A SUPPLEMENTARY STUDY ON THE CELL DIVISION OF ROOT APICAL CELLS IN SOME PTERIDOPHYTES[1]

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Abstract: A study of the root apical meristems of three species of ferns, *Ophioglossum petiolatum* Hook., *Ceratopteris thalictroides* (L.) Brongn. and *Marsilea crenata* Pr. has been made. These ferms were cultured in both the presence and absence of colchicine. The apical cells of some colchicine treated roots showed either a multinucleate condition or other abnormalities, whereas the apical cells of all non-treated roots were mononucleate. All the roots of these three species formed tumors after treatment. The typical c-metaphasic nucleus which has frequently been observed in angiosperms was not seen in this experiment. Apparently the dividing cells of pteridophytes show a different pattern from those in angiosperms in response to the action of colchicine.

It is clear that the root apical cells in these three species always divide more frequently than their adjacent cells. Among these, the root apical cells in *Ceratopteris* divides more frequently than the other two species. The presence of a quiescent center in the root apical meristem in these ferns has not been verified.

INTRODUCTION

In a previous paper by the senior author (Chiang, 1972) the observations of earlier workers was confirmed that the root apical cell in some pteridophytes divides more frequently than its adjacent cells (Avanzi and D'Amato, 1967; Chiang and Gifford, 1971; Gifford, 1960). In studying the division rate of the root apical cell, it has been necessary to analyze the behavior of the apical cell. Studies have been made on the spatial arrangement of the cells in the meristem (Bartoo, 1929, 1930; Bower, 1889; Chiang and Gifford, 1971; Clowes, 1961; Pal and Pal, 1962); the growth rate of lateral roots (Chiang and Gifford, 1971); the frequency of mitotic division of the apical cells (Buvat and Liard, 1953); the measurement of their DNA content (Avanzi and D'Amato, 1967; Clowes, 1956; D'Amato and Avanzi, 1965; Gifford, 1960); and the effects of the application of colchicine (Chiang, 1972) were studied. Each of these factors has its interpretative value in the explanation of the behavior of the apical cell. Some workers believe that the root apical cell in pteridophytes divides infrequently whereas others considered it as an actively dividing cell. (Buvat and Liard, 1953; D'Amato and Avanzi, 1965).

Colchicine has long been used as a tool for investigation of cell division. Although there have been several papers published reporting the effect of colchicine on fern gametophytes but little has been reported on the effects of colchicine on fern sporophytes (Chiang, 1972). It was reported in a previous paper by senior author (Chiang, 1972) that the formation of multinuclei in the cells of the root apical meristem is the most conspicuous phenomenon following colchicine application. The approximate division rate of the root apical cell in *Ceratopteris pteridoides* was

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estimated by counting the number of nuclei per cell.

In the present study a report is made on three other species of pteridophytes. The authors desired to obtain further evidence to confirm observations reported in the previous paper (Chiang, 1972), and to seek to gain a better general picture of the reaction of colchicine on the fern sporophytes.

MATERIALS AND METHODS

Three widely differing species of ferns were chosen for investigation, namely, *Ophioglossum petiolatum* Hook., *Ceratopteris thalictroides* (L.) Brongn., and *Marsilea crenata* Pr. The period of treatment with colchicine for *Ophioglossum* was in March, for *Marsilea* was from June to July and for *Ceratopteris* was from July to September.

The plants were moved from the greenhouse to the laboratory 24 hours before colchicine treatment, then grown in tap water near a window. The roots were dipped into 0.05% colchicine solution by transfering the whole sporophyte into a container of colchicine solution. The time of colchicine treatment was 48 hours for all species. The root tips were then fixed in FAA (Purvis, Collier and Walls, 1966). Materials were prepared for paraffin sectioning by a tertiary butanol series, and stained by tannic acid and iron alum with safranin and orange G (Sharman, 1943). Cytological study and photomicrographs of the root apical meristems and especially the apical cells were made by a Nikon semiautomatic photomicroscope.

RESULTS

1. Outer morphology of the roots

The roots of all three species formed tumors following colchicine treatment. It is seen by microscopic observation that the tumor is caused by a pattern of longitudinal growth and radial expansion of the cells of the root apical meristem (including the apical cell). The expansion of the cells in the tumor region in the isodiametric direction is more conspicuous than those growing in the longitudinal direction. The root tumors in *Ceratopteris* were more conspicuous than those formed in *Marsilea* or *Ophioglossum*, and the roots of *Marsilea* had larger tumors than those of *Ophioglossum*. The diameter of the treated roots were 2.51 times more than those of the non-treated root in *Ceratopteris* and 1.89 times greater in *Marsilea* and 1.76 times greater in *Ophioglossum*. (Table 1).

Table 1. The diameter* of roots before and after colchicine treatment

Plant	** diameter of non-treated roots in mm (C)	*** diameter of treated roots in mm (T)	T/C
Ophioglossum	0.91	1.60	1.76
Ceratopteris	0.57	1.43	2.51
Marsilea	0.36	0.68	1.89

* average of 10 roots

** measured at the corresponding region on a treated root

*** the longest diameter of a tumor

2. Multinucleate condition in the apical meristem

The most conspicuous change occurring in the nucleus of the root apical cells was the formation of a multinucleate stage. A typical c-mitosis did not appear.

The multinucleate condition was common in the apical cells of all species treated. The increase of the number of nuclei in the different species will be discussed separately in the following paragraphs:

Ophioglossum: As shown in Table 2, four out of 15 apical cells of the colchicine treated roots were seen to be binucleate (Fig. 1a). No apical cells were found which possessed three or more than three nuclei. The close derivatives of the apical cells in both treated and non-treated roots which were examined were in a mononucleate stage (Fig. 1a). In other words, all of the cells except four apical cells, in the colchicine treated roots were in a mononucleate stage. But all the treated roots formed root tumors. Apparently the binucleate condition played little part in tumor formation. The apical cell always divides more frequently than its adjacent cells, because a multinucleate condition can be accepted as evidence for subsequent mitoses.

Table 2. The diameter* and the numbers of nuclei in apical cells from roots of *Ophioglossum*

root no.**	in water		root no.**	in colchicine	
	diameter of apical cell (μ)	no. of nuclei		diameter of apical cell (μ)	no. of nuclei
1	48.83	1	1	59.11	1
2	48.83	1	2	59.11	1
3	51.40	1	3	61.68	1
4	53.97	1	4	61.68	1
5	53.97	1	5	64.25	1
6	53.97	1	6	64.25	1
7	53.97	1	7	79.67	1
8	53.97	1	8	79.67	1
9	53.97	1	9	82.24	1
10	56.54	1	10	82.24	1
11	56.54	1	11	82.24	2
12	59.11	1	12	89.95	1
13	59.11	1	13	89.95	2
14	61.68	1	14	92.52	2
15	69.39	1	15	102.80	2

* measured from the transverse section

** arranged in order of the size of the diameter

Ceratopteris: All of the apical cells in colchicine treated roots (21 roots) were found possessing either multinuclei or ameboid nuclei, whereas there were no multinucleate apical cells in the non-treated roots (Table 3). The number of nuclei in a single treated apical cell ranged from three to several (Figs. 2a, 2b). Some of the treated roots had from one to three large ameboid nuclear masses (Figs. 2c, 2d). Micronuclei were very common in the apical cells and these had more than four nuclei. The multinucleate stage was seen in the adjacent derivatives of the root apical cell. The nuclei ranged from one to two in a single adjacent cell. No adjacent cells were found which possessed more nuclei than its apical cell. Evidently the root apical cell never divides less frequently than its close derivatives in *Ceratopteris.*

Table 3. The diameter* and the number of nuclei in apical cell from the roots of *Ceratopteris*

root no.**	in water		root no.**	in colchicine	
	diameter of apical cell (μ)	no. of nuclei		diameter of apical cell (μ)	no. of nuclei
1	23.13	1	1	64.25	3
2	25.70	1	2	64.25	***
3	28.27	1	3	64.25	3
4	28.27	1	4	66.82	***
5	28.27	1	5	82.24	4
6	28.27	1	6	82.24	3
7	28.27	1	7	82.24	***
8	30.84	1	8	84.81	***
9	30.84	1	9	87.38	***
10	30.84	1	10	87.38	***
11	30.84	1	11	87.38	***
12	30.84	1	12	87.38	***
13	30.84	1	13	89.95	6
14	33.41	1	14	92.52	***
15	33.41	1	15	92.52	***
16	33.41	1	16	95.09	3
17	33.41	1	17	95.09	***
18	35.98	1	18	95.09	***
			19	95.09	***
			20	95.09	***
			21	97.66	***

* measured from the transverse seciton.

** arranged in order of the size of the diameter.

*** micronuclei and ameboid.

Marsilea: *Marsilea* produces the thinnest roots of these three species (Fig. 3). Eight out of 16 apical cells of the treated roots were found to have two nuclei (Fig. 1b), and eight were observed to have only one nucleus (Table 4). No apical cells were seen which had three or more than three nuclei in the colchicine treated material. All untreated roots had apical cells with only one nucleus (Table 4).

It was obvious that colchicine treated roots of all species examined possessed an apical cell with more nuclei than its close derivatives. This fact shows that the root apical cells in these three species always divide more frequently than their adjacent cells. Among these three species, the root apical cell in *Ceratopteris* divides more frequently than in the other two genera. The multinucleate condition is very common in both the apical cell and its derivatives (Table 3). The apical cells of *Ceratopteris* receive more nucleotoxic effects than others, because most of the apical cells in the treated roots (15 out of 21 roots) had many micronuclei or ameboid nuclei.

The data obtained in this experiment is not valid for measuring the rate of cell division, because too much mitotic poison was taken up in the treated roots of

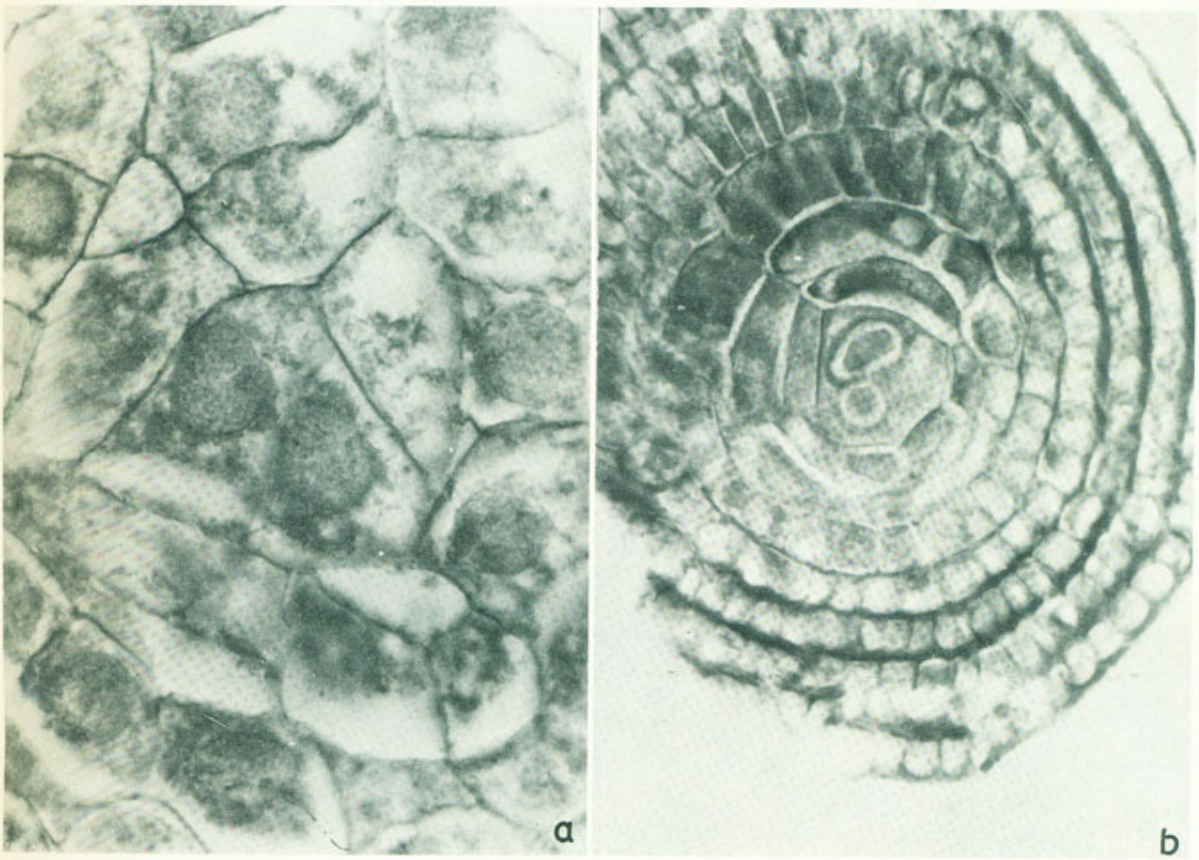

Fig. 1a. A binucleate apical cell of *Ophioglossum* from the transverse section of a root, ×500.
Fig. 1b. Transverse section from the root of *Marsilea* through the apical meristem, showing the tetrahedral apical cell in a binucleate stage, × 500.

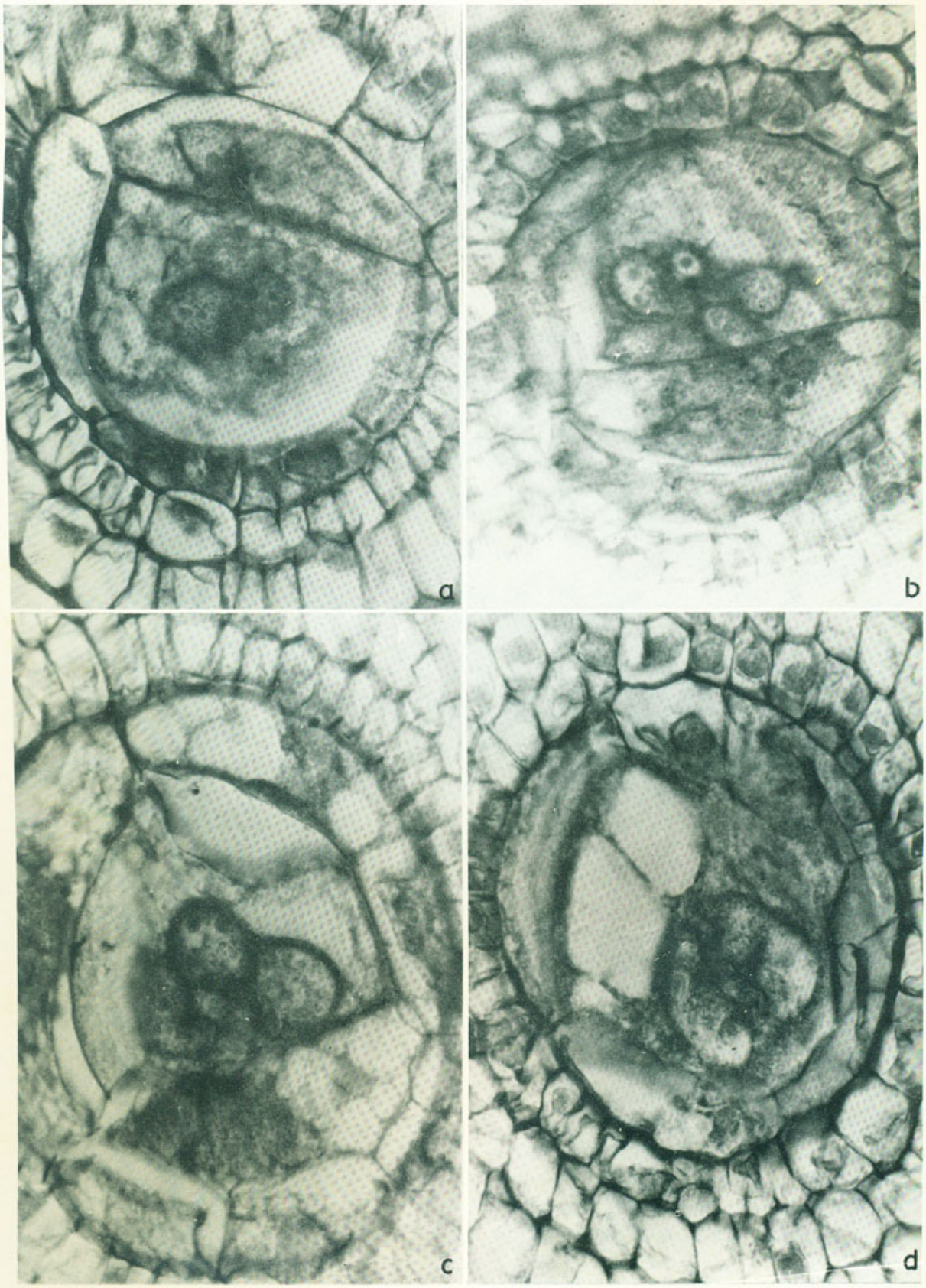

Fig. 2. Transverse sections from the roots of *Ceratopteris* through the apical meristem, all ×500.
2a. Apical cell in trinucleate stage.
2b. Apical cell with more than six nuclei.
2c & 2d. Apical cells with several ameboid nuclei.

Ceratopteris. The multinucleate stage in the apical cells of *Ophioglossum* and *Marsilea* was not very common. No apical cells with three or more than three nuclei were found in either *Ophioglossum* or *Marsilea.* Though things are probably more complicated, it can not be denied that the apical cells in these three species always divide more frequently than their adjacent cells in the same root.

3. Rate of root elongation

Roots of about 4–5 cm were chosen to measure the rate of elongation in a 5 day period. The non-treated roots of *Ceratopteris, Marsilea* and *Ophioglossum* increased 7.35, 3.90 and 0.18 cm in length respectively (Fig. 4). The roots treated with colchicine did not increase in length, and it is apparent that those with apical cells which divide most frequently have receive greater nucleotoxic effect.

4. Size of the apical cell

There is an increase in the volume of the root apical cell as is shown by measuring the diameter of the apical cells (Table 2–4 and Fig. 3). Treated apical cells which possess either mononuclei or multinuclei and other anomalous nuclei tend to be larger than those in non-treated roots. The roots of *Cphioglossum* and *Marsilea* also show an increase in cell volume corresponding with an increase in its nuclear content (Tables 2, 4). Binucleate apical cells are somewhat larger in volume. But the relationship between the size of apical cell and the number of nuclei in the root apical cells of *Ceratopteris* is not clear because of the formation of micronuclei and ameboid nuclei. (Table 3).

Table 4. The diameter* and the number of nuclei in apical cell from the roots of *Marsilea*

root no.**	in water		root no.**	in colchicine	
	diameter of apical cell (μ)	no. of nuclei		diameter of apical cell (μ)	no. of nuclei
1	20.50	1	1	35.98	1
2	23.13	1	2	35.98	1
3	23.13	1	3	38.55	1
4	23.13	1	4	38.55	1
5	23.13	1	5	38.55	1
6	25.70	1	6	38.55	1
7	25.70	1	7	38.55	2
8	25.70	1	8	41.12	1
9	25.70	1	9	41.12	2
10	28.27	1	10	41.12	2
11	28.27	1	11	43.69	1
12	28.27	1	12	43.69	2
13	30.84	1	13	46.26	2
14	30.84	1	14	46.26	2
15	30.84	1	15	48.83	2
16	35.98	1	16	51.40	2
17	38.55	1			

* measured from the transverse section.

** arranged in order of the size of the diameter.

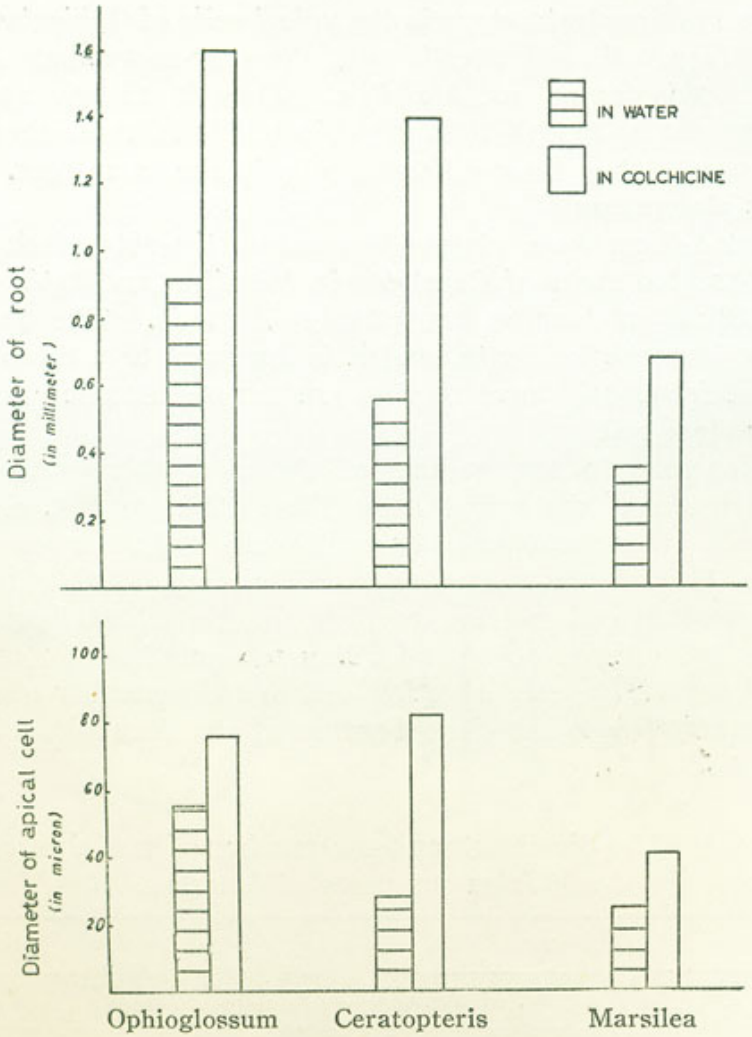

Fig. 3. Histogram showing the diameter of the apical cells from the roots which had been grown in water and in 0.5% colchicine solution for two days. An average of more than 15 apical cells of each treatment is shown in this histogram.

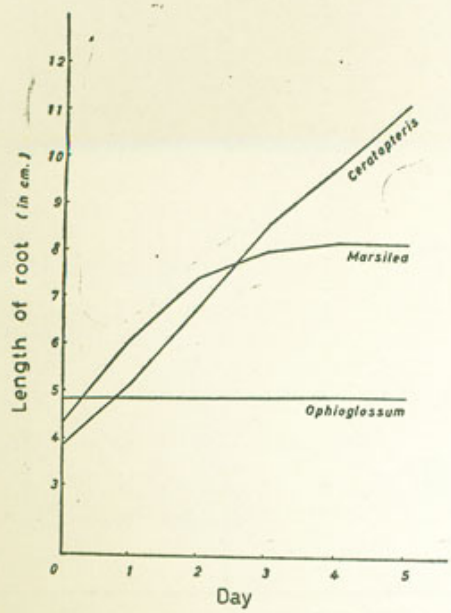

Fig. 4. Changes in root length during five days, all non-treated roots.

DISCUSSION

The single root apical cell of pteridophytes behaves as an initial cell for all the root tissues. Some workers have reported that this single apical cell always divides more rarely than its surrounding cells (Avanzi and D'Amato, 1967; Buvat and Liard, 1953; Clowes, 1961; D'Amato and Avanzi, 1965), whereas others have stated that it divides very frequently (Chiang, 1971; Chiang and Gifford, 1971; Gifford, 1960). In studying the rate of division in a given cell such as the root apical cell, the time of making the collection is very important if the data is based on counting mitotic figures. The time control in colchicine-treated method is more convenient than that in the counting-mitotic-figure method.

The present investigation showed that the root apical cells of *Ophioglossum petiolatum*, *Ceratopteris thalictroides* and *Marsilea crenata* divide more frequently than their adjacent cells in the meristem of the actively growing roots. As pointed out in the previous report (Chiang, 1972), though numerous papers have been published dealing with the apical cells of ferns, there has been little published regarding the presence of a multinucleate condition in the apical cell. We have found that after the application of colchicine the multinucleate condition is common in actively growing roots of the ferns which we have been studying. Evidently the multinucleate condition can be considered to be one of the important consequences of mitosis in colchicine treated roots.

C-tumors were formed in the roots of all the species examined, but no actively dividing mitotic figures were seen during this investigation. Though the DNA content in the apical cell and its surrounding cells were not measured in the present study, the formation of multinuclei and the large volume of nuclear material suggest that endomitosis was very common. The occurrence of endopolyploid is usually considered to be differential in growth (Partanen, 1965; Stange, 1965) or an evidence for a subsequent mitosis. The large volume of the apical cell and endoduplication have been described by earlier workers (Buvat and Liard, 1953; Avanzi and D'Amato, 1967; D'Amato and Avanzi, 1965; Chiang, 1972; Gifford, 1960). The endoduplication is considered to be a result of subsequent mitoses rather than requiring some special process of differentiation as reported by other workers. Any direct relationship between multinuclei and a c-tumor can not be proved from our data. However the formation of a c-tumor is mainly caused by the expansion of the cells in the apical meristem (including the apical cell), and the multinucleate apical cells are always larger than the mononucleate ones. Therefore it seems logical to think that the multinucleate apical cell must be intimately correlated with the formation of c-tumors. Levan and Östergren (1943) stated that tumor formation and the polyploidy were independent processes. But Partanen (1956) pointed out that tumor growth might be due to high polyploidy caused by endomitotic reduplication in fern prothallia.

When we started the previous work (Chiang, 1972) as well as in the present work, we expected to find an arrested metaphase in treated tissues. But our experiments have not revealed any such condition. One of the actions of colchicine usually attributed to it is that of stopping mitosis in the metaphase stage which did not occur in the ferns we investigated. It has been suggested by many experimenters that the action of colchicine on nuclear mitosis is specific and selective (cf. Eigst and Dustin, 1957). It seems to the authors that ferns have a common specificity in response to the colchicine application. The most conspicuous action of colchicine treatment on fern tissues is the formation of a multinucleate stage, but

cytoplasmic division fails to reach completion. In some treated animal tissues, the micronuclei were formed by means of swelling the chromosomes before the metaphase (Brues and Jackson, 1937). But in *Ceratopteris pteridoides* micronuclei and ameboid nuclei were formed only when the time of colchicine application was prolonged (Chiang, 1972). Micronuclei were formed after the appearance of the mitotic figure.

Though a quiescent center has been identified by different workers in many angiosperms (Clowes, 1956, 1968; Phillips and Torrey, 1971a, 1971b, 1972; Thompson and Clowes, 1968), the presence of a quiescent region in the root apical meristem of pteridophytes has not been verified in the ferns we examined.

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