INSOLUBLE CARBOHYDRATES IN THE SHOOT APICAL MERISTEM OF *ADIANTUM CAPILLUS-VENERIS* L.*

SU-HWA TSAI CHIANG and BAI-LING LIN**

Abstract: The amount of insoluble carbohydrates in the shoot apical meristem of *Adiantum capillus-veneris* L. shows an annual periodic change. The accumulation of the insoluble carbohydrates starts in the early spring (February to March), reaches its maximum in April, then decreases gradually, reaching its lowest content in winter (January). A zonation pattern can be seen in the apical region based on both the cell lineage and the PAS distribution in most specimens. The zone of surface initials always contains more PAS granules than in the other cells of the promeristem. Though the apical zone of the winter collected materials had the lowest amount of PAS particles, yet the apical cell constantly contains more PAS stained particles than its neighboring cells. The significance of the apical cell is emphasized in the present report.

INTRODUCTION

The structure of the shoot tip of ferns has been studied for more than a hundred years (Bower, 1889, 1923). It differs from other vascular plants in the presence of a conspicuous apical cell. The orderly segmentation pattern of the apical cell has also been described by many other workers (Bierhorst, 1977; Clowes, 1961; Ford, 1902; Guttenberg, 1966; McAlpin & White, 1974). These workers were able to explain the division of the apical cell and follow its differentiation to various tissues. In recent studies, in addition to the behavior of the apical cell, the apical morphology of pteridophytes has been viewed at the tissue level (McAlpin & White, 1974; Stevenson, 1976a, b). Authors have treated the whole apical area (including the apical cell together with the other cells existing in the apical region) as an unit of operating tissue, and some emphasized a zoned pattern in pteridophytes as applied to the higher vascular plants (McAlpin & White, 1974; Stevenson, 1976b). They seem to disregard the special meaning of the single apical cell which was previously accepted. Those who believe in the zonation concept of the fern apex and ignore the significance of the apical cell appear to have little confidence in the methods reported in the older literatures. The analysis of the pattern of cell lineages in fern shoot apices which was most commonly applied in the older literatures is so orderly that one can not disregard them by merely examining a few slides to observe the distribution of some chemical compounds, such as: DNA, RNA and protein.

This study is to reemphasize the fact that the single apical cell in the fern stem apex plays an important role in the organization and development of the stem growth. The observations have been made mainly on cytochemically stained specimens which are also used as a basis by other authors who believe in the zoned pattern of the shoot tip.

MATERIALS AND METHODS

Shoot tips of *Adiantum capillus-veneris* L. growing in the Taipei area were studied. About eight plants, bearing six to eight well developed fronds on each plant, were randomly harvested

* Supported by the National Science Council Grant NSC-67B-0201-02(07), Republic of China.

** (江蔡淑華) Professor and (林白朗) teaching assistant of Botany Department, National Taiwan University.

at monthly intervals from October, 1976 to February, 1978. Collections were made on 5th of each month. Some additional collections such as: very tiny specimens, were also made in addition to the regular harvests. Shoot tips were fixed in FPA (formalin-propionic acid-alcohol), infiltrated and embedded in tissuemat according to the traditional paraffin methods. Sections were cut at 8 μ longitudinally. Some preparations were stained by periodic acid-Schiff's (PAS) reaction (Jensen, 1962) for insoluble polysaccharides detection, and other preparations by tannic acid and iron alum with safranin and orange G (Sharman, 1943) for histological studies. Observations were centered mainly on the PAS granules which were present inside the cells, though the cell walls were also well stained.

RESULTS

General Morphology

More than 150 shoot tips were studied during the present investigation. It was found that the shape of the apical region in all the specimens appears to be uniform. The top of the shoot apex is rather flat and slightly concave at the very submit of the apex surface where the single apical cell is located. The degree of the flattened pattern appears to be more evident in the stems that have a wider apex surface (Figs. 1a, 2c). The thinner apex is more conical in shape (Fig. 3c). A conspicuous single apical cell which always possesses a larger nucleus can be recognized in all the specimens examined. Based on cell lineage, the three zoned pattern i. e. a zone of surface initials; a zone of subsurface initials; and a cup-shaped zone, as described by Stevenson* (1976b) can be identified in the specimens which have wider apex surfaces (Fig. 1a). But in plants which possess a narrower apex these three zones can hardly be distinguished (Fig. 3a). In addition to these three zones a fourth zone, the outer cup-shaped zone (peripheral zone by McAlpin & White, 1974) located outside the cup-shaped zone is present (Fig. 1a). The cells in this fourth zone appear to be smaller than those in the outer three zones in median longisections. The cells in the surface initials are distinguished by their large size and being elongated, but not all of the cells in the surface zone are vacuolate as is seen in the preparations stained with either tannic acid, iron alum, safranin and orange G, or PAS staining (Figs. 1a, 1b). The cells in the center region (including the apical cell) are always densely stained (Figs. 1a, 1b).

Seasonal Variation of the Distribution and Amount of Insoluble Carbohydrates

The shapes of PAS particles existing in the apical meristem appear to be spherical to amorphous. Although exact counts were not made of the particles in the cells of the meristem yet these particles seemed to decrease in number in the meristematic zone when the temperature decreased. In plants collected in January, the starch was very scanty in all the shoot tips (Figs. 3a, 3b, 3c), whereas in the tips collected in late spring (April), the PAS particles were greater in size and distributed throughout the whole meristematic region (Figs. 5a, 5b, 5c). All the materials, other than those collected in January and April showed a series of widely differing amounts of PAS particles in the materials collected on the same day. In other words, the amount of PAS particles present in the apex ranged from very few to quite numerous in the members collected on a given day (Figs. 2, 4). The materials collected in December for example (Figs. 2a, 2b, 2c), Fig. 2a has the fewest PAS granules as compared to Figs. 2b and 2c which were harvested on the same day as Fig. 2a. And Fig. 2c shows the greatest, Fig. 2b median content quantitatively.

In spite of the different number of starch particles existing in the meristematic zone of the different plants in the same season, the distribution of the PAS granules exhibited a

* For descriptional convenience, the three zoned terminology is used in the present report.

zonational pattern in all the slides examined. In the specimens collected in April, the tips have almost the same amount of the PAS density in all the slides prepared (Figs. 5a—5c), and it appears that the PAS content of the cells in the submit of the shoot spex is always more abundent than that in other zones, and the PAS particles in the surface initials always appeared to be larger in size than those existing in other parts. The zone of the surface initials contained either only one conspicuous apical cell (Fig. 5c), or more than one cell, i. e. the apical cell together with its immediate derivatives (Figs. 5b, 5c). It is not invariably the case that the wider surface area (the distance between two newest leaf primordia) consists of a greater area of surface initials. In Fig. 4c and Fig. 5c for example, the surface area in the former is definitely wider than that of the latter, but the densely stained surface initial zone is smaller. The periphery of the zone of the surface initials together with the zone of subsurface initials and the cup-shaped zone usually showed a lighter staining, especially the periphery of the zone of the surface initials and subsurface initials. The zonational pattern is more obscure in the specimens which have less PAS granules in the promeristem (Fig. 2a). However, the apical cell constantly exhibits a more dense staining even in the materials of January which exhibit the fewest PAS particles throughout the whole meristematic zone than those collected in other seasons (Figs. 3a, 3b, 3c), and in no case were the PAS particles completely absent in the apical cell. In general, the cells in the peripheral arc contain more PAS granules than in any other part of the stem (Fig. 1b). So that, at first glance as observed at lower magnification, the median part of the top of the shoot shows as a lighter region in PAS staining except for the very submit where the apical cell is located (Fig. 1b). The outer cup-shaped zone contains more PAS granules than the other zones. They decrease in number as the cells become elongated. Consequently the elongated pith meristem and cortical meristem in the lower part of the shoot appears to be a less stained region (Fig. 1b).

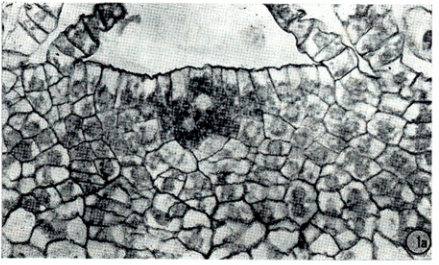
Leaf Primordia

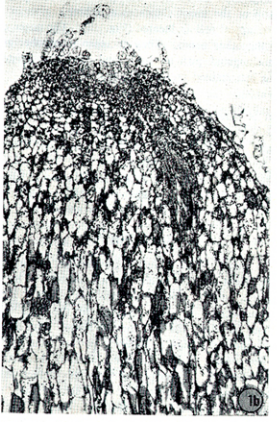
The leaf primordium is arranged in an orderly manner in the periphery of the meristematic region. It initiates at the cup-shaped zone (Fig. 2b, arrow), and can be easily recognized by its cell arrangement and the presence of the PAS particles. The leaf primordium has a prominent apical cell at its tip. Both the apical cell and its adjacent cells have more PAS particles in the early stages of development (Fig. 2b). However the apical cell of the leaf primordium becomes the cell having more abundant PAS granules as it develops (Fig. 3c, arrow).

DISCUSSION

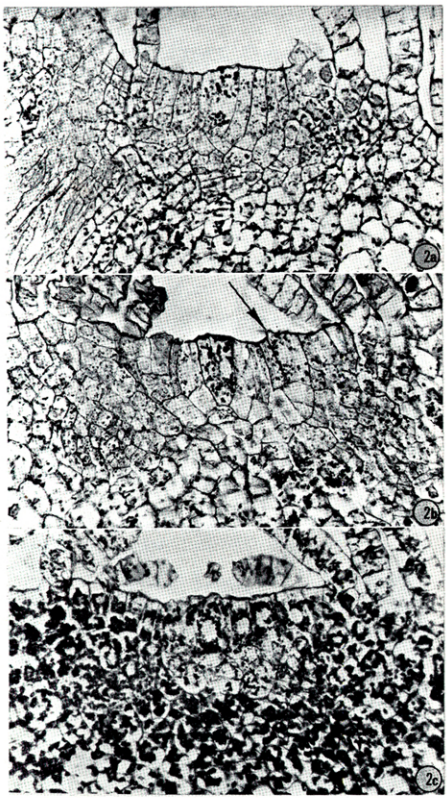
The uniformity of the shape of the apical region in the majority of the materials saved much labor in seeking the right sections under the microscope. The apical cell is constantly observed in all the specimens and the zonational fashion is visible in most tips. The zonational pattern in the median longisection is more distinct in the PAS stained preparation when that is compared with those based on the cell lineage alone. The distribution of the insoluble carbohydrates in this plant showed a slightly different pattern from that obtained in *Botrychium* (Stevenson, 1976b). In *Botrychium*, the rib meristem exhibits less PAS staining than does the peripheral zone. But in *Adiantum* the peripheral zone of surface initials together with the zone of the subsurface initials and the cup-shaped zone show lighter staining (Fig. 1b). The lighter zone is occasionally interrupted by the leaf primordia. The leaf primordia contain more PAS particles when they are still located within the cup-shaped zone (Fig. 2b). The outer cup-shaped zone, including the rib meristem, always stained densely (Fig. 1b).

Though the exact change of the environmental conditions such as: temperature and rainfall were not recorded, yet the amount of the insoluble carbohydrates in the apical meristem

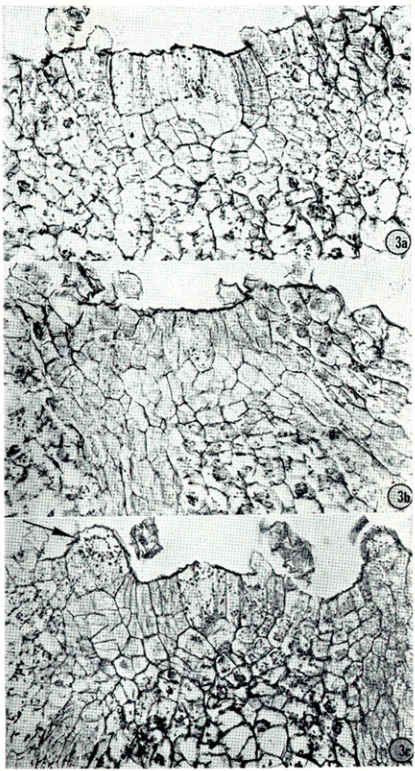




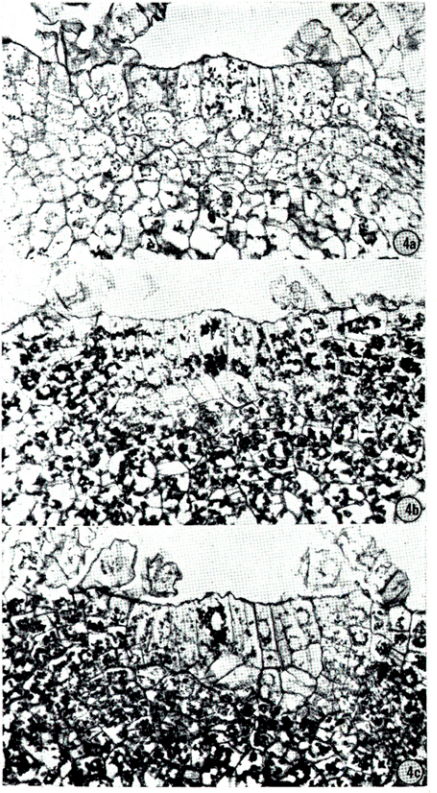
Figs. 1. Median longisections of shoot tips; (a) stained with tannic acid & iron alum with safranin & orange G, ×350; (b) PAS staining, ×88.



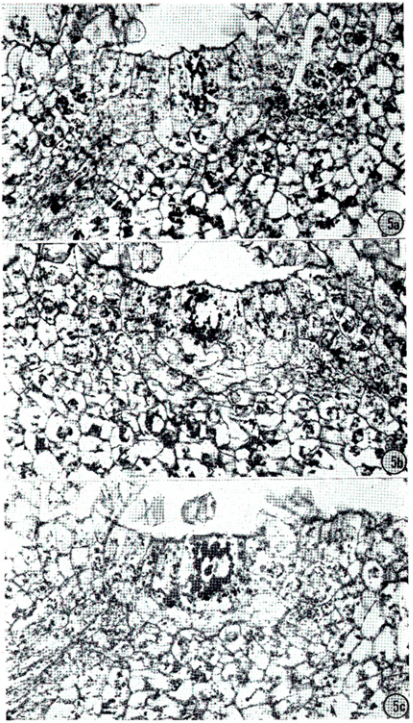
Figs. 2. Median longisections with PAS stained, collected in December, ×350.



Figs. 3. Median longisections with PAS stained, collected in January. ×350.



Figs. 4. Median longisections with PAS stained, collected in March. ×350.



Figs. 5. Median longisections with PAS stained, collected in April, ×350.

is apparently affected by the seasonal change. In the beginning of the growing season (April), the apical region of almost all of the specimens collected contained more numerous PAS granules (Figs. 4, 5) whereas in the plants harvested in winter (January to February), the whole apical zone appeared to be very light in PAS tests (Fig. 3). In the apical region of the plants collected in other seasons, some of them were lighter and some were denser (Fig. 2). This observation reveals that the accumulation of the carbohydrates started in the early growing season (February to March), and reached its maximum amount in April, then decreased gradually, dipping to its lowest level in winter (January). The individual variations of the PAS particles in the intermediate seasons (the seasons between the growing and resting periods) show that some plants contained more and others less particles when observed in the specimens collected on the same day. This result represents a different distributional pattern as observed in *Dryopteris aristata* obtained in England (Frazer, 1946). The authors have intended to correlate the changes in PAS distribution and density with the leaf formation and sporogenesis. But there was no way to do it, however, leaf and spore formation occurred almost all the year round in the plants of considerable size. Nevertheless starch distribution on the shoot apices of *Dryopteris* and *Adiantum* at least have one common agreement in that there is no time of the year when the apical cell is found to be free from numerous starch grains.

Both the apical cell of the shoot tip and of the leaf primordium exhibit denser staining (Figs. 2b, 3c). It is clear that the apical cell plays some common important role in the development of both the shoot and the leaf tissues. The division rate of the apical cell has been predicted based on several categories such as: relative cell wall thickness; asymmetry of the apical cell; counting of the mitotic figures; reactions to the colchicine application and to the radioactive isotopes etc. (Avanzi & D'Amato, 1967; Bierhorst, 1977; Chiang, 1972; Clowes, 1961; D'Amato & Avanzi, 1965; Gifford, 1960). But there is still no well-accepted explanation of the other roles in addition to the division rate, of the apical cell. When the histochemical test was applied, the apical cell shows some similar properties with its neighboring cells (McAlpin & White, 1974; Stevenson, 1976b). Consequently some workers begin to suspect the special meaning of the existence of the apical cell which has long been thought to be the most important cell in the apical meristem of the fern in older literatures (Guttenberg, 1966; Hanstein, 1868).

The apical cell in ferns always is seen as a large and highly vacuolated cell as observed by many workers (Bierhorst, 1977; Clowes, 1961; Croxdale, 1976; McAlpin & White, 1974). But it is not a constant case in *Adiantum*. The apical cell of *Adiantum* is larger in size as described by them, but not always highly vacuolated when compared with the cells surrounding it. On the contrary, the apical cell is very often the most darkly stained cell (cf. all Figs.). This implies that the apical cell of *Adiantum* differs from the other cells. And it indicates that the apical cell is not just an ordinary cell, like those located near it. The degree of similarity between the apical cell and its immediate derivatives should depend upon how recently the apical cell divided, and to what extent its derivatives changed before the next division proceeded in these cells or the preparation was made. So that this result can not be explained by saying that the similarity of the degree of the PAS density only indicates the relative age of the cells in this region. The present result agrees with the concept of Bierhorst (1977) in that he reemphasizes the significance of the apical cell of ferns. As a matter of fact, both the traditional study and the histochemical tests prove that one cannot discuss the apical meristem of the ferns without attaching great importance to the apical cell. It is important to note that the amount of the insoluble carbohydrates in the shoot apical meristem of *Adiantum* shows an annual periodic change. One cannot jump to a conclusion regarding the apical cell by merely observing some specimens without due consideration of such factors as: seasonal changes, developmental stages of the individual plant, etc.

LITERATURE CITED

AVANZI, S. & F. D'AMATO. 1967. New evidence on the organization of the root apex in leptosporangiate ferns. Caryologia **20**: 257-264.

BIERHORST, D. W., 1977. On the stem apex, leaf initiation and early leaf ontogeny in filicalean ferns. Amer. J. Bot. **64**: 125-152.

BOWER, F. O., 1889-1990. The comparative examination of the meristems of ferns as a phylogenetic study. Ann. Bot. **3**: 305-392.

BOWER, F. O., 1923. The ferns (Filicales). Vol. 1. Cambridge Univ. Press, Cambridge.

CHIANG, S. H. T., 1972. The time of mitosis in the root apical cell of *Ceratopteris pteridoides*. Taiwania **17**: 14-26.

CHIANG, S. H. T. & C. Y. LU, 1972. A supplementary study on the cell division of root apical cells in some pteridophytes. Taiwania **17**: 230-238.

CLOWES, F. A. L., 1961. Apical meristems. F. A. Davis Co., Philadelphia.

CROXDALE, J. E., 1976. Origin and early morphogenesis of lateral buds in the fern *Davallia*. Amer. J. Bot. **63**: 226-236.

D'AMATO, F. & S. AVANZI, 1965. DNA content, DNA synthesis and mitosis in the root apical cell of *Marsilea strigosa*. Caryologia **18**: 383-394.

FORD, S. O., 1902. The anatomy of *Ceratopteris thalictroides* (L.) Brongn. Ann. Bot. **16**: 95-121.

FRAZER, H. L., 1946. Seasonal changes in the shoot apex of *Dryopteris aristata* Druce. Ann. Bot. N. S. **10**: 391-408.

GIFFORD, E. M. JR., 1960. Incorporation of H^3-thymidine into shoot and root apices of *Ceratopteris thalictroides*. Amer. J. Bot. **47**: 834-837.

GUTTENBERG, H. VON, 1966. Histogenese der pteridophyten. In Linsbauer, Handbuch der pflanzenanatomie. Band. 7, Teil 2. Gebrüder Borntraeger, Berlin.

HAINSTEIN, J., 1868. Die scheitelzellgruppe im vegetationspunkt der phanerogamen. Festschrift niederrgein. Ges. Nat.-u. Heilkunde: 109-143.

JENSEN, W. A., 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco.

MCALPIN B. W., & R. A. WHITE, 1974. Shoot organization in the Filicales: the promeristem. Amer. J. Bot. **61**: 562-579.

SHARMAN, B. C., 1943. Tannic acid and iron alum with safranin and organge G in studies of the shoot apex. Stain Technol. **18**: 105-111.

STEVENSON, D. W., 1976a. Shoot apex organization and origin of the rhizome borne roots and their associated gaps in *Dennstaedtia cicutaria*. Amer. J. Bot. **63**: 673-678.

STEVENSON, D. W., 1976b. The cytohistological and cytohistochemical zonation of the shoot apex of *Botrychium multifidum*. Amer. J. Bot. **63**: 852-856.