

ULTRASTRUCTURE OF THE SHOOT APEX OF *CHENOPODIUM ALBUM* AND CERTAIN OTHER SEED PLANTS

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ABSTRACT

The ultrastructure of cells of the vegetative shoot apices is described for *Chenopodium album*, *Kalanchoë blossfeldiana* and *K. laxiflora*, *Bryophyllum daigremontianum*, *Nicotiana rustica*, and *N. tabacum* (Maryland Mammoth), and *Ginkgo biloba*. A less intensive study was made of the last three listed. The structures and organelles usually associated with meristematic cells were observed: dictyosomes, plastids (in various stages of development), mitochondria, endoplasmic reticulum (ER), vacuoles, lipid droplets, and plasmalemma. In addition, spherosome-like structures were observed in all zones of the shoot apices. Also, multivesicular bodies were observed in *C. album* and *B. daigremontianum*. Ribosome density is greater in cells of the flank meristem. Proplastids, plastids with prolamellar bodies, or grana have a differential distribution in the apex, characteristic for a particular species. Confirmation could not be given to the concept that vacuoles arise as a series of local dilations in long extensions of the so called "smooth ER." The tonoplast and ER are distinguishable at the time of inception of a vacuole, although the tonoplast may arise from the ER. Rapid growth of a vacuole and/or fusion with other vacuoles may result in irregularly shaped pre-vacuoles. No vacuoles were observed to originate from cisternae of dictyosomes in the species studied.

INTRODUCTION

The volume of literature on the histology of shoot apices is large, and the major controversial issues regarding structure have been reviewed recently in some detail (Clowes, 1961; Cutter, 1965; Nougarede, 1965). Several studies on the ultrastructure of meristematic cells have appeared, but most of these have pertained to investigations of specific organelles, rather than to an analysis of cell structure of the entire shoot apex. A significant body of literature concerning the ultrastructure of the meristematic cell, particularly that of the root, has been contributed by Whaley and his associates (Whaley, Mollenhauer, and Leech, 1960; Whaley, Kephart, and Mollenhauer, 1964), and that of the shoot, by Buvat (1958), Lance (1958), and Bowes

(1965 *a, b*). The endoplasmic reticulum, dictyosomes, and mitochondria are basic components of meristematic cells. Proplastids are identifiable in shoot and root apices, but some investigators do not consider the distinction between proplastids and mitochondria to be clear, although the two structures undoubtedly have their own origin and lineage (Frey-Wyssling and Mühlethaler, 1965). Lance (1958) related the specific structure of mitochondria to the particular stage of development of meristematic cells. According to Lance, an elaborate internal organization is related to a more specialized role of a meristematic cell, e.g. the cell of a young leaf primordium. Some authors have described a stage of plastid development in meri-

stematic cells of the bud in which a prolamellar body or primary grana can be seen (e.g., *Chlorophyton*: Strugger, 1953; *Agapanthus*: Strugger, 1954). Buvat (1958) and Lance (1958) were unable to demonstrate prolamellar bodies in meristematic cells of the shoots of *Elodea* and *Chrysanthemum*, respectively. It is now known that prolamellar bodies are formed in the absence of light (Frey-Wyssling and Mühlethaler, 1965). However, it should be profitable to examine young plastids in various known zones of the shoot apex and in leaf primordia. The penetration of light into the shoot apical region and young leaf primordia should not differ significantly in many plants.

The process of vacuole formation, although described in some detail by several investigators, has not been resolved completely. One group of investigators considers the vacuole to be surrounded by a membrane, from the time of vacuole inception. Buvat (1957, 1958) originally described vacuoles as originating through local differentiation of the endoplasmic reticulum (ER). Although ribosomes do not occur on that portion of the ER (smooth type) which is reported to form a vacuole, and although the latter membrane is only rarely observed in continuity with the "rough" ER, Buvat and Mousseau (1960) and others (Poux, 1961, 1962 *a*, 1962 *b*; Lance, 1958) have held to the original concept. Marinos (1963) described the formation of vacuoles in barley shoot apex as occurring through the enlargement of entire intramembranous spaces of dictyosome cisternae. Increase in surface of the tonoplast is described as being brought about by the formation of narrow

protrusions or extensions of the tonoplast. These descriptions stand in contrast with the concept of Mühlethaler (1960) who described vacuole formation in the root meristem as consisting of a localized hydration of plasma proteins without any relation to preexisting structures or membranes. In non-meristematic cells (hair cells) of *Glechoma*, Bowes (1965*b*) reports the formation of vacuoles from the nucleoplasm and modified mitochondria or plastids.

MATERIALS AND METHODS

Chenopodium album, *Kalanchoë blossfeldiana*, *Kalanchoë laxiflora*, *Bryophyllum daigremontianum* were grown in the greenhouse under long-day conditions so that we could assure that the plants remained in a vegetative condition. *K. laxiflora*, presumably a day neutral species, did not flower under these conditions. The normal day-length was extended by light from incandescent bulbs so that the plants were exposed to 16 hr of light and 8 hr of darkness daily. Shoot apices of *Chenopodium album* were collected from vegetative plants, 8-14 in. in height, and from small plants, 2-4 in. in height, corresponding to Stage II of Gifford and Tepper (1962 *b*). The apices from the other herbaceous species were taken from mature vegetative plants ranging from 9 to 16 in. in height. Apices of *Ginkgo biloba* were taken from dwarf shoots, both in winter and during the flush of growth in the spring.

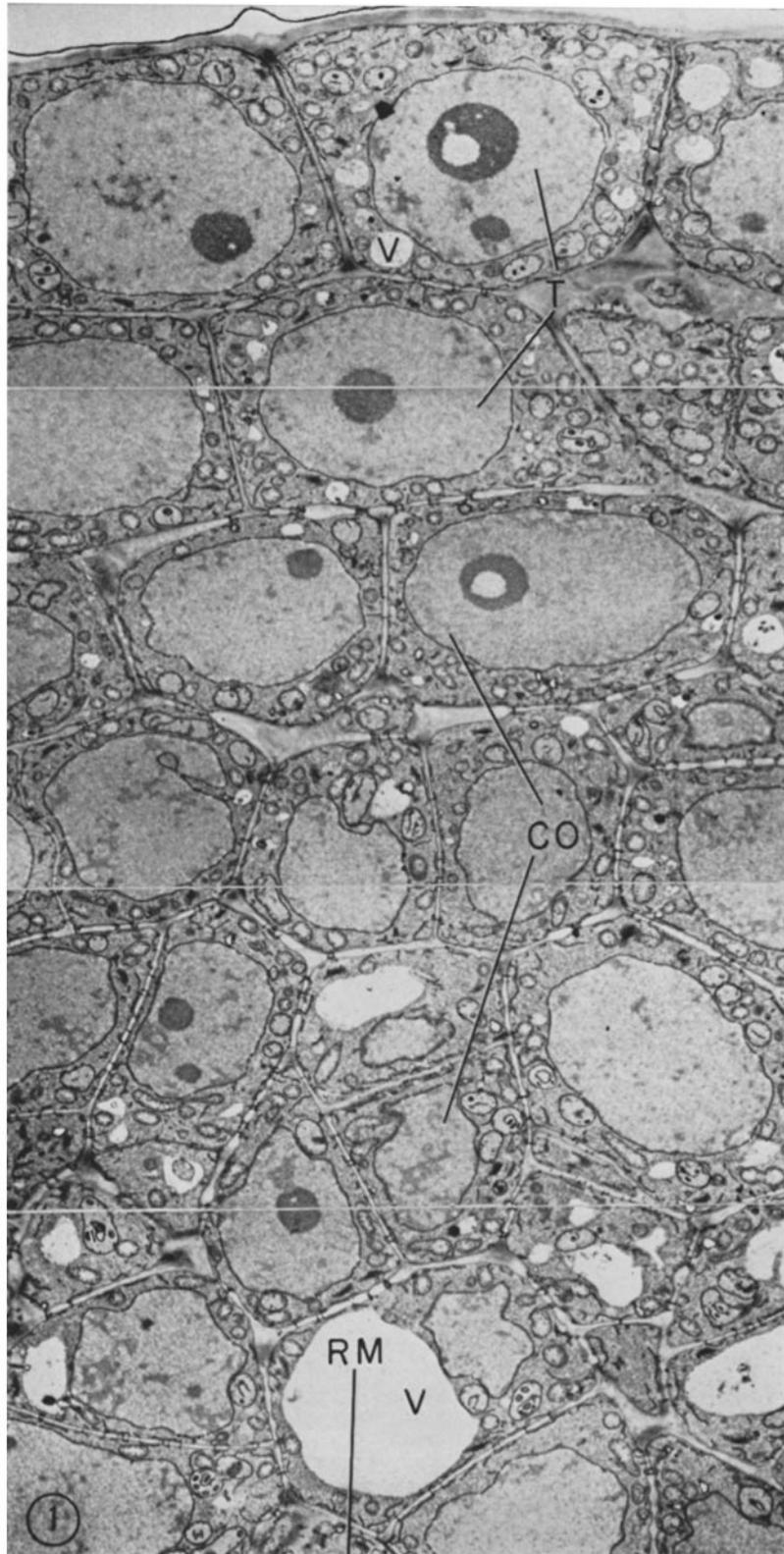
In all cases, whole apices with two to six of the youngest leaf primordia still attached were removed for fixation. Further dissection usually causes severe mechanical damage to the cells of the shoot apex. Root tips of *Chenopodium album* also were fixed.

The shoot and root apices were fixed either in cold (ice bath) 1% OsO₄ in 0.1 M phosphate buffer at pH 6.8 or in cold, unbuffered 2% KMnO₄. The

Abbreviations

<i>CO</i> , corpus	<i>PH</i> , phenolic body or phenolic substance
<i>CW</i> , cell wall	<i>PP</i> , proplastid
<i>ER</i> , endoplasmic reticulum	<i>RM</i> , rib meristem
<i>L</i> , lipid droplet	<i>ST</i> , starch
<i>M</i> , mitochondrion	<i>T</i> , tunica
<i>N</i> , nucleus	<i>TO</i> , tonoplast
<i>NE</i> , nuclear envelope	<i>V</i> , vacuole
<i>NU</i> , nucleolus	

FIGURE 1 Composite micrograph of median longitudinal section through shoot apex of *Chenopodium album* showing cells of tunica layers (*T*), corpus (*CO*), and rib meristem (*RM*). Note presence of vacuoles (*V*) in all cells (clear areas in cytoplasm) and development of large vacuoles in cells of rib meristem. Details of representative cells shown in other figures. Glutaraldehyde-KMnO₄. × 3,700.



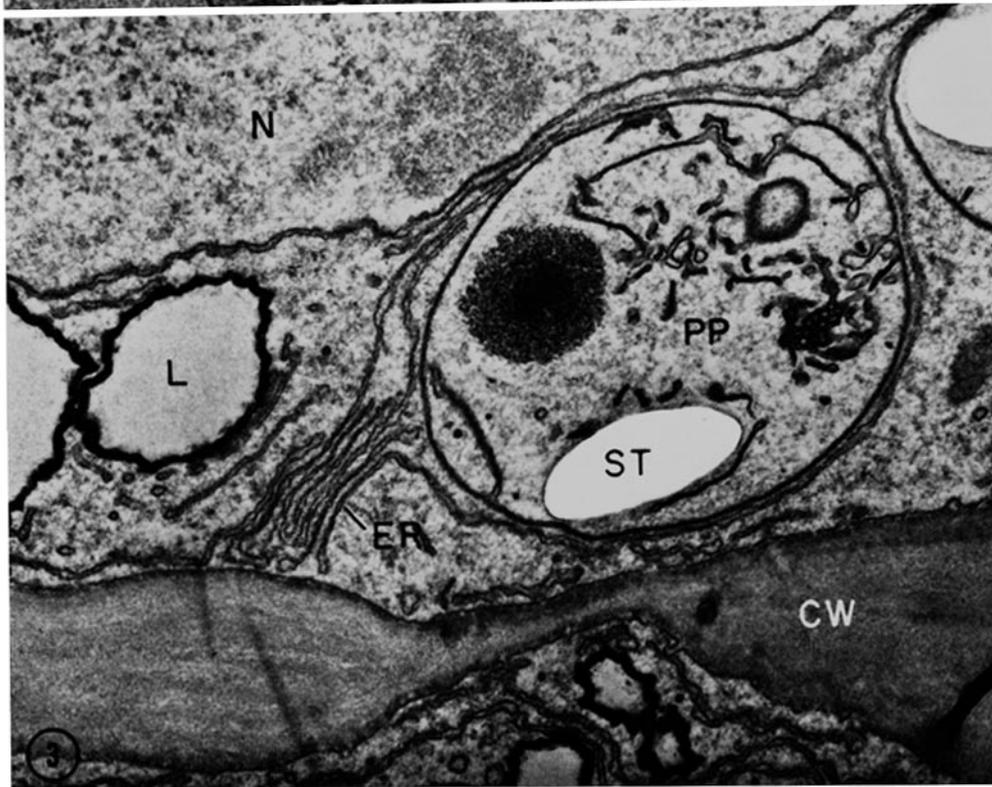
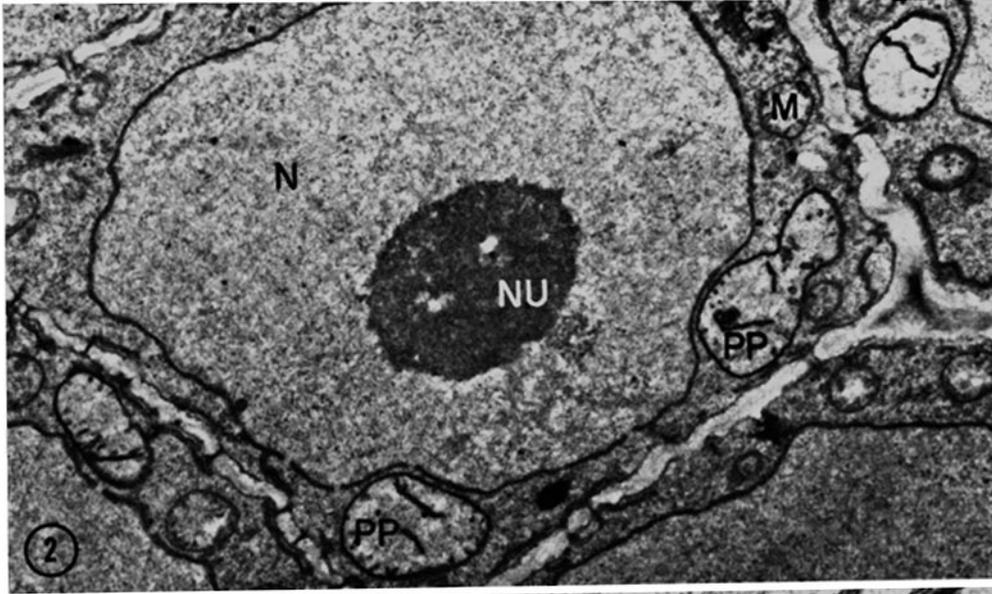


FIGURE 2 Cell from corpus region of *Chenopodium album* showing proplastids (PP) with minimum development of internal membranes. Glutaraldehyde-KMnO₄. $\times 15,000$.

FIGURE 3 *Ginkgo biloba*. Portions of two adjacent cells from the shoot apex in which cisternae of endoplasmic reticulum (ER) surround a proplastid. KMnO₄. $\times 32,000$.

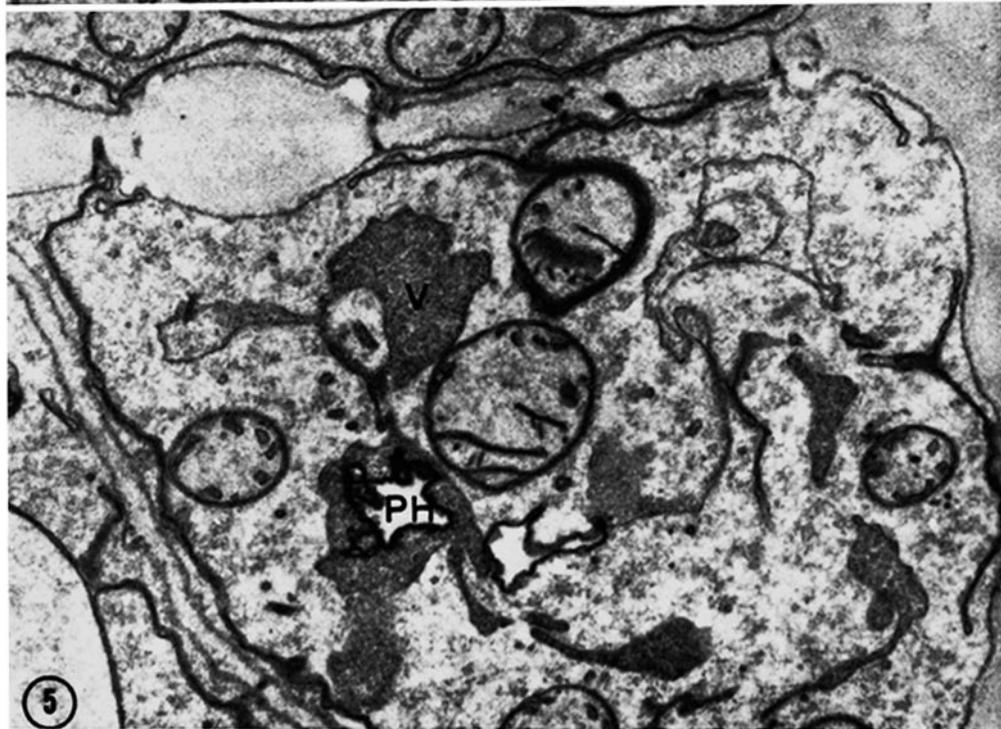
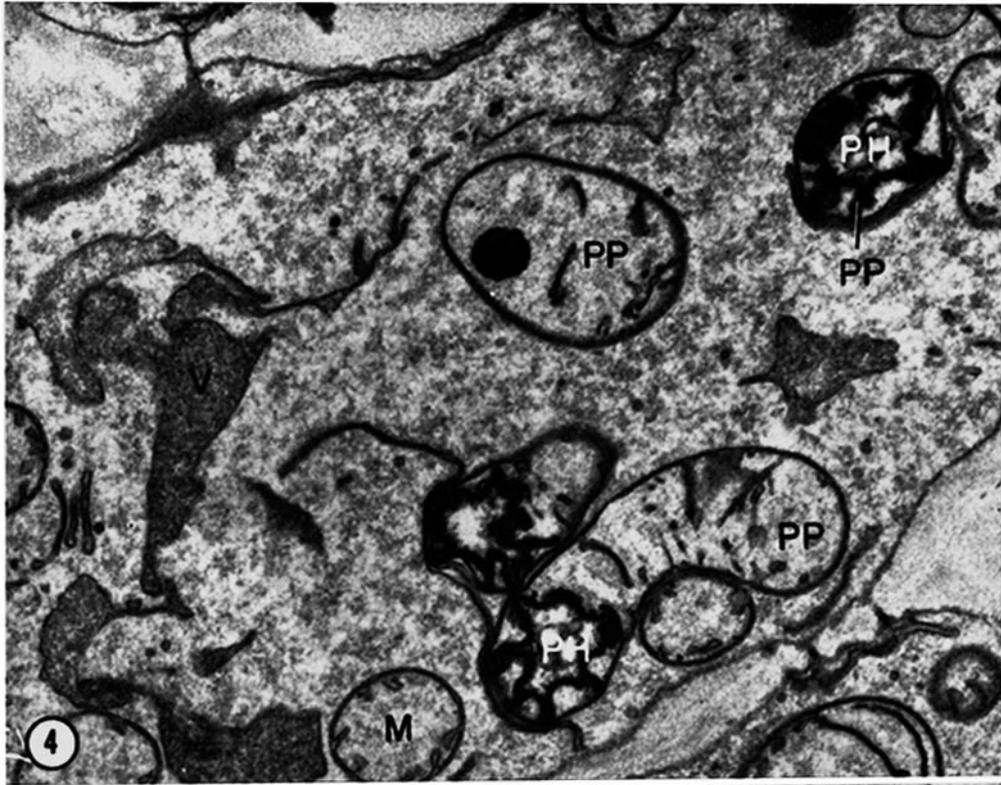


FIGURE 4 Portion of cell from corpus of *Bryophyllum daigremontianum* showing presence of phenolic bodies (PH) in proplastids (PP). KMnO_4 . $\times 31,000$.

FIGURE 5 Corpus cell of *Bryophyllum daigremontianum* showing irregularly shaped vacuoles (V) and presence of phenolic substance (PH) in a vacuole. KMnO_4 . $\times 30,500$.

OsO₄ and KMnO₄ solutions were used both as single killing and fixing agents or as a postfixation after killing with cold 4% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8.

In all cases, the material was dehydrated in a graded series of ethanol and, after being treated with propylene oxide, was embedded in 70% Maraglas-30% cardolite.

The blocks were sectioned with a diamond knife, and the sections were collected on 100- or 200-mesh copper grids coated with carbon-strengthened Formvar. The material was examined with a Hitachi HU-11 electron microscope operated at 75 kv.

OBSERVATIONS

General Organization and the Shoot Apex of Chenopodium album

Zonation of the vegetative shoot apex of *Chenopodium album* changes during ontogeny when grown under conditions unfavorable to flowering (Gifford and Tepper, 1962 *b*). The apex of a young plant (Stage II, 17–21 leaves having been produced; Gifford and Tepper, 1962 *b*) exhibits a definite cytological zonation, in that the central region of the apex stains lightly with pyronin for the identification of ribonucleic acid (RNA). Zonation becomes less obvious in older plants, and the nucleoli of the axial tunica cells increase in size and are larger than those in other regions of the apex. This change in apical structure is comparable to that described by Nougarede, Gifford, and Rondet (1965) for *Amaranthus retroflexus* grown under photoperiods unfavorable for flowering. The apex of such a plant has been termed "intermediate" by the last cited authors. The apex is "intermediate" in the sense that the organization is intermediate between juvenile stages and flowering. Fig. 1 represents a comparable stage for *Chenopodium album*. There is considerable uniformity of cell organization throughout the apex, except

for large vacuoles in the upper part of the rib meristem (Fig. 1) which will form the pith. The structures and organelles usually associated with meristematic cells were observed: dictyosomes, plastids (in various stages of development), mitochondria, endoplasmic reticulum, vacuoles, lipid droplets, and plasmalemma. Contrary to some reports (Frey-Wyssling and Mühlethaler, 1965) that vacuoles do not occur in meristematic cells, we have never observed meristematic cells that did not possess vacuoles or "prevacuoles," although perhaps small in size and number.

Development and Structure of Plastids

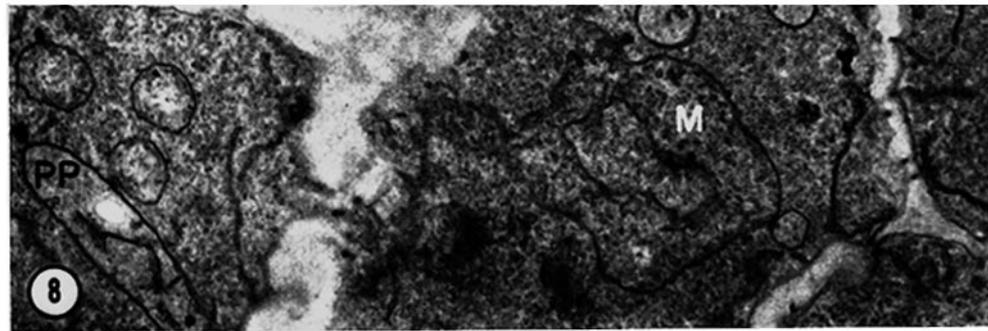
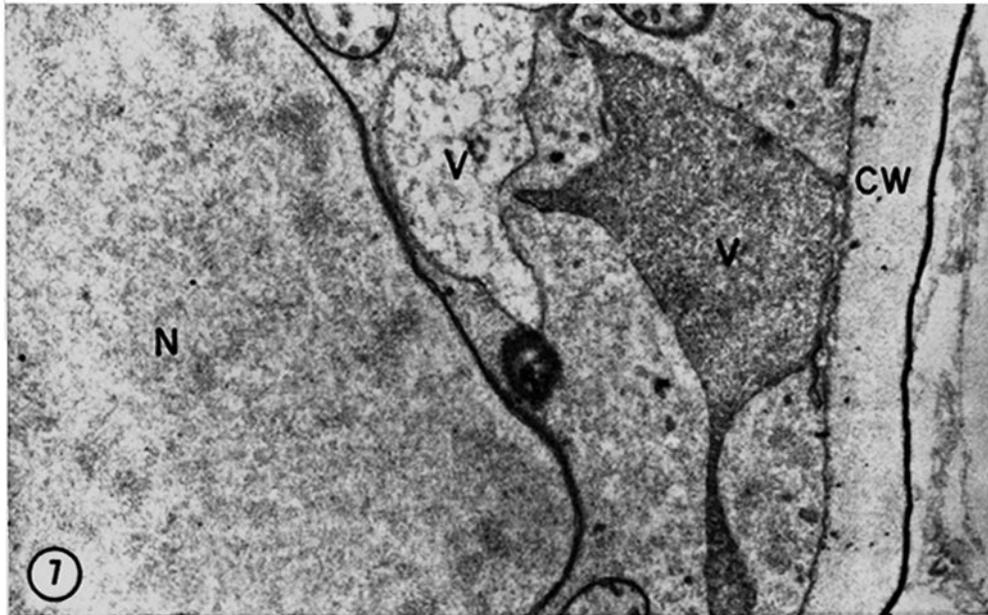
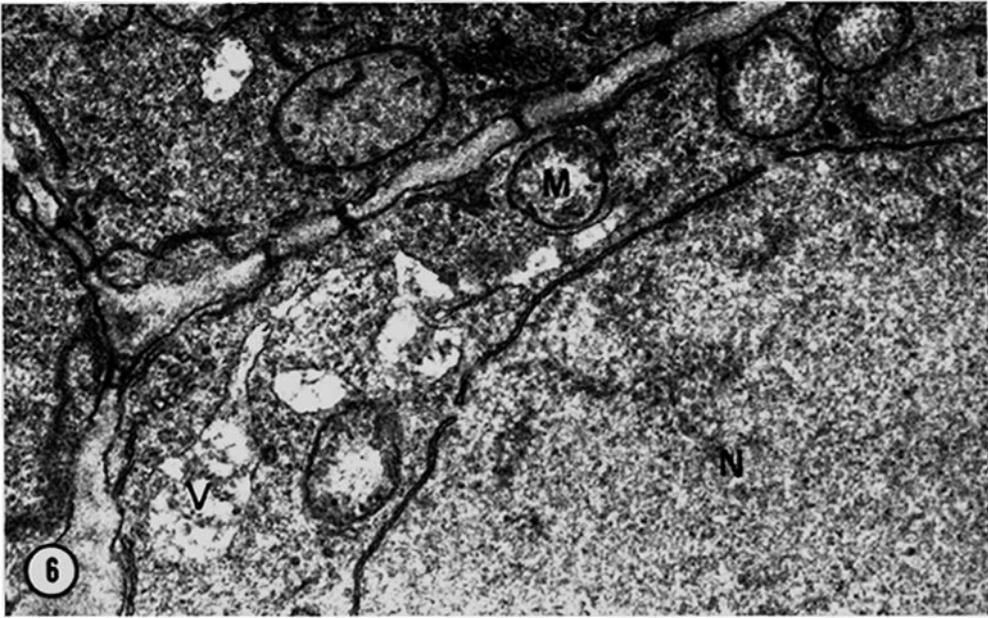
Proplastids with little development of internal membranes are present in the upper central portion of the apex in *C. album* and *Nicotiana rustica* (Fig. 2). In the flank and pith rib-meristems, prolamellar bodies and subsequently grana make their appearance in plastids. Plastid development (formation of prolamellar bodies and grana) in cells of the first tunica layer (T₁) in *Nicotiana rustica* lags somewhat behind that in T₂ and the underlying cells of the flank meristem. In *Bryophyllum daigremontianum* and *Kalanchoë blossfeldiana* the plastids remain as proplastids in the apex and in leaf primordia that are from 0.5 to 0.7 mm in height. Plastids form starch in cells of all of the apices studied, and in *C. album* this is the only definable inclusion. In addition to starch, the plastids of *K. blossfeldiana* and *laxiflora* and of *B. daigremontianum* contain phenolic substances (darkly staining single membrane bodies with KMnO₄ fixation; Fig. 4) as determined by histochemical tests (formaldehyde-ferric chloride fixation; Jensen, 1962). The phenolic substances are first produced in proplastids and then become transferred, presumably, to vacuoles. This sequence will be discussed in detail in a forthcoming publication.

Plastids appear to divide by constriction, except

FIGURE 6 Portions of three cells from corpus of shoot apex of *Chenopodium album* showing electron-transparent vacuoles (V) and "prevacuoles" after preliminary fixation in glutaraldehyde followed by treatment with KMnO₄. × 24,000.

FIGURE 7 Portion of outer tunica cell, shoot apex of *Bryophyllum daigremontianum*, showing the presence of both electron-opaque and electron-transparent vacuoles following KMnO₄ fixation. × 26,000.

FIGURE 8 Curved, filiform mitochondrion (M) from cell of second tunica layer along median axis of shoot apex of *Chenopodium album*. Glutaraldehyde-KMnO₄. × 18,000.



for *Ginkgo*. The mode of division in *Ginkgo* is uncertain, but some micrographs provide evidence for division of plastids by an inward growth of the inner membrane of the plastid envelope.

Endoplasmic Reticulum

The endoplasmic reticulum (ER) is associated invariably with ribosomes in the meristematic cells of the shoot apex (O_2SO_4 fixation). With $KMnO_4$ fixation, the ER can be seen as a continuation of the double nuclear envelope or as an extension of the outer membrane of the double nuclear envelope. The two types may be seen in cells of the same apex and occasionally in the same cell, although the outer nuclear membrane connection is, by far, the most common condition. The ER is occasionally observed in connection with the tonoplast.

In interphase, most of the ER occurs as lamellae lying parallel to, and in close proximity to the plasmalemma. During mitosis, the ER is more generally distributed in the cell and is usually seen as rather short lengths in sectional view. There is no recognizable nuclear envelope, as distinguished from ER, at metaphase.

Continuous with the ER, the plasmodesmata are distributed evenly and singly over the cell walls of *C. album*, but the strands are aggregated into circular groups (as seen in face view) in *Kalanchoë* and *Bryophyllum*.

In the shoot apex of *Ginkgo*, the plastids often are surrounded by 1 or 2 cisternae of ER (Fig. 3). Also, dictyosome-like stacks of ER occur in cells of the apex of *Ginkgo*, and the dictyosomes proper are often loosely organized so that it is difficult (in $KMnO_4$ -fixed material) to distinguish between ER and dictyosomes, in many cases. The dictyosome-like stacks of ER suggest two possibilities: (1) dictyosomes may be formed by the ER or (2) these profiles of ER are an indication of some special secretory function of the ER apart from that of the dictyosomes. The endoplasmic reticulum that surrounds plastids is usually seen in connection with dictyosome-like stacks of ER. This relationship is reminiscent of the plastid-encircling ER in the secretory resin cells of *Pinus pinca* (Wooding and Northcote, 1965).

Mitochondria

Mitochondria of the shoot apex of *Chenopodium album* are usually rod-shaped, and usually in the distal cells they are somewhat larger in diameter,

greater in length, and more curved or bent than in other cells of the apex (Fig. 8). This observation corroborates a light microscope investigation of *C. album* (Gifford and Tepper, 1962 a) and of other plants (Lance, 1957). Mitochondria appear to multiply by constriction and division of preexisting ones. No promitochondria (as described by Frey-Wyssling and Mühlethaler, 1965) have been observed or recognized as such in the shoot and root apices of *C. album*. Furthermore, no promitochondria have been observed in cells of the embryonic shoot apex of ungerminated seeds of *Nicotiana tabacum* or in these cells when the seed is germinated. There is no evidence for de novo development of mitochondria in somatic meristematic cells in those plants investigated in this study.

Vacuoles

In meristematic cells, very small vacuoles are angular and elongate in sectional view and have densely staining contents after $KMnO_4$ fixation (Fig. 4). Whaley (1960) refers to these as prevacuolar bodies. In *C. album*, both the prevacuolar bodies and the large vacuoles in tunica layers T1, T2 and in the flank regions have uniformly darkly staining contents when $KMnO_4$ is used as the sole fixing agent. When glutaraldehyde is used as a preliminary killing solution before $KMnO_4$, both types of vacuoles are more electron transparent with some densely staining material occurring as scattered particles (Fig. 6).

In *B. daigremontianum*, the small prevacuolar bodies have densely staining contents after $KMnO_4$ fixation, and the large prevacuoles or vacuoles may contain densely staining material or may be electron transparent; both types occur in the same cell and throughout the apex (Fig. 7). If comparable material is prefixed in glutaraldehyde prior to staining with $KMnO_4$, the vacuoles are more apparent and usually spherical, and perhaps are more representative of the true form of the vacuoles (Fig. 9).

In *Nicotiana rustica*, $KMnO_4$ -fixed material contains dark prevacuolar bodies and large vacuoles that may be electron opaque or electron transparent. The electron-transparent vacuoles (usually circular in sections) are found at the tip of the apex (in a zone five to seven cells broad and two or three cells deep). Small prevacuolar bodies occur in these cells also. The electron-opaque, larger vacuoles occur in the flanks of the apex, along with

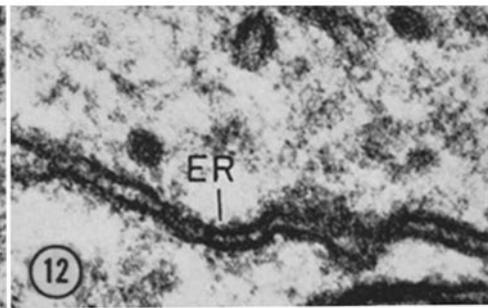
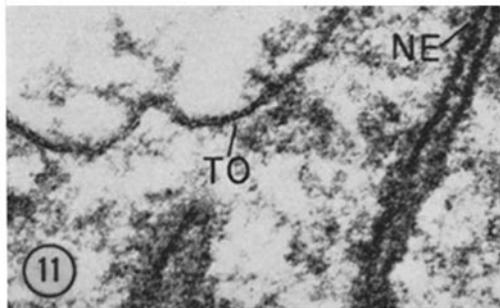
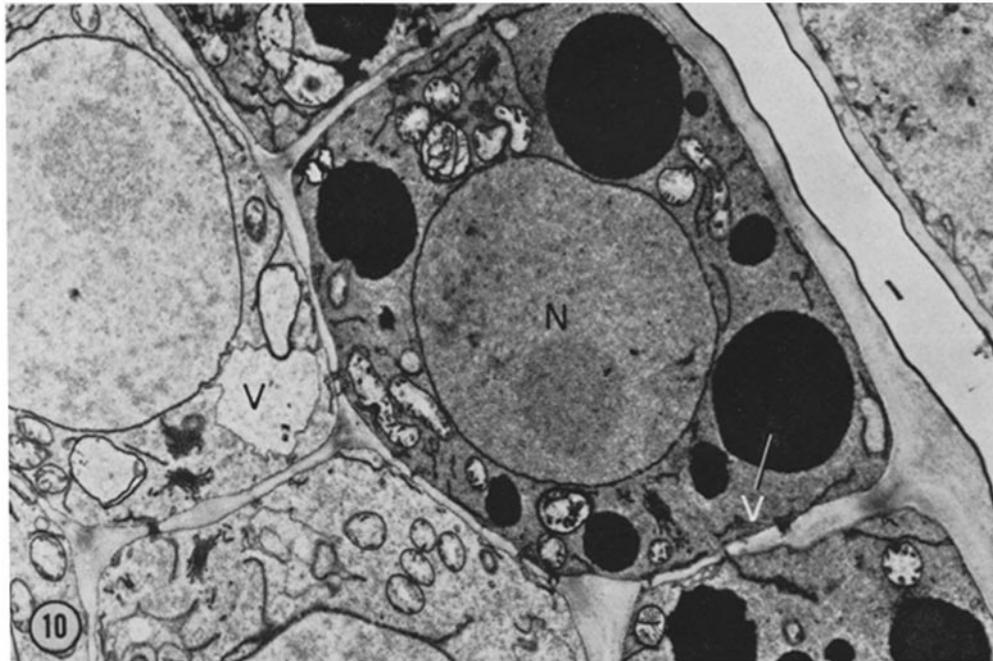
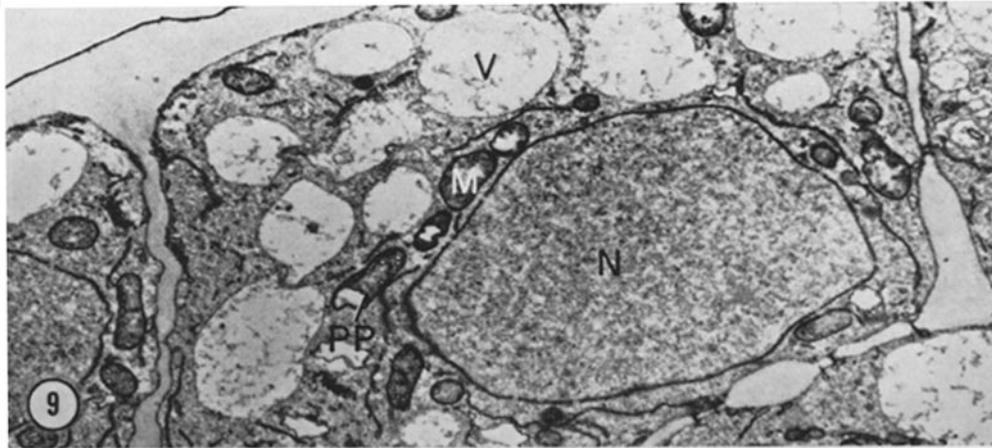


FIGURE 9 Portions of adjacent outer tunica cells of the shoot apex of *Bryophyllum daigremontianum*. Material was prefixed in glutaraldehyde followed by KMnO_4 . Under these conditions all vacuoles tend to be spherical; compare with Fig. 10. $\times 11,000$.

FIGURE 10 Portions of cells of the tunica layers of *Kalanchoë blossfeldiana* following fixation with KMnO_4 . The density of vacuoles in cells of the outer tunica layer is due, presumably, to the presence of an anthocyanin pigment. $\times 8,000$.

FIGURE 11 Tonoplast and segment of nuclear envelope. Unit structure of tonoplast is visible. glutaraldehyde- KMnO_4 . $\times 110,000$.

FIGURE 12 The ER from same micrograph negative as Fig. 11. $\times 110,000$.

small prevacuolar bodies. In contrast to the electron-transparent vacuoles, the large, dense vacuoles are usually somewhat angular and elongated in sectional view. It is possible that the large, dark, angular vacuoles represent vacuoles that are actively enlarging and fusing with other vacuoles arising from prevacuolar bodies, and that the electron-transparent, spherical vacuoles at the top represent a less active condition. Vacuoles in cells of the pith rib-meristem soon lose their electron-opaque contents (perhaps by dilution of darkly staining material).

Aside from the electron-opaque contents present in the prevacuolar bodies, some (but not all) of the larger vacuoles in the apex of certain species, such as *Pharbitis nil* (Healy, 1964) and *K. blossfeldiana*, contain extremely electron-opaque contents (Fig. 10). The nature of this vacuolar substance has not been demonstrated conclusively, but it is likely that the substance is anthocyanin. Although such vacuoles do not appear to be strictly localized in *Pharbitis nil*, they are usually restricted to the outer tunica layer in *K. blossfeldiana*. During fixation, the tonoplast of these dense vacuoles is often ruptured, and the loss of vacuolar contents causes a part or all of the ground plasma to be stained darkly, almost black. In *K. blossfeldiana*, the ground plasma of the outer tunica layer is almost uniformly darker than that of other cells of the apex (Fig. 10). Bowes (1965 *a*) reports this condition in the single tunica layer of *Glechoma hederacea* and speculates that the density of the ground plasma here is a result of the high protein content of these cells. It is quite possible that the dense contents in the cells of the tunica in *Glechoma hederacea* represent the result of ruptured anthocyanin vacuoles, for no such differentially staining cells have been observed in any of our plants except those in which this type of vacuole occurs.

In addition to the dense substances found in the vacuoles of some species, the vacuoles of *K. blossfeldiana*, *K. laxiflora*, and *B. daigremontianum* contain darkly staining bodies (with KMnO_4 fixation) that have been identified as phenolic substances (Fig. 5). The phenolic substances are first produced in the proplastids in cells of the apex and then presumably are transferred to the vacuoles (Fig. 4). This is to be discussed more fully in a separate publication.

Other Organelles and Structures

The results of most histochemical studies on vegetative shoot apices, with light microscopy, have shown that cells of the flank meristem and pith rib-meristem tend to stain more intensely with pyronin and azure B; this condition is indicative of a high concentration of RNA (Lance, 1957; Gifford and Tepper, 1962 *a*; Healy, 1964; and others). The electron micrographs support these observations, in that free ribosomes have a greater density per unit area in the regions mentioned above than in other cells of the shoot apex.

Lipid droplets have been observed in cells of all shoot apices examined; they do not appear to show a preferential distribution.

Spherosome-like organelles limited by a single membrane have been observed in cells of all zones of the shoot apex.

Multivesicular bodies similar to those reported by Jensen (1965) in the nucellar cells of cotton have been observed in cells of the shoot apices of *C. album* and *B. daigremontianum*. Multivesicular bodies were not observed, however, in cells of the root tip in *C. album*. Such multivesicular bodies have been reported in the outer root cap cells of maize (Mollenhauer, Whaley, and Leech, 1961), but we are not aware of their existence in the meristematic cells of the root tip in any investigated species.

We have no new information on structure, function, or occurrence of dictyosomes that has not been discussed extensively in the literature on electron microscopy of the root tip (Whaley, Mollenhauer, and Leech, 1960).

DISCUSSION

Despite the demonstrated effects of the presence or absence of light on the internal development of plastids, attention should be given to development of these organelles during their normal ontogeny in plant meristems. In *C. album* and *Nicotiana rustica*, plastids develop grana within cells of the apex itself (cells of the flank meristem and pith rib-meristem), but in *B. daigremontianum* and *K. blossfeldiana* the plastids remain as proplastids even within young leaf primordia. The buds of *Kalanchoë* and *Bryophyllum* are quite open as compared with those of *Chenopodium* or *Nicotiana*, and no doubt receive as much or more light than the apex and young leaves of the latter two genera. The condition in *Bryophyllum* and *Kalanchoë* is perhaps an indication that, although prolamellar bodies and

grana develop in response to darkness and light, respectively, they are susceptible to these stimuli during normal development only at certain developmental stages of tissues of a specific plant. Plastid development and specialization may be controlled primarily by position in the plant rather than by a simple correlation with the presence or absence of light.

Other investigators have related vacuole origin in meristematic cells to dictyosomes (Marinos, 1963), endoplasmic reticulum (Buvat, 1957, 1962), or to a type of de novo origin in the cytoplasm (Mühlethaler, 1960). We have never observed or been able to associate vacuole formation with dictyosomes in the material investigated. We agree with Buvat (1962) and Poux (1962 *a*) that the origin of vacuoles is intimately associated with the ER, but disagree with the latter authors on the general sequence of development. According to Buvat (1965) and Poux (1961, 1962 *a*), vacuoles are the result of *local* dilations of ER, resulting, in some cases, in a concatenate series of vacuoles. Dilations occur in ER cisternae which result in vacuole formation. The above authors report a system of membranes which resembles the ER but lacks ribosomes (so called smooth ER). Fine prolongations from vacuoles are reported to be indicative of ER continuity, but the above authors admit that these prolongations are only rarely observed in continuity with ER even though they often are contiguous. The concept of rough ER (associated with ribosomes) and smooth ER (lack of associated ribosomes) is fairly well entrenched in the literature on ultrastructure, but we propose that the term ER should be restricted to the rough type for meristematic cells of plants. The so called smooth type in meristematic cells represents, in our opinion, the tonoplast already associated with a definable vacuole, however small and narrow in profile. We agree with Manton (1962) that, once a vacuole is formed, the tonoplast could grow rapidly and thus could account for the presence of small vacuoles with tonoplast profiles that

closely approach each other. Healy (1964) was unable to relate vacuoles with ER in *Pharbitis nil*. Also, vacuoles with fine extensions could be the result of partial collapse of a vacuole during fixation (cf. appearance of vacuoles in Figs. 4 and 9). The material of Manton (1962, young sporophytes of *Anthoceros*) would appear to show the result of partial collapse of vacuoles.

The tonoplast is a membrane similar in appearance to the plasmalemma (Frey-Wyssling and Mühlethaler, 1965). In our KMnO₄-fixed material, the tonoplast resembles the plasmalemma in appearance and the ER membranes resemble the membranes of the nuclear envelope. The unit structure of the tonoplast (Fig. 11) and plasmalemma is easily visible at relatively low magnifications. The membranes of the ER, however, are coarser and more difficult to resolve into unit membranes (cf. Figs. 11 and 12, which are parts of the same micrograph negative). In most cases, the tonoplast is distinguishable from the ER membranes in KMnO₄-fixed material because of its different appearance, and it is distinguishable in OsO₄-fixed material because ribosomes are associated with the true ER membranes. We have not identified smooth ER as separate from rough ER in the meristematic cells of the shoot apex. Although occasionally the ER membranes are observed to be continuous with the tonoplast, we must disagree with Poux (1962 *a*) and Bowes (1965 *b*) who state that the membranes surrounding developing vacuoles are indistinguishable from ER membranes. Close examination of our material reveals that two closely lying, parallel membranes leading to and connected with the tonoplast, or occurring between two vacuoles, have the appearance of the tonoplast and not of ER. We do agree with Poux (1962 *a*), however, that new membranes can form during vacuolation of the cell and that the ER undoubtedly presents variable patterns in cells of different plants as cited by that author.

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