

**Studies on the leaf structure**  
**in some species of Leucobryaceae. I.**  
***Octoblepharum albidum* Hedw. (Octoblephareae)**

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*Riassunto*

I filloidi del muschio *Octoblepharum albidum* (Octoblephareae) sono costituiti, per la loro intera lunghezza, da vari strati di leucocisti e da uno strato intermedio di clorocisti. Le leucocisti, a maturità, sono cellule morte e prive di contenuto protoplasmatico. Le loro pareti interne sono perforate da ampi pori, la cui frequenza e distribuzione non variano significativamente lungo il filloide. Pori sono anche presenti sulle pareti esterne delle leucocisti superficiali, ma solo sulla faccia abassiale dei filloidi, raccolti prevalentemente all'apice e alla base. In generale le pareti esterne delle leucocisti sono assai più spesse e robuste di quelle interne. Nel complesso le leucocisti dei filloidi di *O. albidum* costituiscono un sistema di cavità capillari capace di assorbire una notevole quantità d'acqua e di trattenerla per periodi relativamente prolungati. Appare verosimile che questo tipo di organizzazione strutturale rappresenti un adattamento alle notevoli escursioni nel grado di umidità ambientale che caratterizzano, in generale, l'habitat naturale della specie. Le clorocisti sono cellule vive e provviste di cloroplasti. Dall'apice alla base del filloide l'ultrastruttura delle clorocisti varia sensibilmente, suggerendo l'esistenza di una certa diversificazione funzionale di queste cellule.

INTRODUCTION

The Leucobryaceae have been well known for a long time because of the peculiar structure of their leaves, that consist of

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hyaline dead cells, the leucocysts, and green living cells, the chlorocysts, arranged separately in regular layers. The leaves of most Leucobryaceae seem to be nerveless at first sight. According to CARDOT (1900), they are mostly or entirely composed of the enormously enlarged nerve, the lamina being reduced to a thin margin or absent at all.

An apparent similarity with the Sphagnales results from this structural pattern (MOZINGO *et al.*, 1969; CASTALDO & DI MARTINO, 1970; FABRE, 1973). However, functional analogies between the two groups seem to be rather feeble (CASTALDO *et al.*, 1979).

An early investigation by CARDOT (1900) reports a noticeable variability in the leaf structure of the Leucobryaceae, including number and arrangement of the leucocyst layers, presence of a sterome, and structure and arrangement of the chlorocysts. These features may vary considerably even in the relatively restricted ambit of the single genus, and constitute the main taxonomic characters for the classification of these mosses.

The Leucobryaceae have a very wide geographical distribution, and are reported from regions with different climatic regimes (see WIJK VAN DER *et al.*, 1959-1969). This suggests that the environmental requirements of these mosses may be somewhat diversified, especially as far water availability is concerned. The question is raised whether and to what extent changes in the leaf structure may be related with that ecological diversification.

A recent review by PROCTOR (1979) emphasizes the use of electron microscopy techniques for studies of eco-physiological adaptations in bryophytes. However, studies on the ultrastructure of Leucobryaceae are very scarce (FAVALI & BASSI, 1978; CASTALDO *et al.*, 1979). Thus, in order to contribute to a better understanding of the adaptive potential expressed in this family, a comparative SEM and TEM study was done on species from different habitats.

The first paper of this series deals with the leaf structure in *Octoblepharum albidum*.

## MATERIAL AND METHODS

Plants of *Octoblepharum albidum* Hedw. (Octoblephareae) were collected in the Botanical Gardens of the University of Ibadan (Nigeria), where this moss grows spontaneously.

Fully-developed leaves were kindly detached from gametophyte stems and carefully washed before fixing with 3% glutaraldehyde in 0.065 M phosphate buffer at pH 7.2 for 2 at room temperature. The leaves were then post-fixed with 2% OsO<sub>4</sub> in the same buffer at room temperature for 1.5 h, dehydrated with ethanol and propylene oxide, and embedded in the Spurr's resin. The dehydration was successfully performed according to the procedure of COLQUHOUN & RIEDER (1980) for enhancing the contrast. Ultrathin sections cut with diamond knives were stained with uranyl acetate and lead citrate and viewed by a Siemens Elmiskop 1A electron microscope. For scanning electron microscopy glutaraldehyde-fixed leaves were dehydrated with ethanol and critical-point dried in a Bomar SPC-900 apparatus. They were then coated with about 20 nm of gold and observed with an Autoscan U1 ETEC Siemens scanning electron microscope at an accelerating voltage of 20 KV. Thick sections of resin-embedded leaves were obtained with glass knives, stained with 0.1% toluidine blue, and observed with a Leitz Dialux 20 EB light microscope.

The ultrastructural observations were performed at the Centro di Studio di Microscopia Elettronica of the Faculty of Sciences (University of Naples, Italy).

## RESULTS

The leaves of *O. albidum* have a typical *heterostrosic* structure (CARDOT, 1900), being composed of several layers of leucocysts and one middle layer of chlorocysts (Figs. 1-3).

At maturity the leucocysts are devoid of any protoplasmic content; their diameters are 20-40  $\mu\text{m}$ . The leucocyst walls abutting on the outside are much thicker (1-1.5  $\mu\text{m}$ ) than the inner

ones (Fig. 10). Large pores are common on both surface and inner walls of leucocysts. Surface pores are found only on the abaxial leaf side, where they are mostly gathered at the tip and at the base (Figs. 4-7). In the apical region of the leaf the surface pores are either distributed singly or in small groups and display a circular profile (Figs. 4, 8); at the base of the leaf the surface pores are much more numerous than at the tip and have an irregular shape (Figs. 6, 7). In any case no thickening is present around their edges (Fig. 9). The pores piercing the inner leucocyst walls (Fig. 10) appear to be evenly distributed throughout the leaf.

The chlorocysts contain several relatively large chloroplasts usually located at the cell periphery and provided with a well developed grana-fretwork system (Fig. 11). Transverse septa between adjacent chlorocysts are slightly oblique or perpendicular to the main axis of cells, and are pierced by a great number of plasmodesmata (Fig. 12). In the both apical and middle region of the leaves, plasmodesmata also occur on longitudinal walls of neighbouring chlorocysts (Fig. 13). At the leaf base, however, the longitudinal walls have no plasmodesmata, and may be coated with an additional layer (Figs. 14, 16). The chlorocysts at the basal region are also characterized by having large starch deposits in their plastids, and numerous lipid droplets closely associated to microbodies (Fig. 16).

## DISCUSSION

As reported by EGUNYOMI (1978, 1979), in Western Nigeria *O. albidum* is annually subjected to a prolonged dry season (November-March) alternating with a predominantly rainy season (April-October). During the dry season the moss loses most of its water content, ceases growing, and survives in a quiescent dry state thus exhibiting a high tolerance to desiccation. During the wet season water is on the whole abundant. Temporary water deficiency, however, may occur frequently, because of the uneven seasonal distribution of the rainy days (OLARINMOYE, 1974).

There is an apparent relation between this type of water

regime and the peculiar pattern of leaf structure in *O. albidum*, that accounts for the following features of the moss:

a) *High water-holding capacity.* It is due to the presence of several layers of leucocysts in the leaves; these cells are filled with water when it is available. The water content of fully hydrated plants of *O. albidum* may exceed 800% of their dry weight (EGUNYOMI, 1979).

b) *Relatively high resistance to water loss.* At the relative humidity of 62-68% fully hydrated plants of *O. albidum* averagely lose only about 28% of their fresh weight per day (EGUNYOMI, 1979). Probably the thick outer walls of the surface leucocysts reduce the water loss by evaporation. The total absence of pores from the adaxial surface of leaves, which is directly exposed to the sun irradiance, could also be important in reducing transpiration. In addition, the compact growth form of *O. albidum* undoubtedly lessens the average water loss rate (see PROCTOR, 1979).

It is pertinent to note that the water-storing system of *O. albidum*, composed of intra-organ capillary spaces (the leucocysts) with average diameters of about 30  $\mu\text{m}$ , should drain under tensions around 0.1 bar, a value decidedly higher than that averagely estimated for the inter-organ capillary systems (DILKS & PROCTOR, 1979), that are the main water-storing site in most ectohydric bryophytes (BUCH, 1945, 1947).

c) *Rapid absorption of water.* Partially dry leaves of *O. albidum* are able to fill with water almost instantaneously if wetted. It is likely that the numerous pores present on their abaxial surface are responsible for this. The localization of these pores on the apex and the base of the leaves might be related to the fact that the meteoric water mostly gathers on these regions, especially in the capillary channels between the stem and the sheathing bases of the leaves. Probably the absorbed water is distributed throughout the leaf through the inner pores. The leaf capability to absorb water rapidly allows the growing moss to utilize even minimal precipitations, and might be particularly effective for the exploitation of the dew, which is the only water source in the rainless days. The growth rate of *O. albidum* on the bark of trees is significantly higher at low levels

than at high levels above the ground (EGUNYOMI, 1978). This might be related to the more abundant precipitation of dew at the ground level, thus suggesting that this water source might play an important role in the ecological strategy of *O. albidum*. Indeed the common occurrence of this moss in exposed sites such as rocks in dry savanna areas of Western Nigeria might be largely due to its ability to utilize the nocturnal precipitations of dew.

The above considerations indicate that the leaf structure of *O. albidum* is able to minimize the water stress during temporary dry periods of the rainy season. This may be of great importance for the adaptive success of the moss. Indeed in bryophytes the photosynthesis seems to be affected to a greater extent than respiration by the water content, whereby even moderate water deficits may bring about negative values of net assimilation (DILKS & PROCTOR, 1979). Further, there is some evidence (PROCTOR, 1972) that repeated drying and wetting may produce cumulative deleterious effects on bryophytes.

BURRELL (1907) emphasizes the persistent growth of unattached cushions of *Leucobryum glaucum* in Norfolk (England), and ascribes it to the capacity of the moss to store great amounts of meteoric water within its leaves. These have several layers of leucocysts in their basal region (CARDOT, 1900). The same leucocyst arrangement is reported for the leaves of *Leucobryum candidum*, which, moreover, show a distribution of external pores quite similar to that described in the leaves of *O. albidum* (CASTALDO *et al.*, 1979).

With regard to the chlorocysts, it is noteworthy that there is a high frequency of plasmodesmata on their transverse walls, comparable to that reported for the end walls of leptoids in some polytrichaceous mosses (see SCHEIRER, 1978). Undoubtedly such a high frequency of plasmodesmata does enhance the rate of photosynthate translocation from the leaf to the gametophyte stem. Those plasmodesmata occurring on longitudinal walls of neighbouring chlorocysts allow a lateral cell-to-cell flow of solutes. In the basal region of the leaf, where lateral symplastic connections are lacking, the photosynthates may be translocated only longitudinally.

The abundance of starch deposits in the plastids of the basal chlorocysts suggests that these cells may provide a temporary pool for surplus metabolites when the rate of production of nutrients by the upper chlorocysts exceeds that of translocation by the basal chlorocysts, or/and when the demand for nutrients by the gametophyte stem is low. This hypothesis is supported by the occurrence of abundant lipid reserves in the cytoplasm of the basal chlorocysts. The intimate association of these lipid inclusions with microbodies is indicative of a gluconeogenic activity (BEEVERS *et al.*, 1974). Similar associations, likely involved with the mobilization of fat reserves, are reported in germinating spores of *Bryum capillare* (PAIS & CARRAPIÇO, 1979), and in the sporophyte transfer cells of *Buxbaumia piperi* (LIGRONE *et al.*, 1982).

The ultrastructural changes seen in the chlorocysts from different regions of the leaf seem to denote a progressive maturation and functional specialization of these cells from the apex to the base. This is in accordance with the observation that the chlorocyst capability to de-differentiate and to form protone-matal filaments will progressively decrease from the tip to the base of the leaf (EGUNYOMI, *et al.*, 1980).

#### SUMMARY

The leaves of the moss *O. albidum* are composed of several layers of leucocysts embedding one middle layer of chlorocysts. The leucocysts are dead empty cells at maturity. Their walls are frequently pierced by large pores, those on inner walls being evenly distributed throughout the leaf length. External pores are present only on the abaxial leaf surface and are numerous at the tip and at the base. The external walls of the outermost leucocysts are much thicker than the inner ones. This pattern of leaf structure enables the moss to rapidly absorb a large quantity of water and to retain it for relatively long periods. It is proposed that these features might be related with the uneven distribution of the rains during the wet season in the natural habitat of the moss. Ultrastructural changes in the chlorocysts from different regions of the leaf seem to denote a functional diversification of these cells.

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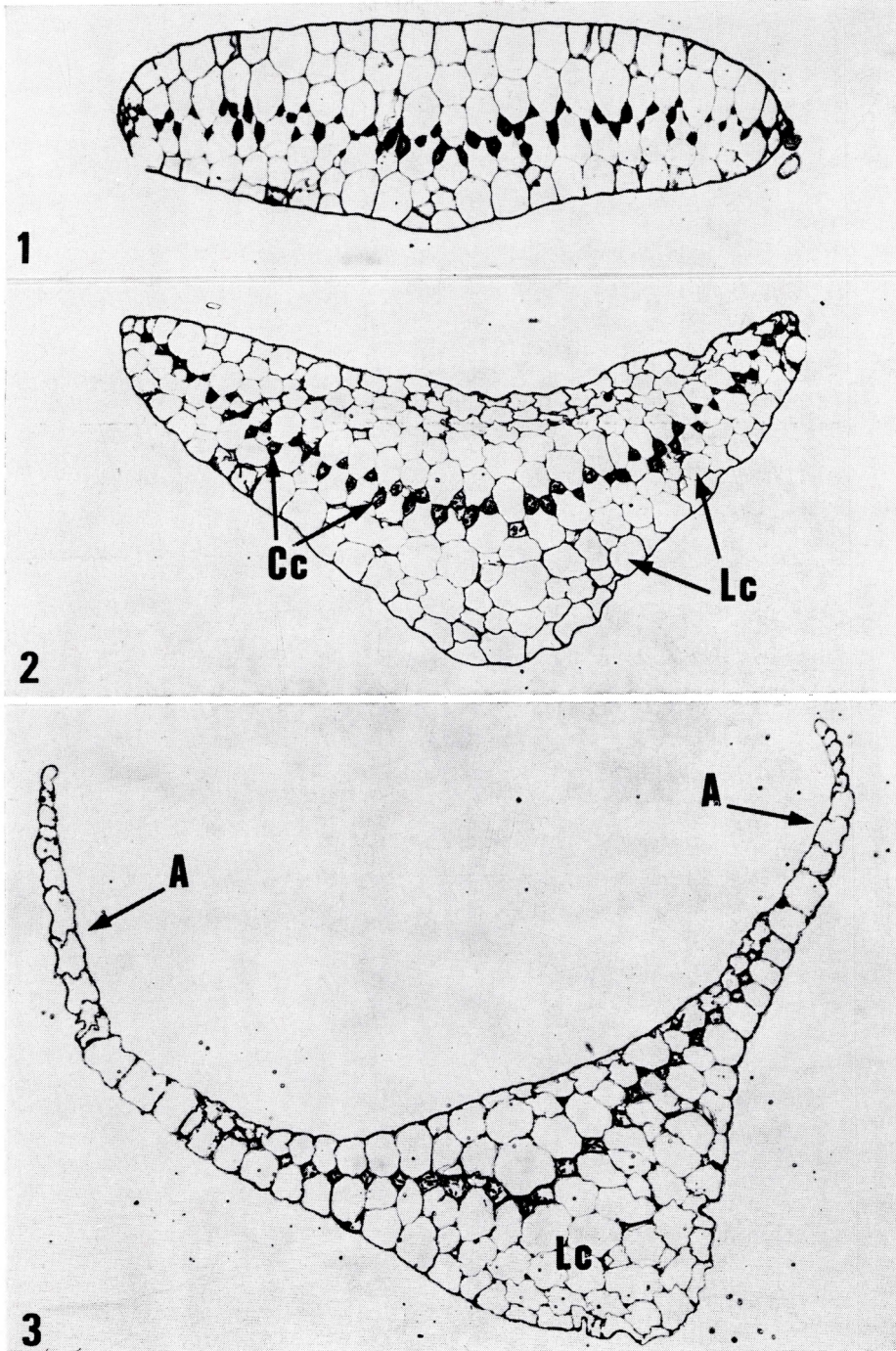


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Plate I

Figs. 1-3. - The leaves of *O. albidum* are entirely composed of leucocysts (Lc) and chlorocysts (Cc). The leaf anatomy changes markedly from the apex to the base. In the apical region (Fig. 1) the leaf has a flattened ellipsoidal profile in transection. In the middle region (Fig. 2) the leaf enlarges along its median longitudinal axis. At the base (Fig. 3) two wide auricles (A) are visible, each composed of a single series of leucocysts. The chlorocysts are arranged in several longitudinal rows connected with one another by frequent anastomoses. In transection the chlorocysts usually show a triangular profile in the apical region (Fig. 1), and a quadrangular one in the basal region (Fig. 3), both types being present in the middle region (Fig. 2). x 200.

Plate I



## Plate II

Figs. 4-7. - SEM details of the leaf surface.

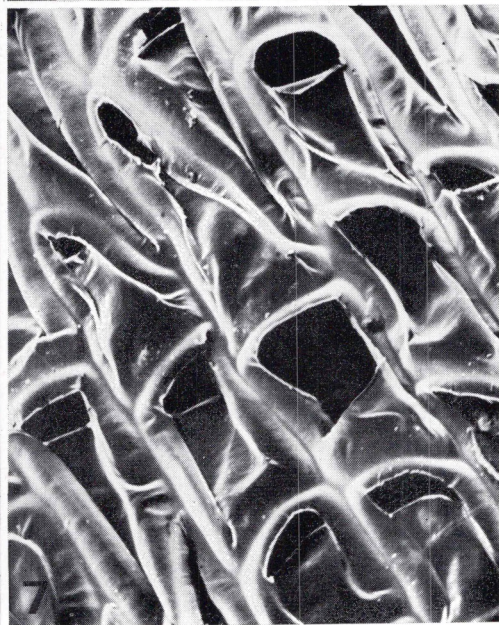
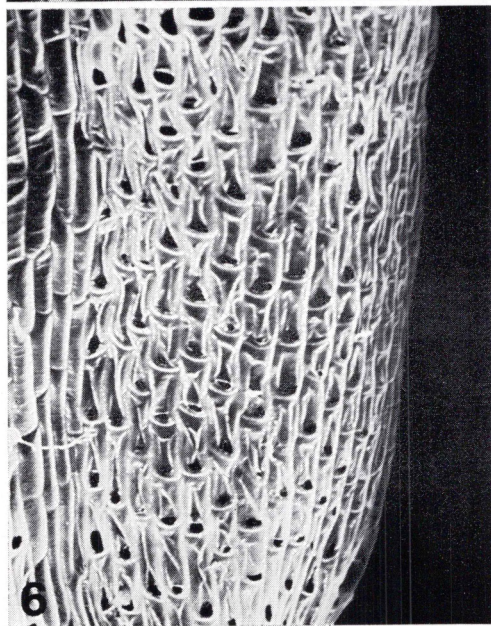
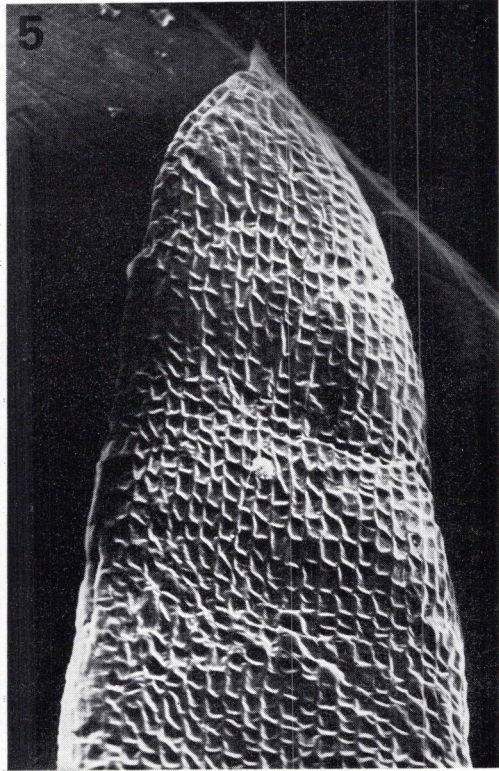
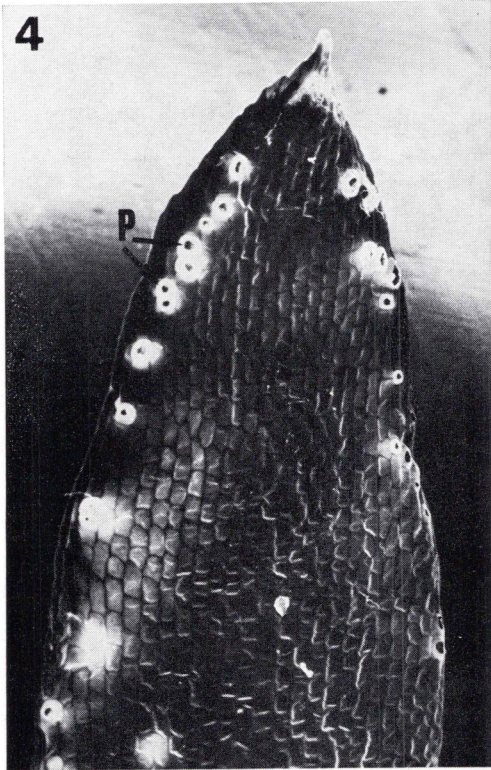
Fig. 4. - Apical region, abaxial side. Along the margins there are several leucocysts whose outer wall is protruding over the leaf surface and is centrally pierced by one circular pore (P) x 100.

Fig. 5. - Apical region, adaxial side. No pore is ever present on this leaf side. x 100.

Fig. 6. - At the base of the leaf the outer wall of most surface leucocysts is pierced by one pore. x 180.

Fig. 7. - The outer pores at the leaf base are irregularly shaped and usually lie near the proximal end of leucocysts. x 700.

Plate II



### Plate III

- Fig. 8. - At high magnification several inner pores (arrows) are visible through the large outer pores of the leaf tip. Altogether the leucocysts form a complex capillary system of cavities communicating with one another and with the outside. x 950.
- Fig. 9. - Outer pores cut transversely. No thickening is present around their edges. x 2200.
- Fig. 10. - Transection of the leaf tip near the margin. The external leucocyst walls are much thicker than the inner ones. An outer pore (P) and some inner pores (arrows) are visible. Chlorocysts (Cc). x 2000.

Plate III

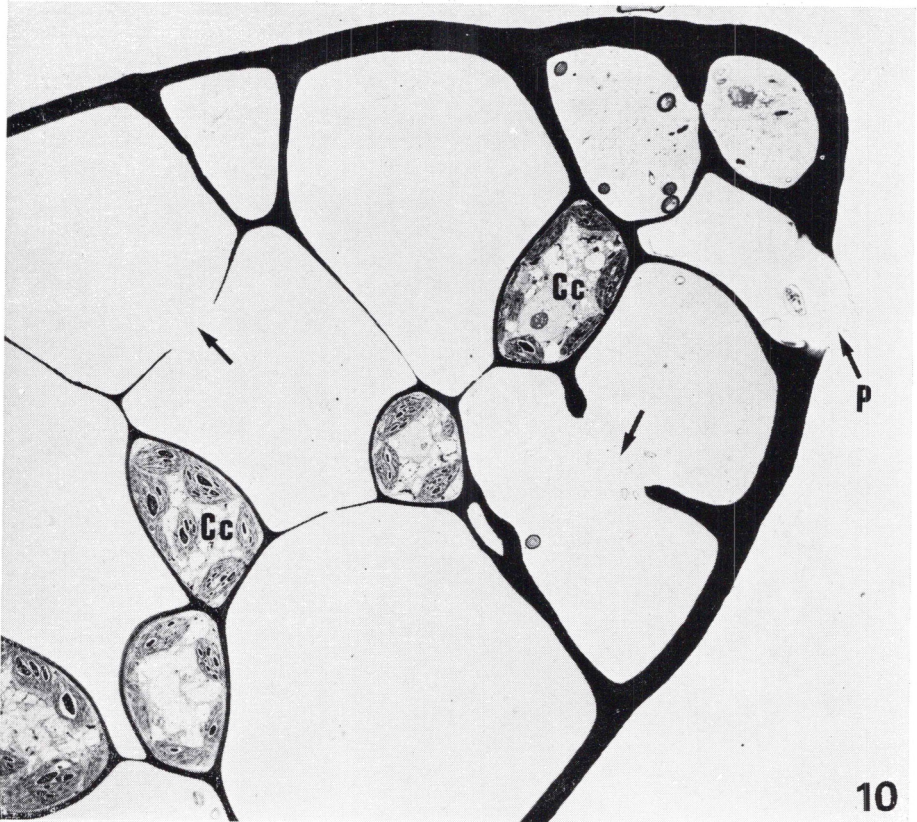
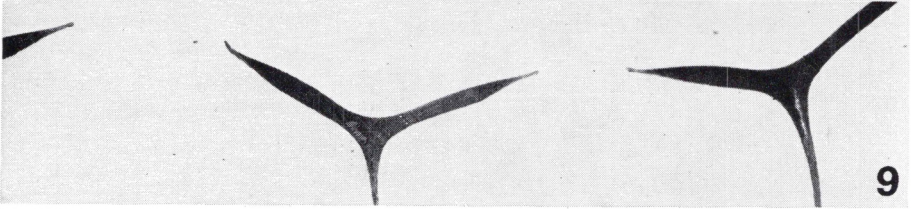
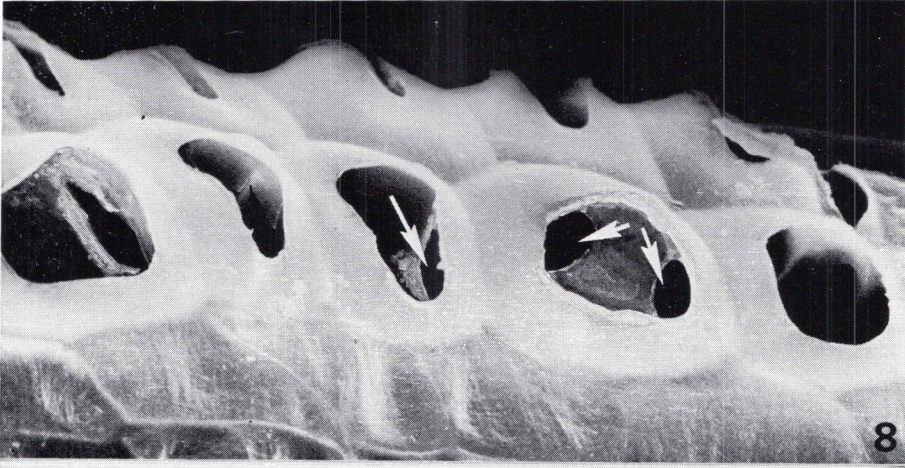
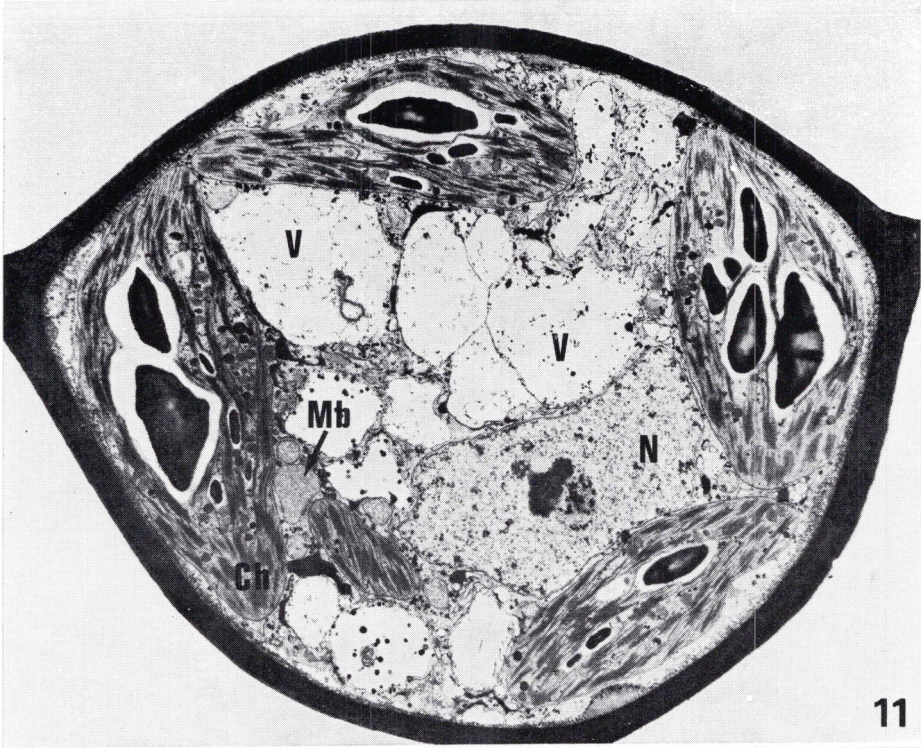


Plate IV

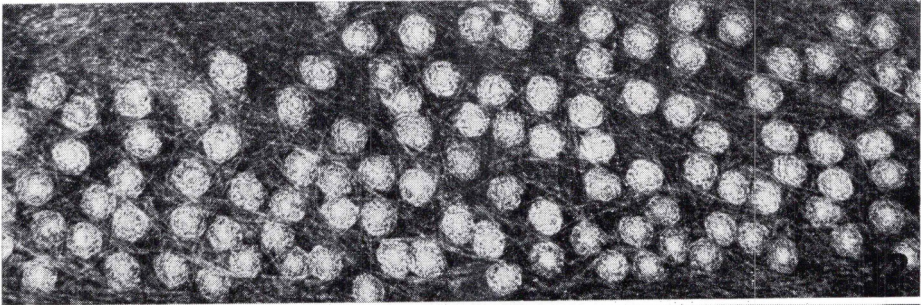
- Fig. 11. - The chlorocysts of the leaf tip have a rather dense cytoplasm and contain several vacuoles (V) with abundant electron-dense precipitates. The chloroplasts (Ch) are rich in starch deposits and plastoglobules. Microbodies (Mb) associated with chloroplasts and mitochondria are commonly present in these cells. They are probably peroxisomes. Nucleus (N). x 10,000.
- Fig. 12. - The transverse walls between adjacent chlorocysts are pierced by a great number of plasmodesmata, each contoured by a light fibrillar sheath. x 34,000.
- Fig. 13. - The chlorocysts at the middle of the leaf are larger in diameter than those at the leaf tip, and usually contain less dense cytoplasm. Arrows indicate plasmodesmata on a longitudinal septum. x 3000.



Plate IV



11



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Plate V

- Fig. 14. - The chlorocysts at the leaf base have a little dense cytoplasm. Their chloroplasts (Ch) are almost entirely filled with starch. Like in the upper regions of the leaf, neighbouring chlorocysts of the base may come into contact with one another along their longitudinal walls. These walls, however, always lack plasmodesmata and are sometimes coated with an additional layer on their protoplasmic side (arrows). x 3000.
- Fig. 15. - At high magnification that coat shows a loose fibrillar structure quite different from that of typical chlorocyst walls. x 10,000.
- Fig. 16. - As a rule the lipid inclusions (L) in the chlorocysts of the leaf base are intimately associated to microbodies (Mb). x 15,000.

Plate V

