THE TIME OF MITOSIS IN THE ROOT APICAL CELL OF *CERATOPTERIS PTERIDOIDES*[1]

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Abstract: This work is concerned with cell division and nuclear abnormalities in the root apical cell and its close derivatives of a water fern, *Ceratopteris pteridoides* (Hook.) Hieron. when grown either in the presence or absence of a colchicine solution.

The apical cells of rapidly growing roots divide mainly after midnight. An important consequence of applying colchicine is the formation of multinucleate cells. The formation of ameboid nuclei and other nucleotoxic effects occur as the time of colchicine is prolonged. By counting the number of nuclei formed after a 24 hour-colchicine-treatment, the nuclear cycle of the apical cells in some of the roots of this fern seems to be very short. It was estimated about as 8 hours or shorter. It never divides less frequently than its adjacent cells.

The concept that the apical cell in pteridophytes is an "unicellular quiescent center" suggested by some workers is also discussed.

INTRODUCTION

The apex of the root has been investigated in a large number of leptosporangiate ferns and reviewed thoroughly by Bower (1889–1890, 1923) as well as by others. It is familiar to botanists that in some pteridophytes all of the root tissues originate from the single tetrahedral apical cell. The root apical cell in many ferns has been reported as dividing in a very regular manner (Bartoo, 1929, 1930; Clowes, 1961; Chiang, 1970, Chiang and Gifford, 1971). Several reports have been made on the rate of cell division in the root meristem of ferns. Some workers have expressed the opinion that the root apical cells in pteridophytes divide but rarely (Buvat and Liard, 1953). Furthermore, D'Amato and Avanzi (1965) reported that the root apical cell of *Marsilea strigosa* as well as some other ferns (Avanzi and D'Amato, 1967) is a mitotically active cell only during the origin and initial organization of the root primordium, and this root apical cell is an "unicellular quiescent center" comparable with the quiescent center of angiosperm roots. They suggested that most of the actual cell divisions in the meristem of these lower vascular plants takes place in the cells just beside or below the apical cell. On the contrary, by labelling DNA with radioactive precursors, Clowes (1956) reported that the apical cells of adventitious roots in *Azolla* showed DNA synthesis which can be accepted as the evidence for subsequent mitosis. Gifford (1960) also reported that the apical cells of both shoot and root in young sporophyte of *Ceratopteris thalictroides* are engaged in DNA synthesis. Recently Chiang and Gifford (1971) by careful analysis on anatomical evidence and growth pattern, have demonstrated that the apical cell in the root of *Ceratopteris thalictroides* divided in a very predictable manner, and that it does not divide less frequently than its neighboring cells.

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In these years the role of colchicine as an agent for the induction of polyploidy and blockadge of mitosis by prolongation of metaphasic chromosomes was conclusively demonstrated (Eigsti and Dustin, 1957). Though colchicine has been tried on the growing prothallia of a number of ferns (Mehra, 1952; Rosendahl, 1941), little information has been provided on the relationship between the root apical cell of pteridophytes and the action of colchicine. This investigation is concerned with the cellular morphology in the root apical cell of *Ceratopteris pteridoides* after exposure to a colchicine solution. This provides a better evidence for the view that the root apical cell of pteridophytes divides frequently.

MATERIALS AND METHODS

Sporophytes of about 8–10 cm height of the annual water fern, *Ceratopteris pteridoides* (Hook.) Hieron., were moved to the laboratory from the greenhouse in the Depratmant of Botany, National Taiwan University and cultured in a tank (with tap water) two days before the colchicine treatment. The tank was kept in the labortory near the window, and the water was changed every morning. The room temperature during the experiment at noon was 32°C±2°C.

The roots were treated in a 0.05% colchicine solution by transferring the whole plant to the solution. The time for colchicine treatment for the first group was 4, 8, or 12 hours. These are grouped in the text as "day-time-treatment". All of the materials indicated in the day-time-treatment were transferred into colchicine solution at 8 a. m.. The second group of experimental materials which were grouped as "night-time-treatment" were transferred into colchicine solution at 8 p. m.. The third group which is grouped as "day & night-treatment" was transferred into colchicine solution at 8 a. m.. The materials of day & night-treatment were treated in the colchicine for 24, 48 or 72 hours. The root tips and the petiole bases were collected and fixed in Craff III (Sass, 1958) immediately after the desired period of treatment. The fourth group which was not exposed to the colchicine solution was collected at the same time of the colchicine treated groups. Cytological studies were carried out on the materials sectioned using the paraffing method and stained by tannic acid and iron alum with safranin and orange G (Sharman, 1943).

RESULTS

The roots used in this experiment were the adventitious roots emerging from the petiole bases. Since the root of about 5 cm in length was identified as at its grand period of growth in *Ceratopteris thalictroides* (Chiang and Gifford, 1971), the roots which were longer than 6 cm were not used in the present investigation.

All the root tips examined were obtained from three groups of different lengths or ages of roots. They were embedded roots (still embedded in the petiolar tissue), 0.3–0.5 and 3–5 cm in length, respectively, representing root initials (at the period of root initiation), young roots (its lateral roots not visible to the naked eyes), and developing roots (its lateral roots being visible to the naked eyes, some of them were emergent) (Tables 1, 2, 3, 4).

(A) Tumor formation

Conspicuous root tumors were regularly formed in the roots (excluding the embedded roots) which were treated in the colchicine solution for 24 hours or longer. In the 3-day-treated roots, tumors formed at the region about 0.05 cm away from

the extreme tip of the root. The cells located at the extreme tip of the root were identified as cap cells. Observations of various planes of the sections, showed that the tumor formation was mainly caused by the isodiametric enlargement or swelling of the cells rather than cell division in the root tips. Apparently, the cap cells, as compared with the other cells in the tip, have taken a rather small part in the formation of tumors. In the non-treated roots, the cells which are located at the region corresponding to the tumor region of the treated root, elongate in the direction parallel to the root axis.

Tumors were more conspicuous in the meristematic region. The size of the tumor was proportional to the duration of exposure to the colchicine solution in the first three days. Maximum swelling was found in the 72-hour-treated material. The root tip showed withering brown to black spots when the plants were placed in the colchicine solution longer than 72 hours. This shows that the cells in the meristematic region are more sensitive, and the prolongation of the treatment causes some toxic effects that arrest cellular growth.

(B) Mitotic figure found in non-treated root

From the data presented in Table 1, of about one eighth (25 out of 194) of the root apical cells were found in the process of cell division at the period from midnight to 8 a.m.. Mitotic figures are rarely found in the roots collected in the daytime. All interphasic cells in the root apical cells from this non-treated material are mononucleate.

(C) Mitotic figure found in treated roots

The dividing apical cell is very rarely seen even in the colchicine treated roots. Only three metaphasic nuclei were seen among all the treated materials (Tables, 2, 3, 4). No mitotic figure other than the metaphase has been found. All the metaphasic chromosomes were arranged in the equatorial plate. No nuclear envelop was seen surrounding the orderly oriented chromosomes (Fig. 1). Two chromosome groups from the treated metaphases which were separated by the equatorial plate were more compactly oriented, whereas the metaphasic chromosomes from the non-treated apical cell were more loosely arranged and the individuality of the free ends of each chromosome were more distinguishable.

It is familiar to biologists that colchicine has specific action upon mitosis, especially the inhibition of spindle fibers (Nebel and Ruttle, 1938). But the spindle behavior during the mitosis was not studied in the present work, since neither anaphasic nuclei nor telophasic ones were seen in the present investigation.

(D) Multinucleate stage in the apical cell and its close derivatives

The most conspicuous change occurring in the treated roots at the microscopic level is the multinucleate condition of the apical cell. The nucleus in the root apical cell of treated adventitious roots were observed in increasing numbers. The number of nuclei per single apical cell ranged from one to several. Only one out of 323 apical cells receiving the "day-time-treatment" was in the multincleate stage (binucleate), whereas the remaining 322 roots had mononucleate apical cells (Table, 2). No mitotic figure was found in "day-time-treated" materials. All the non-treated materials collected at the same time of each corresponding treated material possessed interphasic mononucleate apical cells (Table, 1).

More multinucleate apical cells were found in the "night-time-treatment" roots (Table, 3). Bi- or trinucleate apical cells were very common in the 8- and 12 hour treated roots which were collected at 4 a.m. and 8 a.m. respectively. No multinucleate apical cell were found in the materials collected at 12 midnight. The root

Table 1. Number of apical cells found in different phases of the mitotic cycle in non-treated roots

Length of root (cm)	Time of harvest	*Corresponding group of treated materials	No. of roots examined	Phases in mitotic cycle				
				Mitosis				Interphase
				Prophase	Metaphase	Anaphase	Telophase	Mono-nucleate
embedded root	12 (noon)	4 hr day-time treatment	25	0	0	0	0	25
0.3-0.5	12 (noon)	4 hr day-time treatment	21	0	0	0	0	21
3-5	12 (noon)	4 hr day-time treatment	32	0	0	0	0	32
embedded root	4 pm	8 hr day-time treatment	18	0	0	0	0	18
0.3-0.5	4 pm	8 hr day-time treatment	22	0	0	0	0	22
3-5	4 pm	8 hr day-time treatment	30	0	0	1	0	29
embedded root	8 pm	12 hr day-time treatment	14	0	0	0	0	14
0.3-0.5	8 pm	12 hr day-time treatment	18	0	0	1	0	17
3-5	8 pm	12 hr day-time treatment	31	0	0	0	0	31
embedded root	12 (night)	4 hr day-time treatment	12	0	0	0	1	11
0.3-0.5	12 (night)	4 hr day-time treatment	22	1	0	0	0	21
3-5	12 (nigat)	4 hr day-time treatment	33	1	1	1	0	30
embedded root	4 am	8 hr day-time treatment	15	0	1	1	1	12
0.3-0.5	4 am	8 hr day-time treatment	20	1	2	2	1	14
3-5	4 am	8 hr day-time treatment	21	2	2	3	2	12
embedded root	8 am	12 hr day-time treatment 24 hr day-time treatment 48 hr day-time treatment 72 hr day-time treatment	7	0	0	1	0	6
0.3-0.5	8 am	〃	28	0	1	0	1	26
3-5	8 am	〃	36	0	0	0	1	35

* cf. Tables 2, 3, 4.

Table 2. Number of root apical cells found in different phases of the mitotic cycle after colchicine treatment (day-time-treatment)*

**Length of root (cm)	***Duration of treatment (hour)	No. of roots examined	Phases in mitotic cycle											
			Mitosis				Interphase							
			Pro-phase	Meta-phase	Ana-phase	Telo-phase	Mono-nucleate	2-nucleate	3-nucleate	4-nucleate	5-nucleate	6-nucleate	7-nucleate	8-nucleate
embedded root	4	32	0	0	0	0	32	0	0	0	0	0	0	0
0.3-0.5	4	41	0	0	0	0	41	0	0	0	0	0	0	0
3-5	4	41	0	0	0	0	40	1	0	0	0	0	0	0
embedded root	8	36	0	0	0	0	36	0	0	0	0	0	0	0
0.3-0.5	8	37	0	0	0	0	37	0	0	0	0	0	0	0
3-5	8	37	0	0	0	0	37	0	0	0	0	0	0	0
embedded root	12	30	0	0	0	0	30	0	0	0	0	0	0	0
0.8-0.5	12	23	0	0	0	0	23	0	0	0	0	0	0	0
3-5	12	46	0	0	0	0	46	0	0	0	0	0	0	0

* Treated in 0.05% colchicine solution.
** Measured at the time of harvet.
*** All the materials were transferred into the colchicine solution at 8 a. m.

Table 3. Number of root apical cells found in different phases of the mitotic cycle after colchicine treatment (night-time-treatment)*

**Length of root (cm)	***Duration of treatment (hour)	No. of roots examined	Phases in mitotic cycle											
			Mitosis				Interphase							
			Pro-phase	Meta-phase	Ana-phase	Telo-phase	Mono-nucleate	2-nucleate	3-nucleate	4-nucleate	5-nucleate	6-nucleate	7-nucleate	8-nucleate
embedded root	4	20	0	0	0	0	20	0	0	0	0	0	0	0
0.3-0.5	4	24	0	0	0	0	24	0	0	0	0	0	0	0
3-5	4	21	0	0	0	0	21	0	0	0	0	0	0	0
embedded root	8	—	—	—	—	—	—	—	—	—	—	—	—	—
0.3-0.5	8	37	0	0	0	0	31	5	1	0	0	0	0	0
3-5	8	43	0	0	0	0	34	6	3	0	0	0	0	0
embedded root	12	66	0	1	0	0	52	6	7	0	0	0	0	0
0.3-0.5	12	41	0	0	0	0	39	2	0	0	0	0	0	0
3-5	12	39	0	0	0	0	33	6	0	0	0	0	0	0

* Treated in 0.05% colchicine solution.
** Measured at the time of harvest.
*** All the materials were transferred into the colchicine solution at 8 p. m.

Table 4. Number of root apical cells found in different phases of the mitotic cycle after colchicine treatment (day & night-time-treatment)*

**Length of root (cm)	***Duration of treatment (hour)	No. of roots examined	Phases in mitotic cycle											
			Mitosis				Interphase							
			Pro-phase	Meta-phase	Ana-phase	Telo-phase	Mono-nucleate	2-nucleate	3-nucleate	4-nucleate	5-nucleate	6-nucleate	7-nucleate	8-nucleate
embedded root	24	45	0	0	0	0	43	1	1	0	0	0	0	0
0.3-0.5	24	66	0	0	0	0	48	11	7	0	0	0	0	0
3-5	24	32	0	1	0	0	15	5	6	(3)****	(2)	0	0	0
embedded root	48	34	0	0	0	0	23	7	4	0	0	0	0	0
0.3-0.5	48	112	0	0	0	0	10	24	53	(20)	(3)	(2)	0	0
3-5	48	45	0	0	0	0	0	2	11	(16)	(11)	(2)	(2)	(1)
embedded root	72	17	0	0	0	0	0	0	1	(5)	(7)	(3)	(1)	0
0.3-0.5	72	34	0	0	0	0	0	0	2	(6)	(10)	(4)	(4)	(8)
3-5	72	26	0	0	0	0	0	0	0	(1)	(11)	(11)	(2)	(1)

* Treated in 0.05% colchicine solution.
** Measured at the time of harvest.
*** All the materials were transferred into the colchicine solution at 8 a. m.
**** ( ), with great range in the nuclear size, some of the nuclei counted were micronuclei.

Table 5. Nuclear division in root apical cells(*1)

Duration of colchicine treatment (hour)	No. of roots examined	Mononucleate	2-(*2)nucleate	3-(*3)nucleate	4-(*4)more-nucleate	Mitosis occurred (%)
24	143	106	17	14	(6)	44.01
48	191	33	24	53	(81)	142.93
72	77	0	0	3	(74)	296.10

(*1) Derived from the data presented in Table 3
(*2) The cells are considered as the consequence of one nuclear division
(*3) The cells are considered as the consequence of two nuclear divisions
(*4) The cells are considered as the consequence of three nuclear divisions

Table 6. The number and percentage of multinucleate apical cells in 24-hour-treated roots

Length of root (cm)	No. of roots	No. of multinucleate apical cells	% of multinucleate apical cells
embedded root	45	2	4.44
0.3-0.5	66	18	27.20
3-5	32	16	50.00

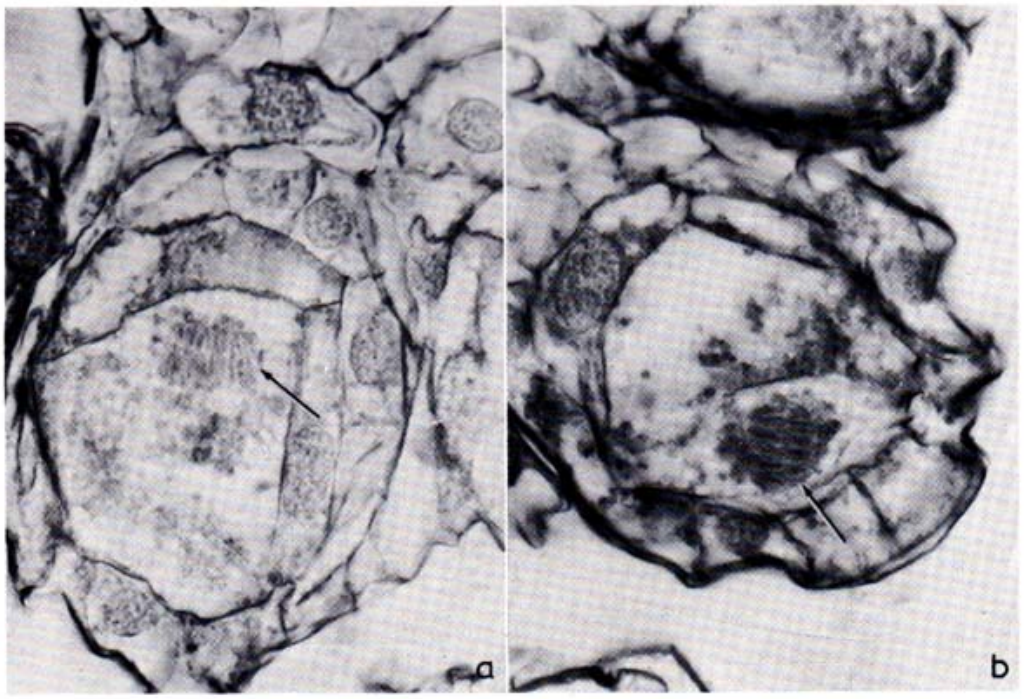

Fig. 1. Metaphasic chromosomes (arrows) in apical cell (a) and cap initial (b) from the embedded roots after 12 hours in colchicine solution, ×800.

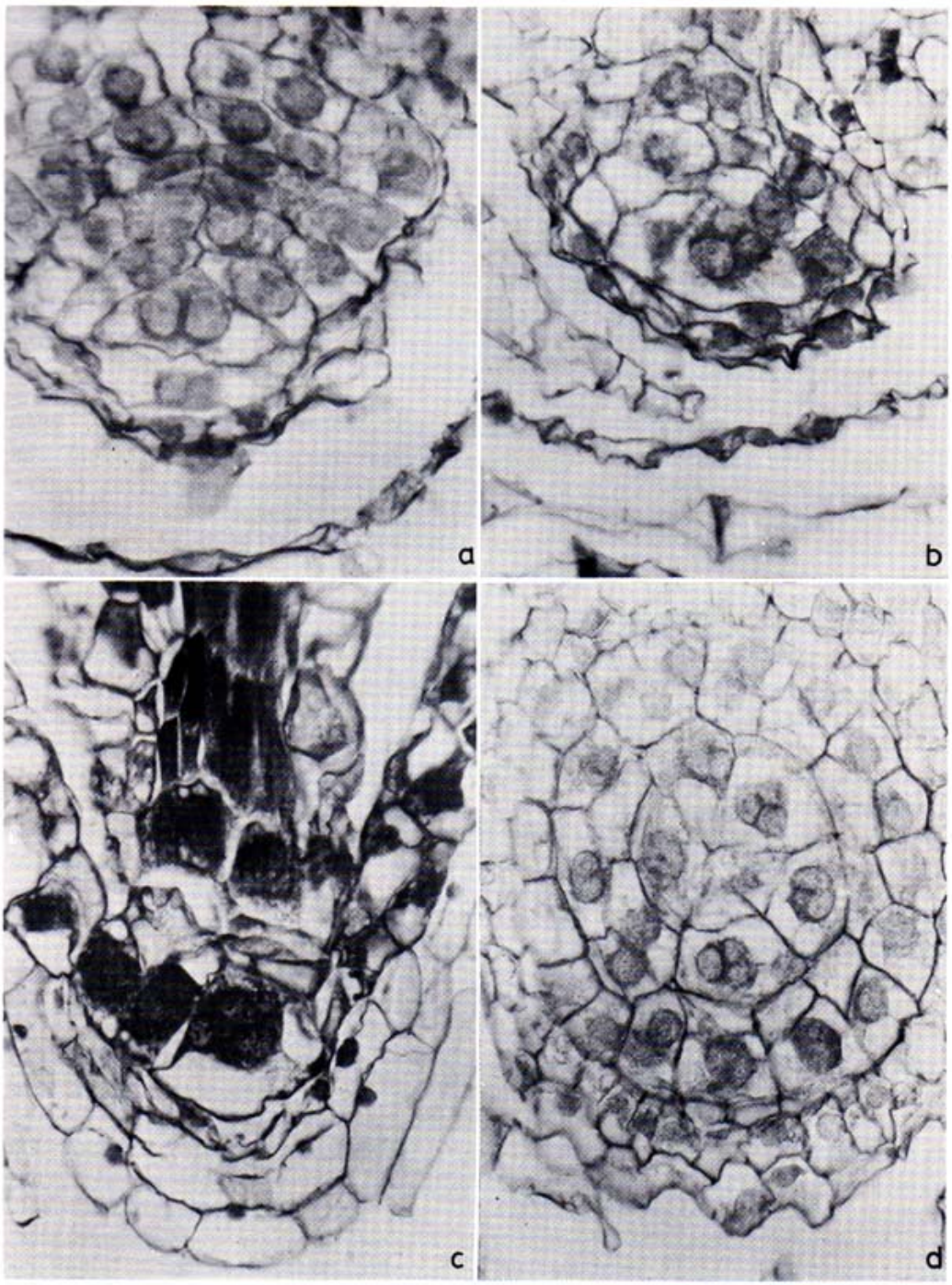

Fig. 2. a—c, Longitudinal sections of colchicine treated roots through the apical cells, ×400.

a. 48 hours after treatment, showing binucleate apical cell, binucleate cap initial and several multinucleate close derivatives of the apical cell.

b. 48 hours after treatment, showing trinucleate apical cell.

c. 72 hours after treatment, showing 7-nucleate apical cell, some of them are micronuclei.

d. Transverse section through the root about 40 μ away from the apical cell, showing many cells in the multinucleate stage, ×400.

apical cells which possessed four or more nuclei were not seen in "night-time-treatment" materials. This group had been in contact with colchicine for 4 to 12 hours at night.

All the members in the "day & night-time-treatment" materials were either mononucleate or multinucleate root apical cells (Table 4; Figs. 2a, 2b). They ranged from one to eight nuclei in a single apical cell. There were no mononucleate apical cells in the roots exposed to the colchicine solutions for 72 hours or more. All of them possess more than two nuclei. The number of nuclei per single apical cell tended to be proportional to the duration of exposure to the colchicine solution (Tables 3, 4). This is more apparent in the "day & night-time-treatment" group (Table 4). It is clear that the multinucleate apical cells can easily be obtained in roots which have been grown in the colchicine solution for more than eight hours; and this eight hours must include one late midnight period.

Though the multinucleate apical cells are very common in the night-time-treatment materials, the multinucleate stage is rare in its close derivatives. The multinucleate condition in the close derivatives of the apical cells increases as the time of colchicine treatment is prolonged. Most of the close derivatives of the apical cells possess two, or more than two nuclei in the roots which have been dipped in the colchicine solution for more than 24 hours (Fig. 2d). Accurate data for the frequency of the multinucleate cells other than the root apical cell was not obtained in the present investigation.

The nuclei in bi- or trinucleate apical cells as well as its close derivatives are almost the same in size (Fig. 2). But the size of the nuclei in a single apical cell which has four (or more than four) nuclei always differ from each other. Some of them can be referred to micronuclei (Fig. 2c) (Brues and Jackson, 1937). Other abnormalities of nuclei other than the multinucleate condition in the treated roots were seen, such as large ameboid nuclei and fusing nuclei (Figs. 2d, 3).

(E) Mitotic rate in apical cell

It is found that nucleotoxic effects occur during mitosis in the roots of *Ceratopteris pteridoides* after colchicine application. The colchicine solution causes multinucleate formation in the earlier stage of drug application, and further effects, such as large ameboid nucleate cells are the important results of applying the colchicine for a prolonged period.

In addition to the nucleotoxic effects mentioned above, the submicroscopic changes could have occurred during, or before mitosis. However, the fact remains that the formation of multinucleate cells are one of the important consequences induced by mitosis under the presence of colchicine. The increase of one nucleus could be the outcome of one mitosis, or of more mitoses if other factors are taken into consideration. Colchicine might stop mitosis during prophase and turn the process back to interphase (Gavaudan, Dodé and Poussel, 1945). In order to simplify the analyzing data obtained, the increase of one nucleus in one single apical cell is considered as the consequence of one mitosis. Though more than three mitoses might occur in the cells possessing four (or more than four) nuclei, it is considered that they are the consequence of three nuclear divisions. Therefore the mitosis depicted in Table 5 is a conservative number. Actually it could divide more times than the data presented shows. Only 6 out of 106 apical cells have four (or more than four) nuclei in a single apical cell in the 24-hour-treated group, so that the nuclear division counted in this group is more acceptable than in any other group. Because they receive less nucleotoxic effects of colchicine than any other groups.

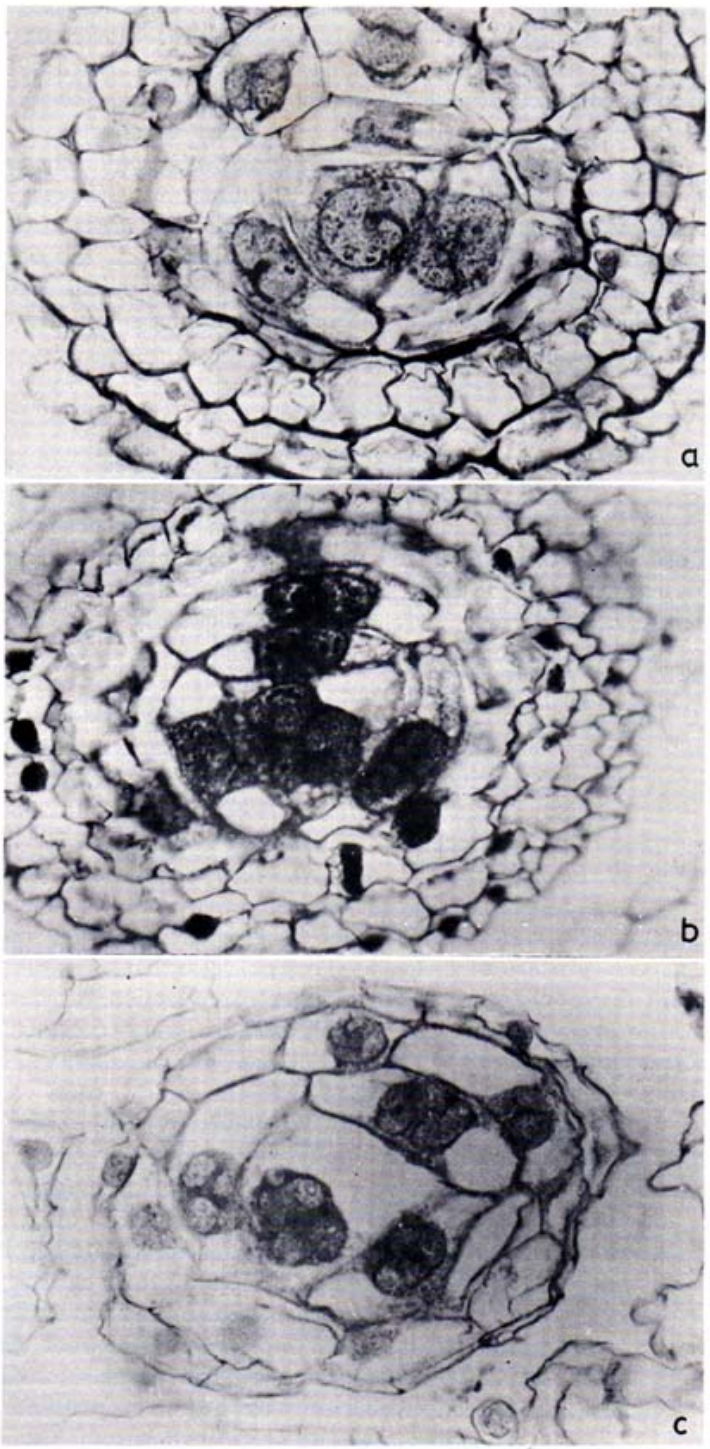

Fig. 3. Transverse sections of roots through the apical cells after 72 hours treatment. Apical cell and its close derivatives possess either multinuclei or ameboid nuclei, ×400.

Apparently, the nuclei in 17 apical cells (binucleate) have divided once, 14 (3-nucleate) have divide twice, 6 (4- or more nucleate) three or more times within 24 hours (Table 5). If nuclear division occurs three times within 24 hours, the nuclear cycle for the cell would be 24/3=8 (hours). So that, the duration of the mitotic cycle in some root apical cells would be 8 hours or shorter.

The grand period of root growth in *Ceratopteris thalictroides* has been measured when the roots were about 3–5 cm in length (Chiang & Gifford, 1971). In the present work, in 24 hour- treated materials, 4.44% of root initial (embedded root), 27.20% the young root (0.3–0.5 cm in length) and 50.00% of the developing roots (3–5 cm in length) were found to possess multinucleate apical cells (Table 6). Obviously the apical cells in the roots of about 3–5 cm length divide more frequently than other two groups of roots.

DISCUSSION

The present studies on the root tip of *Ceratopteris pteridoides* reveal that mitosis in the apical cell usually occurs between midnight and early morning and since it occurs in the wee hours of the morning, it has been seldom observed. Therefore some investigators have concluded that the apical cell seldom divides. Out of 194 non-treated roots examined 25 of them contained mitotic figures. It is unreliable to decide whether the cells divide frequently or infrequently upon the basis of how many cells are observed in the process of cell division. It is very important that the time of observation be taken into consideration. It is more reliable if the time between each collection is short. In the present work, the root tips were collected every four hour during the time of experimentation. Earlier workers stated that the root apical cells of some pteridophytes divides infrequently (Wetmore in Sinnott, 1960; Buvat & Liard, 1953; D'Amato & Avanzi, 1965), that was because they rarely observed any apical cell in the process of division. Furthermore, D'Amato & Avanzi (1965) suggested that the root apical cell of *Marsilea strigosa* and possibly of other pteridophytes is an unicellular quiescent center in which the apical cell rarely divides. Their statements were mainly based on the fact that they had rarely seen an apical cell in the process of mitosis. This concept that the apical cell divides infrequently might have changed if their times of collection (or observation) had been different. Mitotic figures in the apical cell are very commonly seen if the time of collection is appropriate.

It is clear from the present investigation that the apical cells in the actively growing roots of *Ceratopteris pteridoides* divide frequently. This observation agrees with the phenomenon in some other pteridophytes described by Avanzi and D'Amato (1967). They mentioned that the root apical cell of *Marsilea strigosa* (1965) and of other pteridophytes (1967) behave as a mitotically active cells only in the origin and early organization of the root primordium. In later stages, its becomes quiescent, comparable to the "unicellular quiescent center". The mitotic activity is retained in the cells surrounding the apical cell. All the species used by earlier workers as well as the present author have no secondary growth. The root grows actively only in the early stage. In later stages, all the root tissue, including the apical cell, gradually becomes "quiescent". According to the data presented by Avanzi and D'Amato (1967). It may be stated that the apical cell becomes quiescent earlier than its surrounding cells or other cells.

Most of the pteridophytes have a single apical cell in the root meristem. This

single apical cell behaves as a typical initial for all of the root tissues, *i.,e.* the apical cell gives rise to all of the root tissues. It divides according to a definite sequence in a given species (Bartoo, 1929; 1930; Chiang, 1970; Chiang & Gifford, 1971). The apical cell is the "original initial" of all the root tissues. When it becomes inactive (or quiescent) first, the other cells of the root, other than the apical cell, will become inactive mitotically.

Finally no cells, such as cambial cells, retain mitotic activity. So that the root apical cell remains more "quiescent" than its surrunding cells only for a limited period. The "quiescent center" used here is somewhat different from that applied to the root tip of angiosperms (Clowes, 1961).

Though numerous papers concerning apical cells of ferns have been published, there is little information in them dealing with the presence of a multinucleate condition in the apical cell. Evidently the multinucleate condition is caused by the application of colchicine. It is well known that colchicine has specific actions upon mitosis, such as the inhibition of spindle fibers (Nebel & Ruttle, 1938); the formation of an arrested metaphase (Eigsti, 1938); polyploidy, multinucleate cells and ameboid nucleate cells (Eigsti, Dustin & Gay-Winn, 1949). Colchicine causes the formation of the multinucleate stage and the unequal division of nucleus in the cells of root apical meristem including the apical cell of *Ceratopteris pteridoides.*

It is possible to measure the mitotic cycle from the number of nuclei present in a single apical cell after a definite period of colchicine treatment in the early stage of drug application. Because nucleotoxic effects appear as the time of application is prolonged, the data obtained from the later stages of drug application are not valid for this purpose. The mitotic cycle of the apical cell in the some roots of *Ceratopteris pteridoides* was shown to be 8 hours or even shorter. The adjacent cells of the apical cell divide less often than the apical cell in actively growing roots. This is based on the fact that the multinucleate condition appears in the adjacent cells after it occurs in the apical cells of colchicine treated roots.

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