the root cap initials undergoes very rapid cellular differentiation. The cessation of DNA synthesis appears to be a very early event in this process. Other changes in the cell include the development of amyloplasts from proplastids, the incorporation of glucose primarily into starch grains within the amyloplast rather than into the cell wall, large increases in the amount of endoplasmic reticulum, changes in the distribution of plasmodesmata within the root cap columella and between adjacent cell populations, and changes in the density of the cell walls. In addition, nucleolar RNA synthesis continues at a high rate.

A lifetime of 6 to 9 days for a cell in the root cap columella would allow for the formation of a relatively stable population of cells of importance in detecting geotropic stimuli and effecting a geotropic response. In addition, this period of time would allow sufficient time for a constant renewal of cells from the root cap initials, thereby compensating for the loss of cells by the sloughing off process as the root grows through the soil. Thus, the root cap appears to be a compromise between a stability needed in detecting geotropic stimuli and a constant turnover of cells in allowing growth.

#### LITERATURE CITED

- AUDUS, L. J. 1969. Geotropism. In: M. B. Wilkins, ed., *Physiology of Plant Growth and Development*. McGraw Hill, London, pp. 205-242.
- BARLOW, P. W. 1970. RNA synthesis in the root apex of *Zea mays*. *J. Exp. Bot.* 21: 292-299.
- CLOWES, F. A. L. 1956. Nucleic acids in root apical meristems of *Zea*. *New Phytol.* 55: 29-34.
- CLOWES, F. A. L. 1958. Development of quiescent centres in root meristems. *New Phytol.* 67: 85-88.
- CLOWES, F. A. L. 1958. Protein synthesis in root meristems. *J. Exp. Bot.* 9: 229-238.
- CLOWES, F. A. L. 1968. The DNA content of the cells of the quiescent centre and root cap of *Zea mays*. *New Phytol.* 67: 631-639.
- CLOWES, F. A. L. AND B. E. JUNIPER. 1964. The fine structure of the quiescent centre and neighboring tissue in root meristems. *J. Exp. Bot.* 15: 622-630.
- DAUWALDER, M., W. G. WHALEY, AND J. E. KEPHART. 1969. Phosphatases and differentiation of the Golgi apparatus. *J. Cell Sci.* 4: 455-497.
- GRIFFITHS, H. J. AND L. J. AUDUS. 1964. Organelle distribution in the stacyte cells of the root tip of *Vicia faba* in relation to geotropic stimulation. *New Phytol.* 63: 319-333.
- HIMES, M. 1966. Nuclear RNA synthesis during differentiation of maize root cap cells. *J. Cell Biol.* 31: 47A.
- IVERSEN, T. 1969. Elimination of geotropic responsiveness in roots of cross (*Lepidium sativum*) by removal of statolith starch. *Physiol. Plant.* 22: 1251-1262.
- JUNIPER, B. E. AND P. W. BARLOW. 1969. The distribution of plasmodesmata in the root tip of maize. *Planta* 89: 352-360.

13. JUNIPER, B. E. AND A. FRENCH. 1970. The fine structure of the cells that perceive gravity in the root tip of maize. *Planta* 95: 314-329.
14. JUNIPER, B. E., S. GROVES, B. LANDAU-SCHACHAR, AND L. J. AUDUS. 1966. Root cap and the perception of gravity. *Nature* 209: 93-94.
15. KONINGS, H. 1968. The significance of the root cap for geotropism. *Acta Bot. Neerl.* 17: 203-211.
16. NORTHCOTE, D. H. AND J. D. PICKETT-HEAPS. 1966. A function of the Golgi apparatus in polysaccharide synthesis and transport in the root cap cells of wheat. *Biochem. J.* 98: 159-167.
17. PHILLIPS, H. L., JR. AND J. G. TORREY. 1971. The quiescent center in cultured roots of *Convolvulus arvensis* L. *Amer. J. Bot.* In press.
18. RABIDEAU, G. E. AND L. W. MERICLE. 1953. The distribution of  $C^{14}$  in the root and shoot apices of young corn plants. *Plant Physiol.* 28: 329-333.
19. THOMAS, D. R. 1967. Quiescent center in excised tomato roots. *Nature* 214: 739.
20. TORREY, J. G. 1954. The role of vitamins and micronutrient elements in the nutrition of the apical meristem of pea roots. *Plant Physiol.* 29: 279-287.
21. WILKINS, M. B. 1966. Geotropism. *Annu. Rev. Plant Physiol.* 17: 379-408.