Phyton (Horn, Austria)	**Vol. 41**	Fasc. 2	227–245	28. 12. 2001

The Ultrastructure of Mucilage Secreting Trichomes of *Zebrina pendula* SCHNIZL.

By

Irmtraud THALER, Manfred GAILHOFER and Günther ZELLNIG*)

With 19 Figures

Received June 18, 2001

Key words: *Zebrina pendula, Commelinaceae*, mucilage secreting trichomes, rough endoplasmic reticulum, Golgi apparatus, protein bodies.

Summary

THALER I., GAILHOFER M. & ZELLNIG G. 2001. The ultrastructure of mucilage secreting trichomes of *Zebrina pendula* SCHNIZL. – Phyton (Horn, Austria) 41(2): 227–245, with 19 figures. – English with German summary.

The glands of *Zebrina pendula (Commelinaceae),* consisting of a foot-, a stalk- and a club formed mucilage secreting head cell, were investigated by using light and transmission electron microscopy. As common in specialized cells with mucilage, containing polysaccharides and protein as components, a very close association of highly developed rough endoplasmic reticulum and the Golgi apparatus is of great importance. Both organelles are involved in the secretion process. In the head cell of the gland of *Zebrina* apparently two different ways of protein transport occur. One is the common way from the rough endoplasmic reticulum with vesicles to the cis-face of the dictyosomes. The other way occurs in a later stage without the participation of the Golgi apparatus. Protein bodies seem to be transported to the vacuole with the help of actin microfilament bundels. In addition, protein crystals and mucilage can be found in the vacuole. Protein bodies might be formed inside "pseudovacuoles" or, if treated with cytochalasin B, also in the rough endoplasmic reticulum. Cytochalasin B also blocks the activity of dictyosomes and thereby the polysaccharide synthesis. The role of leucoplasts in the head cell remains unknown.

Zusammenfassung

THALER I., GAILHOFER M. & ZELLNIG G. 2001. Die Feinstruktur des Schleimhaares von *Zebrina pendula* SCHNIZL. – Phyton (Horn, Austria) 41(2): 227–245, 19 Abbildungen. – Englisch mit deutscher Zusammenfassung.

*) I. THALER, M. GAILHOFER and G. ZELLNIG, Institute of Plant Physiology, University of Graz, Schubertstr. 51, A-8010 Graz, Austria.

Dedicated to Prof. Dr. Otto Härtel on the occasion of his 90th birthday.

Das Schleimhaar von *Zebrina pendula (Commelinaceae)*, bestehend aus einer Fuß-, einer Stiel- und einer schleimbildenden, keulenförmigen Köpfchenzelle, wurde licht- und elektronenmikroskopisch untersucht. Wie in allen spezialisierten Zellen, deren Schleime Polysaccharide und Proteine enthalten, spielt die dichte Assoziation von rauhem endoplasmatischem Reticulum und dem Golgi Apparat eine wichtige Rolle. Beide Organellen sind am Sekretionsprozess beteiligt. In der Köpfchenzelle finden anscheinend zwei Wege des Proteintransportes statt. Der erste ist der übliche vom rauhen endoplasmatischen Reticulum mit Hilfe von Vesikeln zur cis-Seite der Dictyosomen; der zweite Weg findet später und ohne Beteiligung des Golgi-Apparates statt. Proteinbodies scheinen mit Hilfe von Mikrofilamentbündeln zur Vakuole zu gelangen. Man findet sie neben Eiweißkristallen und Schleim in der Vakuole. Proteinbodies können in „Pseudovakuolen" entstehen, aber bei Einwirkung von Cytochalasin B auch im rauhen endoplasmatischen Reticulum. Cytochalasin B hemmt deutlich die Aktivität der Dictyosomen und damit die Polysaccharidbildung. Die Rolle der Leukoplasten in der Köpfchenzelle bleibt weiterhin unbekannt.

Introduction

Glandular cells which are accumulating polysaccharides containing mucilage are commonly characterized by hypersecretory dictyosomes. The synthesis of mucilage occurs in the Golgi apparatus (GA). The endoplasmic reticulum (ER) in these cells is underdeveloped. The mature Golgi vesicles secrete the mucilage by fusion of the vesicle membrane with the plasma membrane (FAHN 1979, DEXHEIMER 1981, SCHNEPF 1993). Besides polysaccharides some glandular cells also contain proteins in their secretion products. A complicated network of rough endoplasmic reticulum (RER) occurs here, which is strongly associated with hypersecretory dictyosomes. This structural association is thought to be connected with the amount of proteins in the secretion (ROBINSON & KRISTEN 1982). Protein secreting tissue of the ligula of *Isoetes lacustris*, the glandular hairs of *Psychotria bacteriophila*, and *Veronica beccabunga* showed a distinct transfer of vesicles between ER and dictyosomes after a high level of protein occurred (KRISTEN 1980, DEXHEIMER 1981, KRISTEN & LOCKHAUSEN 1985, LOCKHAUSEN & KRISTEN 1986).

Mucilage trichomes of *Commelinaceae* which were first documented by STAUDERMANN 1924 are a characteristic feature of this family. The club formed mucilage head cell (MHC) of the hair of *Zebrina* contains an extensive RER membrane system which is closely associated with active dictyosomes. Both components are thought to be involved in secretory processes (THALER & al. 1986).

The lobed, large nuclei of the MHCs contain a DNA amount of 4C and 8C, and it can reach even higher values. The amount of DNA in MHC is always higher than in foot- and stalk cells (GUTTENBERGER 1990).

The present study documents some aspects of the secretion accumulation in the glands of *Zebrina pendula* and especially in the MHC. A

combination of light and electron microscopical methods were used to accomplish our investigations.

Material and Methods

Plant material

Zebrina pendula SCHNIZL. was cultivated in the greenhouse of the institute.

Light microscopy

Coomassie brilliant blue or mercury bromphenolblue were employed to detect protein in secretion material of the glands. Unfixed samples (segments) of the shoot surface were treated with the phosphate buffered (pH 7.5) fluorochrome acridinorange (1 : 10000) to stain vacuoles of the head cell of glands, with ruthenium red to stain pectic polysaccharides with acidic groups, with Sudan black B to stain lipids and with aniline blue to stain callose.

The lectins (Sigma Munich) BS-I (*Bandeira simplicifolia*), Con A (*Canavalia ensiformis*), PNA (*Arachis hypogaea*), SBA (*Glycine max*), WGA (*Triticum vulgare*) were used to detect glycoconjugates of glands. Segments of stems were fixed for 2 hours in 4% formaldehyde and rinsed with phosphate buffer (pH 7.4) followed by a 30 min treatment in lectins labeled with fluorescein isothiocyanate.

Samples were examined with a Leitz Orthoplan fluorescence microscope.

Electron microscopy

Stem segments were fixed in phosphate buffered 3% glutardialdehyde followed by 1% osmium tetroxide. After washing in phosphate buffer the material was dehydrated in an ethanol series and embedded in Spurr's medium. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Philips CM 10 electron microscope.

Pepsin digestion

Ultrathin sections collected in plastic rings were floated on 5% hydrogen peroxide solution for 30 min, washed in distilled water and incubated in 0.5% pepsin in 0.1 N HCl (Fluka AG) for 1–8 hours. After washing the sections were stained with uranyl acetate and lead citrate (cf. GAILHOFER 1983a).

3,3' – Diaminobenzidine (DAB) treatment

DAB medium contained 1 mg/ml 3,3'- diaminobenzidine.4 HCl (Serva, Heidelberg), 1.10^{-4} M 3-(3,4 dichlorophenyl)-1,1-dimethylurea (Serva, Heidelberg), 1 mM phosphate buffered (pH 7.2) sodiumazid (cf. NIR & SELIGMAN 1970, GAILHOFER 1983b). Glutaraldehyde fixed tissue was incubated in the DAB medium 60 min at room temperature and after washing in phosphate buffer (pH 7.2) postfixed in phosphate buffered 1% osmium tetroxide, dehydrated and embedded in Spurr's resin.

Cytochalasin B treatment

The incubation medium contained 50 µg/ml cytochalasin B (Sigma Munich) in 1% DMSO. Glands were incubated for 15, 30, 45 and 60 min in the medium, controls were incubated in 1% DMSO or in tap water followed by fixation and embedding for the electron microscope.

Staining for polysaccharides, PAT-Ag Prot-reaction (cf. THIÉRY 1967)

Ultrathin sections, collected in plastic rings, were floated for 20 min on 1% periodic acid before washing on water. Controls for the selectivity of the oxidation were performed by substituting the periodic acid treatment with 10% H_2O_2 for 20 min. This treatment does not specifically oxidize glycols, poysaccharides will not react. After oxidation and washing, the sections were floated at room temperature on 0.2% thiocarbohydrazide in 20% acetic acid (overnight), washed with an aqueous solution of acetic acid with decreasing concentration, washed with water, then floated on 1% aqueous silver proteinate for 30 min in the dark followed by 2 washes with water. Sections were mounted on grids for the electron microscope.

Abbreviations: CB cytochalasin B, ER endoplasmic reticulum, GA Golgi apparatus, MBB membrane bound body, MHC mucilage head cell, PB protein body, RER rough endoplasmic reticulum, TGN trans-Golgi network

Results

Light microscopical observations

The approximately 150 µm long mucilage hairs of *Zebrina* occur on shoots and on both sides of the leaves. They consist of one foot cell, one stalk cell and one club formed MHC which is double the length of the stalk cell (Fig. 1). The foot and the stalk cell are slightly bent so that the head cell lays parallel to the epidermis. Well preserved hairs can be found on the shoot underneath the leaf sheaths. All the other mucilage trichomes show a completely bent, degenerated MHC which remains on the stalk cell.

The MHC contains a large nucleus in the middle of the cell, which is surrounded by approximately 70 leucoplasts. Siphonal vacuoles can be observed in a yellow green fluorescence after treatment with acridin-orange. A strong cytoplasmatic streaming occurs even during the secretory process. The extraplasmatic space and the subcuticular space, which cover two thirds of the MHC, contain a granular substance. This substance can also be found outside the MHC. At the end of the secretory process the cytoplasm degenerates. Induced by the loss of substance, the protoplast lifts off the side wall on its thinnest place located shortly above the transverse wall of the stalk cell. The side wall cracks during this process.

After treatment with mercury bromphenolblue the leucoplasts, the nucleus and the secretions appear blue thus possibly representing proteins. The secretion can also be stained with ruthenium red which could be a sign of the occurrence of pectic polysaccharides. Lipids and callose can not be detected with Sudan black B and anilinblue.

Fixed hairs were treated with FITC conjugated lectins (Con A, BS-I, PNA, SBA and WGA). A strong green fluorescence was observed in the secretions, cell wall and the plasma membrane after the treatment with Con A. All the other lectins showed a negative reaction.

Electron microscopical observations

The cell wall of the MHC shows irregular invaginations in the upper third of the cell (Fig. 2). These invaginations are covered by the plasma membrane which results in an increase in surface area of the protoplast. RER occurs in parallel strands along the cell wall. Some elements lie close to the plasma membrane. In addition, accumulated RER was found inside the cell. It occurs approximately 5 µm underneath the top of the MHC and is always associated with organelles. Often vacuoles are covered with ER cisternae. The ER is not always found in its rough form. The ends of the ER cisternae are dilated and smooth (Fig.3). During degeneration of the cells the ER is enlarged. Dictyosomes are frequently completely surrounded by RER cisternae and a transfer of vesicles between ER and dictyosomes can generally be seen (Fig.4). Terminal ER cisternae are sometimes found lying close to the vesicles of the first cis-cisternae of the dictyosome (Fig.5). In most cases the dictyosomes consist of seven to nine cisternae, in few cases of eleven. They are clearly differentiated in cis-, medial- and trans-cisternae. The cis-cisternae are irregularly shaped, and their lumen shows the weakest contrasts, whereas the lumen of the medial cisternae show the strongest contrasts. The length of the trans-cisternae is a little larger than the length of the medial ones. The 8^{th} cisterna is frequently fragmented into vesicles. Intercisternal elements always occur except between the first and the second cis-cisternae (Fig.6). The vesicles at the cis-side are conspicuous large during their active stage and show no stainable content. Vesicles of the trans-side contain a granular filamentous content. These vesicles are able to fuse with each other and are associated with the fenestrated borders of the trans-Golgi. The trans-Golgi network (TGN) with its, tubulous vesicular structures can be easily distinguished from the trans-cisternae of the dictyosome. In most cases it is separated from the Golgi cisternae (Figs.6, 7). Coated vesicles are always associated with the TGN (Figs.3, 7). They also occur close to the membrane of the RER and at the plasma membrane.

During the late stages of development, after the dictyosomes have lost their activities, the cytoplasm of the MHC contains amorphous, spherical and highly contrasted structures with an average diameter of 0.5 µm. They are generally surrounded by a membrane with polysomes attached on the surface (Fig.10). These structures are suggested to be protein bodies (PB) and they are usually associated with filament bundles which can be orientated to the vacuole (Figs.8, 9). The PBs appear to be integrated into the vacuole, where they are embedded in a mucilaginous network (Fig.18). Additionally, the vacuole contains large numbers of crystals which show different patterns depending on the cutting direction (Fig.19). These crystals are probably protein crystals. A natural degradation of these proteins was not observed. Besides their occurrence in the vacuole the PBs can also

be found near the plasma membrane. The development of PBs appears to change after treatment of MHCs with CB. In untreated MHCs the RER cisternae form limiting layers around areas of cytoplasm where proteins are synthesized and accumulated. These structures are also known as "pseudovacuoles" (Fig.11). MHCs treated with CB show that PBs are developed within the RER (Fig.12).

Besides the well developed RER-Golgi system, a large number of amoeboid leucoplasts, approximately 70, occur in the MHC. They are surrounded by RER-cisternae which are smooth and swollen at the areas where they are lying close to the leucoplasts (Fig.13). This close association could indicate a functional relationship. The leucoplasts have a length of approximately 3.7 μm and a diameter of 1.8 μm. In most cases they contain one or two rounded, amorphous membrane bound bodies (MBB), small transparent vesicles, lamellae, tubules and two to three areas of DNA. Ribosomes occur in completely intact leucoplasts. Thylakoids and starch are not formed. Leucoplasts in MHCs degenerate very fast in comparison to stalk and epidermal cells, where the degeneration is slower. The envelope of degenerated leucoplasts is damaged and only parts of the membrane of the inclusion can be found. During differentiation the MBBs appear amorphous and electron dense, whereas during degeneration the MBBs occur granulated and electron translucent. In some cases the stroma contains large globular inclusions of different size, which are usually not surrounded by a membrane. Such structures can also be observed in the cytoplasm of degenerated cells. The leucoplasts are stained blue with mercury bromphenolblue indicating lysin and glutamin. The MBBs are not sensitive to pepsin but show a positive DAB reaction.

Effects of cytochalasin B (CB) on glands

The effects of CB, an inhibitor of microfilament polymerisation, was studied and subsequently the formation of polysaccharides was observed by using the PAT-AgProt-reaction. After 15 min of incubation changes in the formation of dictyosomes were observed. The cisternae are distended at their rims and vesicles occur in large groups closely together at the trans-side (Fig.17). In areas close to the dictyosomes the RER is transformed into swollen vesicles. After the incubation of CB for 45 min almost all cisternae of the dictyosomes are flattened, the intercisternal elements remain unchanged. Only few and very small vesicles are occurring and vesicles with granulated or filamentous contents are completely missing. Coated vesicles still occur. The trans-side shows some large vesicles which developed by the fusion of small vesicles. The ER close to the cis-side of the dictyosomes collapses into small vesicles, whereas the main part of the RER remains unchanged. After 60 min of incubation with CB no dictyosomes could be found in the samples. Remnants of dictyosomes in the form of single swollen cisternae occur seldom. More often groups of large

vesicles can be seen, which were formed by fusion of small vesicles. The RER is strongly dilated and the mitochondria occur suspiciously accumulated together. No differences between DMSO treated and control MHC were found. By using the PAT-AgProt-reaction a distinct difference between control and CB-treated MHCs is found. In untreated MHCs four to five cisternae and vesicles of the trans-side show a PAT-Ag Prot-staining. However, in CB treated cells only two to three cisternae and vesicles react positive (Figs.14, 15). These results clearly document a decrease in dictyosome activity in CB-treated MHCs. Additionally, polysaccharide containing vesicles are not transported (Fig.17). The cell wall, mucilage, plasma membrane and vesicles within the cytoplasm also show a PAT-Ag Prot- staining (Fig.16).

Discussion

Mucilage secreting trichomes are typical for developing leaves (FAHN 1979). In most cases these hairs degenerate before the leaves finish their growth. The mucilage hairs of *Commelinaceae* always consist of three cells, whereas microhairs of various *Gramineae* usually consist of two cells (STAUDERMANN 1924, AMARASINGHE 1990). Glandular cells and nectaries are often characterized by cell wall protuberances (GUNNING & PATE 1969, DUKE & PAUL 1993). Such "ingrowths" are typical for cells where short distance transport occurs (FAHN 1979).

Two thirds of the cell wall of the MHC of *Zebrina* show ingrowths which increase the surface area for possible secretion. The space between the cell wall and the plasma membrane and the subcuticular space is filled with secretion material. The use of histochemical procedures indicate that the mucilage contains polysaccharides as well as proteins and/or glycoproteins.

Since lectins are carbohydrate binding proteins they react with various carbohydrate structures at the surface of plants (KNOX 1992). Out of five FITC-labeled lectins (BS-I, Con A, PNA, SBA and WGA) used in the present studies, only Con A showed a positive reaction with the secretion, the cell wall and the surface of the plasma membrane in MHCs of *Zebrina*. FITC-labeled Con A binds specifically with the inner, non-reduced terminal α-mannosyl rest and α-mannose is a main constituent of glycoproteins (STRYER 1990). The protrusions and the secreted mucilage of glandular cells of *Nymphoides peltata* react positive with FITC-labeled WGA. However, the mucilage of microhair cells of *Gramineae* do not show a positive reaction with different FITC-labeled lectins including Con A (MEYBERG 1988, AMARASINGHE 1990).

Generally, the polysaccharide mucilage is synthesized in the GA and gets secreted by vesicles (FAHN 1988). During mucilage secretion a large amount of vesicles occur and the highly active dictyosomes are small and

consist of few flat cisternae (SCHNEPF 1993). In some polysaccharide mucilage containing cells like in raphides idioblasts of *Zebrina pendula* or mucilage cells *of Aloe arborescens*, the GA stays inactive (BOUCHET 1982, TRACHTENBERG 1984). It is suggested that the starch in the plastids of *Aloe* is divided and transformed into mucilage monomers. In raphides idioblasts of *Zebrina* the distinct hypertrophical ER is involved in the mucilage synthesis. A degeneration of plastids was also documented by BOUCHET 1982. The mucilage of glandular cells of *Zebrina* contains polysaccharides as well as proteins. It is not clear yet whether the mucilage of raphides idioblasts also contains proteins.

Microhairs of various *Gramineae* secrete polysaccharides, proteins and/or glycoproteins. Thereby the different types of hairs perform different functions. Whereas in the "panicoid" type, the dictyosomes are involved in the secretory processes, in the "chloridoid" and "Enneapogon" types partitioning membranes participate in secretion (AMARASINGHE 1990). A distinct RER like in the MHC of *Zebrina* is missing in the microhairs of *Gramineae*. The RER in MHCs of *Zebrina* is connected with the cis-side of the dictyosomes by direct membrane contact or transitory vesicles. Membrane and particle transport of proteins between ER and dictyosomes is probable. Transitory vesicles between ER and dictyosomes are not common in higher plants (KRISTEN & al. 1988). These plants usually contain a well developed RER which is closely associated with dictyosomes and secrete polysaccharides and proteins. In the MHC of *Zebrina* proteins are transported in two different ways. Besides the protein transport from the RER to the GA and with the help of vesicles to the plasma membrane and the vacuole, a later occurring second transport without GA involvement exists. It is suggested that microfilament bundles are involved in the transport of PBs directly to the tonoplast. In cotyledons of *Sinapsis alba* and in the endosperm of *Triticum*, two forms of storage protein transport are documented (BERGFELD & al. 1980, RUBIN & al. 1992). To our knowledge microfilaments at PBs are not described in the current literature, whereas tubules were found in a mutant of *Zea mays*. PBs and tubules are produced in the RER, but the role of the tubules is unknown at this moment (LENDING 1996).

Storage proteins are synthesized in vegetative tissues as well as in reproductive tissues of higher plants (KRISTEN & BIEDERMANN 1981, THALER & al. 1992, HERMAN 1994). Vacuoles which are storing proteins are called protein storage vacuoles. The final forms of these vacuoles are known as PBs. They are either formed by protein storage vacuoles or they are developed directly by terminal dilatation and vesiculation out of the ER (rev. ROBINSON & HINZ 1997). Different types of PBs are documented in the literature. In vegetative tissues, spindle shaped fibrillar, globular granulated, and irregular shaped PBs occur. All of them derive from the RER.

Spindle and globular shaped PBs are documented in basal cells of the ligula in *Isoetes lacustris* (KRISTEN & BIEDERMANN 1981).

In secretory cells of glandular scales and in short-stalked glandular hairs of *Scutellaria altissima,* spherical PBs with granulated content, which are surrounded by RER membranes, occur. The PBs remain intact in the short-stalked glandular hair, whereas in the peltate hairs they secrete their content (THALER & al. 1992). The spherical shaped PBs in MHCs of *Zebrina* develop in ribosome rich but organelle free areas of cytoplasm which are surrounded by RER cisternae. Apparently, the separation of the developing PBs from the cytoplasm occurs by fusion of ER-membranes. This ER-configurations have also been reported in protein polysaccharide mucilage secreting glands of *Aptenia cordifolia* and *Platythyra haeckeliana.* Such developing cytoplasmic compartments have been named "pseudovacuoles" (KRISTEN 1977). These pseudovacuoles are able to produce and accumulate proteins and have also been found in resin ducts of *Mangifera indica* (JOEL & FAHN 1980). In our research PBs inside the RER are only found after treating the hairs with CB. The MHC contains no pseudovacuoles, which could be due to an early developmental stage. The vacuole of the MHC forms a siphonale network similar to the observed vacuoles in the glandular hairs of *Veronica beccabunga* (KRISTEN & LOCKHAUSEN 1985). The polysaccharides are transported to the vacuoles by Golgi vesicles. The proteins form spherical shaped structures, which are similar in size and structure to PBs, and can also be found in form of protein crystals. Since the pH value of the vacuolar sap is low, it is possible that the polypeptides form hexameres. Similar processes might also occur with phaseolin-polypeptides within storage vacuoles in the seeds of *Phaseolus* (BLAGROVE & al. 1984). The three dimensional structure of Phaseolin was described by LAWRENCE & al. 1990.

It is well documented that leucoplasts in secretory cells are involved in the biosynthesis of monoterpenes (HEINRICH & al. 1980, GLEIZES & al. 1983, CHENICLET & CARDE 1985, THALER & al. 1992). The occurrence of numerous leucoplasts containing MBBs in MHCs of *Zebrina* and their close contact with the ER system lead to the assumption that they are involved in metabolic processes. During advanced degeneration of the MHC, the content of the MBBs appears granulated and electron translucent, and only remnants of the surrounding membrane remain. Similar structural changes of MBBs were described in plastids of the epidermis of *Origanum dictamnus* (BOSABALIDIS 1987). The chemical nature of MBBs appears to vary extremely in different plants. The main constituents are proteins, lipids, lipoproteins, carbohydrates and phenolic substances (cf. GAILHOFER 1983a). The content of MBBs in leucoplasts of *Zebrina* is not soluble with pepsin. Nevertheless, the occurrence of protein components is indicated by the positive reaction of the MBBs with bromphenolblue. A resistance

against pepsin, trypsin and pronase was also observed in MBBs of leucoplasts in laticifers of *Papaver somniferum* and also in plastids in the epidermis of *Ranunculus bulbosus* (NESSLER & MAHLBERG 1979, GAILHOFER 1983a). However, the MBBs in plastids of *Origanum dictamnus* are soluble with pepsin and it is supposed, that catalase is the present protein (BOSABALIDIS 1987).

GAILHOFER 1983b reports an oxidation of DAB, a medium for the localization of photosystem I (NIR & SELIGMAN 1970) by the contents of MBBs in the plastids of *Ranunculus bulbosus*. This could be induced by precursors of the electron transport chain since the MBBs in these plastids are closely connected with the thylakoid system (GAILHOFER 1983a, b). The leucoplasts of *Zebrina* also show a positive DAB reaction. Photochemical activities and possible catalyzing activities of the leucoplasts can be denied since a thylakoid system is missing here. It is possible that the MBB of plastids in MHCs of *Zebrina* persist the protein content and has a still unknown function.

Cytochalasines are known to inhibit the activities of actin (cf. COOPER 1987). A large variety of experiments are documenting the effects of cytochalasines in cells. KRISTEN & LOCKHAUSEN 1983 showed in the ovary glands of *Aptenia cordifolia* that CB completely inhibits the transport of Golgi vesicles to the plasma membrane whereas the intensity of the vesicle production remains unchanged. A fusion of Golgi vesicles is supposed to be improbable. In MHCs of *Zebrina* CB inhibits the production of both Golgi vesicles and polysaccharides in comparison to control cells, thus probably affecting the transport between the ER and the dictyosomes. Stacks and coils of ER cisternae accumulation, as can be found after CB treatment in pollen tubes of *Nicotiana alata* (LANCELLE & HEPLER 1988), did not occur in MHCs of *Zebrina*. In the MHC the ER close to the dictyosomes appears dilated, whereas the RER at the cell periphery and around the nucleus remains normal and parallel to the plasma membrane, even after the 45 min treatment of CB.

Acknowledgement

The authors are grateful to Ing. G. GRAGGABER for technical assistance.

References

AMARASINGHE V. 1990. Polysaccharide and protein secretion by grass microhairs. A cytochemical study at light and electron microscopic levels. – Protoplasma 156: 45–56.

BERGFELD R., KÜHNL R. & SCHOPFER P. 1980. Formation of protein storage bodies during embryogenesis in cotyledons of *Sinapis alba* L. – Planta 148: 146–156.

BLAGROVE R. J., LILLEY G. G., VAN DONKELAAR A., SUN S. M. & HALL T. C. 1984. Structural studies of a french bean storage protein: Phaseolin. – Int. J. Biol. Macromol. 6: 137–141.

BOSABALIDIS A. M. 1987. Origin, differentiation and cytochemistry of membrane-limited inclusion bodies in leucoplasts of leaf epidermal cells of *Origanum dictamnus* L. – Cytobios 50: 77–88.

BOUCHET P. 1982. Étude ultrastructurale des cellules muco-oxalifères d'une Commélinacée : *Zebrina pendula* SCHNIZL. – Bull. Soc. Bot. Fr. 129, Actual. Bot. 1982 (1): 29–35.

CHENICLET C. & CARDE J.-P. 1985. Presence of leucoplasts in secretory cells and of monoterpenes in the essential oil: A correlative study. – Isr. J. Bot. 34: 219–238.

COOPER J. A. 1987. Effects of cytochalasin and phalloidin on actin. (Mini-Rev.). – J. Cell Biol. 105: 1473–1478.

DEXHEIMER J. 1981. Some aspects of the functioning of the dictyosomes of the secretory trichomes of *Psychotria bacteriophila (Rubiaceae)*. – Z. Pflanzenphysiol. 101: 333–346.

DUKE S. O. & PAUL R. N. 1993. Development and fine structure of the glandular trichomes of *Artemisia annua* L. – Int. J. Plant Sci. 154: 107–118.

FAHN A. 1979. Secretory tissues in plants. – Academic Press, London, New York, San Francisco.

— 1988. Transley Review No. 14. Secretory tissues in vascular plants. – New Phytologist 108: 229–257.

GAILHOFER M. 1983a. Die Feinstruktur der Plastideneinschlüsse von *Ranunculus bulbosus*. – Phyton 23: 197–210.

— 1983b. Die Lokalisation von oxidiertem 3,3'-Diaminobenzidin in Plastiden von *Ranunculus bulbosus*. – Phyton 23: 211–219.

GLEIZES M., PAULY G., CARDE J.-P., MARPEAU A. & BERNARD-DAGAN C. 1983. Monoterpene hydrocarbon biosynthesis by isolated leucoplasts of *Citrofortunella mitis*. – Planta 159: 373–381.

GUNNING B. E. S. & PATE J. S. 1969. "Transfer Cells" Plant cells with wall ingrowths, specialized in relation to short distance transport of solutes – their occurrence, structure, and development. – Protoplasma 68: 107–133.

GUTTENBERGER H. 1990. Kern-DNA-Gehalt der Drüsenhaare von *Zebrina pendula* SCHNIZL. – Beitr. Biol. Pflanzen 65: 205–210.

HEINRICH G., SCHULTZE W. & WEGENER R. 1980. Zur Kompartimentierung der Synthese von Mono- und Sesqui-Terpenen des ätherischen Öls bei *Poncirus trifoliata*. – Protoplasma 103: 115–129.

HERMAN E. M. 1994. Multiple origins of intravacuolar protein accumulation of plant cells. – Adv. Struct. Biol. 3: 243–283.

JOEL D. M. & FAHN A. 1980. Ultrastructure of the resin ducts of *Mangifera indica* L. (*Anacardiaceae*). 3. Secretion of the protein-polysaccharide mucilage in the fruit. – Ann. Bot. 46: 785–790.

KNOX J. P. 1992. Molecular probes for the plant cell surface. – (Rev. Article) Protoplasma 167: 1–9.

KRISTEN U. 1977. Auffällige ER-Konfigurationen in Protein-Polysaccharidschleim sezernierenden pflanzlichen Drüsen. – Planta 133: 161–167.

— 1980. Endoplasmic reticulum-dictyosome interconnections in ligula cells of *Isoetes lacustris*. – Eur. J. Cell Biol. 23: 16–21.

— & BIEDERMANN M. 1981. Ultrastructure, origin, and composition of the protein bodies in the ligule of *Isoetes lacustris* L. – Ann. Bot. 48: 655–663.

— & LOCKHAUSEN J. 1983. Estimation of Golgi membranes flow rates in ovary glands of *Aptenia cordifolia* using cytochalasin B. – Eur. J. Cell Biol. 29: 262–267.

— & — 1985. The leaf glands of *Veronica beccabunga* L.: Ultrastructure and a possible pathway of secretion. – Israel J. Bot. 34: 147–156.

— , — & KANDASAMY M. K. 1988. Structural aspects of protein secretion in higher plant cells. – Phyton (Austria) 28: 183–191.

LANCELLE S. A. & HEPLER P. K. 1988. Cytochalasin-induced ultrastructural alterations in *Nicotiana* pollen tubes. – Protoplasma 1988 (Suppl. 2): 65–75.

LAWRENCE M. C., SUZUKI E., VARGHESE J. N., DAVIS P. C., VAN DONKELAAR A., TULLOCH P. A. & COLMAN P. M. 1990. The threedimensional structure of the seed storage protein phaseolin at 3 Å resolution. – EMBO J. 9: 9–15.

LENDING C. R. 1996. A novel tubular array associated with protein bodies in the rough endoplasmic reticulum of opaque-2 maize. – Protoplasma 195: 68–77.

LOCKHAUSEN J. & KRISTEN U. 1986. Dictyosome-endoplasmic reticulum associations in gland cells of *Veronica beccabunga*. – Eur. J. Cell Biol. 42: 328–331.

MEYBERG M. 1988. Cytochemistry and ultrastructure of the mucilage secreting trichomes of *Nymphoides peltata* (*Menyanthaceae*). – Ann. Bot. 62: 537–547.

NESSLER C. L. & MAHLBERG P. G. 1979. Plastids in laticifers of *Papaver*. I. Development and cytochemistry of laticifer plastids in *P. somniferum* L. (*Papaveraceae*). – Am. J. Bot. 66: 266–273.

NIR I. & SELIGMAN A. M. 1970. Photooxidation of diaminobenzidine (DAB) by chloroplast lamellae. – J. Cell Biol. 46: 617–620.

ROBINSON D. G. & HINZ G. 1997. Vacuole biogenesis and protein transport to the plant vacuole : A comparison with the yeast vacuole and the mammalian lysosome. (Rev. article). – Protoplasma 197: 1–25.

— & KRISTEN U. 1982. Membrane flow via the golgi apparatus of higher plant cells. – Internat. Rev. Cytology 77: 89–127.

RUBIN R., LEVANONY H. & GALILI G. 1992. Evidence for the presence of two different types of protein bodies in wheat endosperm. – Plant Physiol. 99: 718–724.

SCHNEPF E. 1993. Golgi apparatus and slime secretion in plants: The early implications and recent models of membrane traffic. – Protoplasma 172: 3–11.

STAUDERMANN W. 1924. Die Haare der Monokotylen. – Bot. Archiv 8: 105–184.

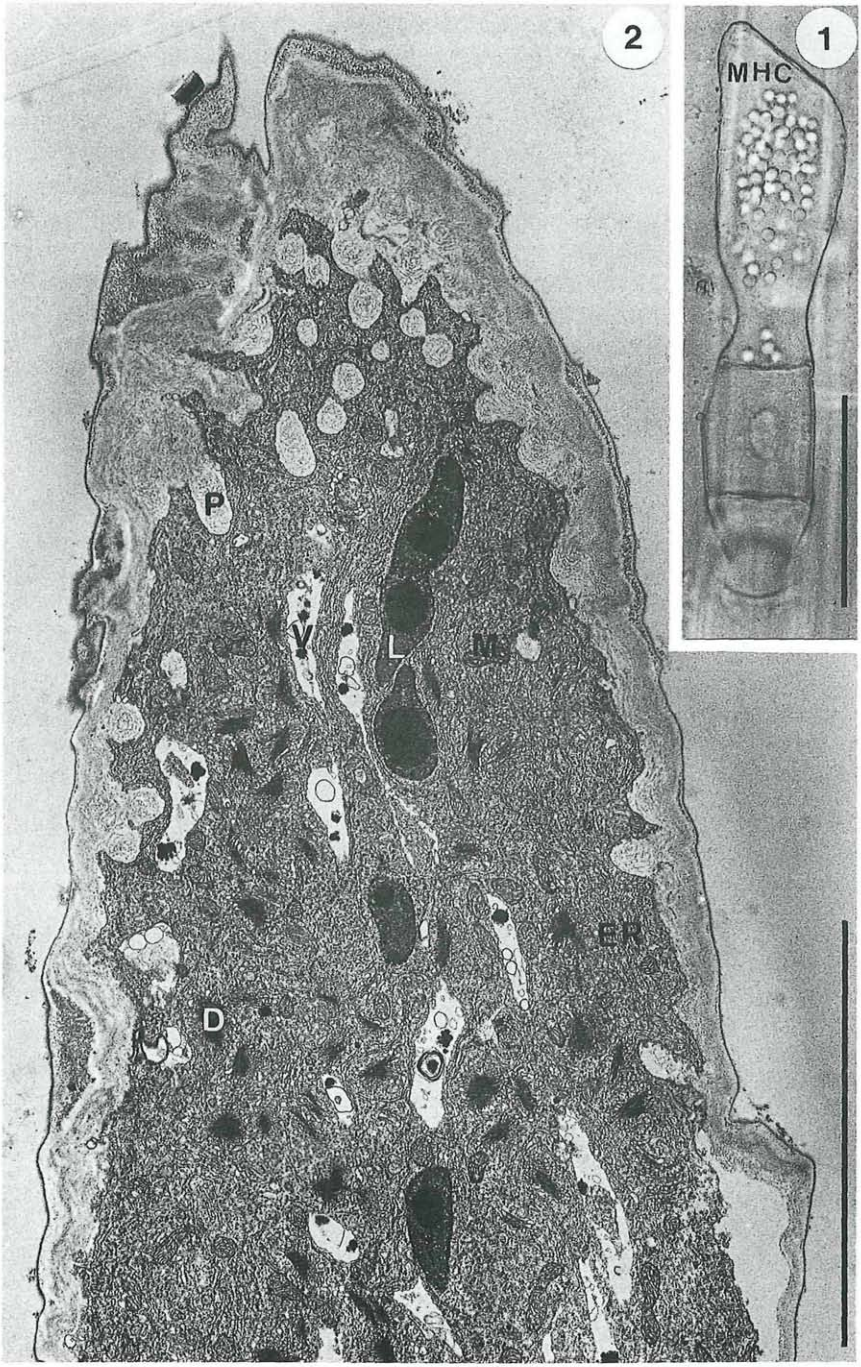
STRYER L. 1990. Biochemie. – 5. Auflage. Spektrum der Wissenschaft Verlagsges., Heidelberg.

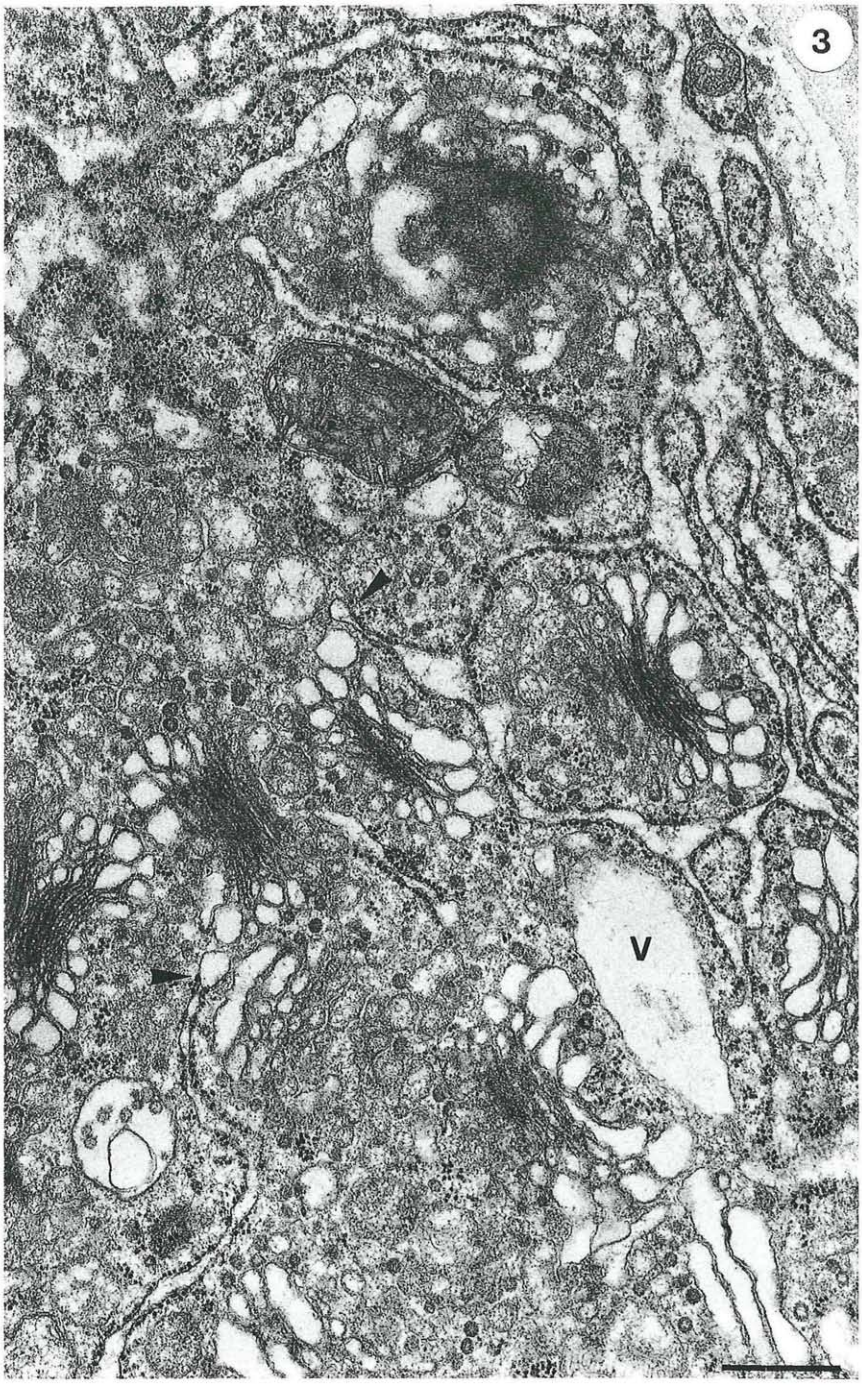
THALER I., GAILHOFER M. & GUTTENBERGER H. 1986. DNA-content and ultrastructure of glandular hair of *Zebrina pendula* SCHNIZL. – 5. Jugoslavenski simpozij iz electronske mikroskopije Plitvička Jezera 27.–30. Maja 1986.

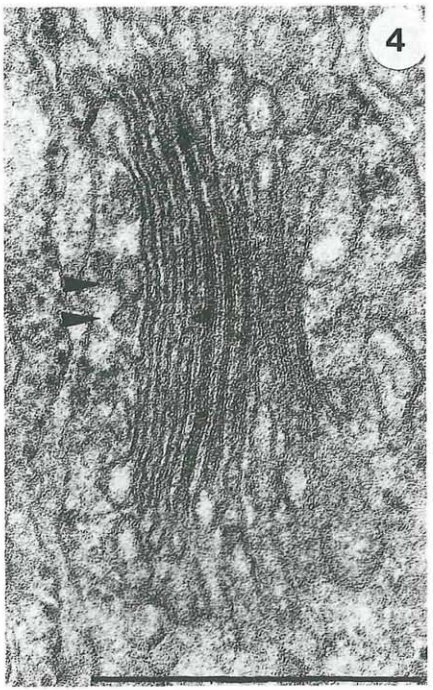
— , — & PFEIFHOFER H. W. 1992. Proteinkörper in Drüsenhaaren von *Scutellaria altissima* (*Lamiaceae*). – Phyton (Austria) 31: 263–280.

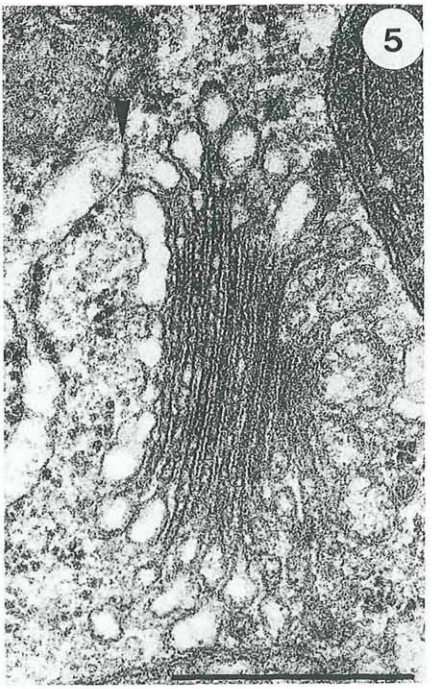
THIÉRY J. P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. – J. Microscopie 6 : 987–1018.

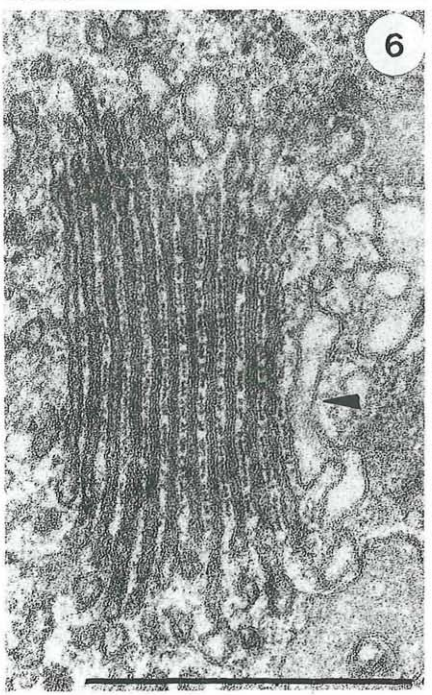
TRACHTENBERG S. 1984. Cytochemical and morphological evidence for the involvement of the plasma membrane and plastids in mucilage secretion in *Aloe arborescens*. – Ann. Bot. 53: 227–236.

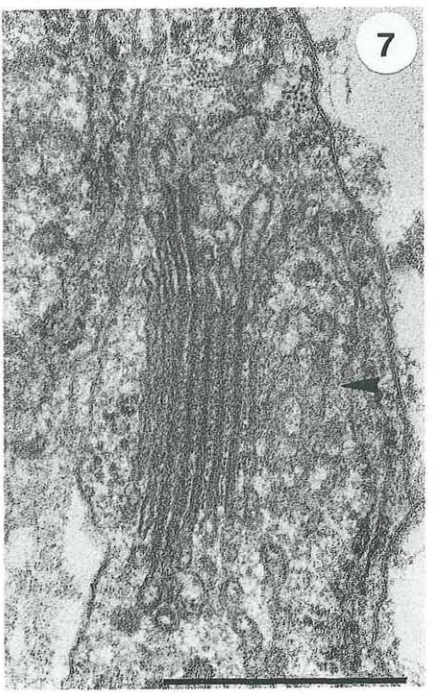


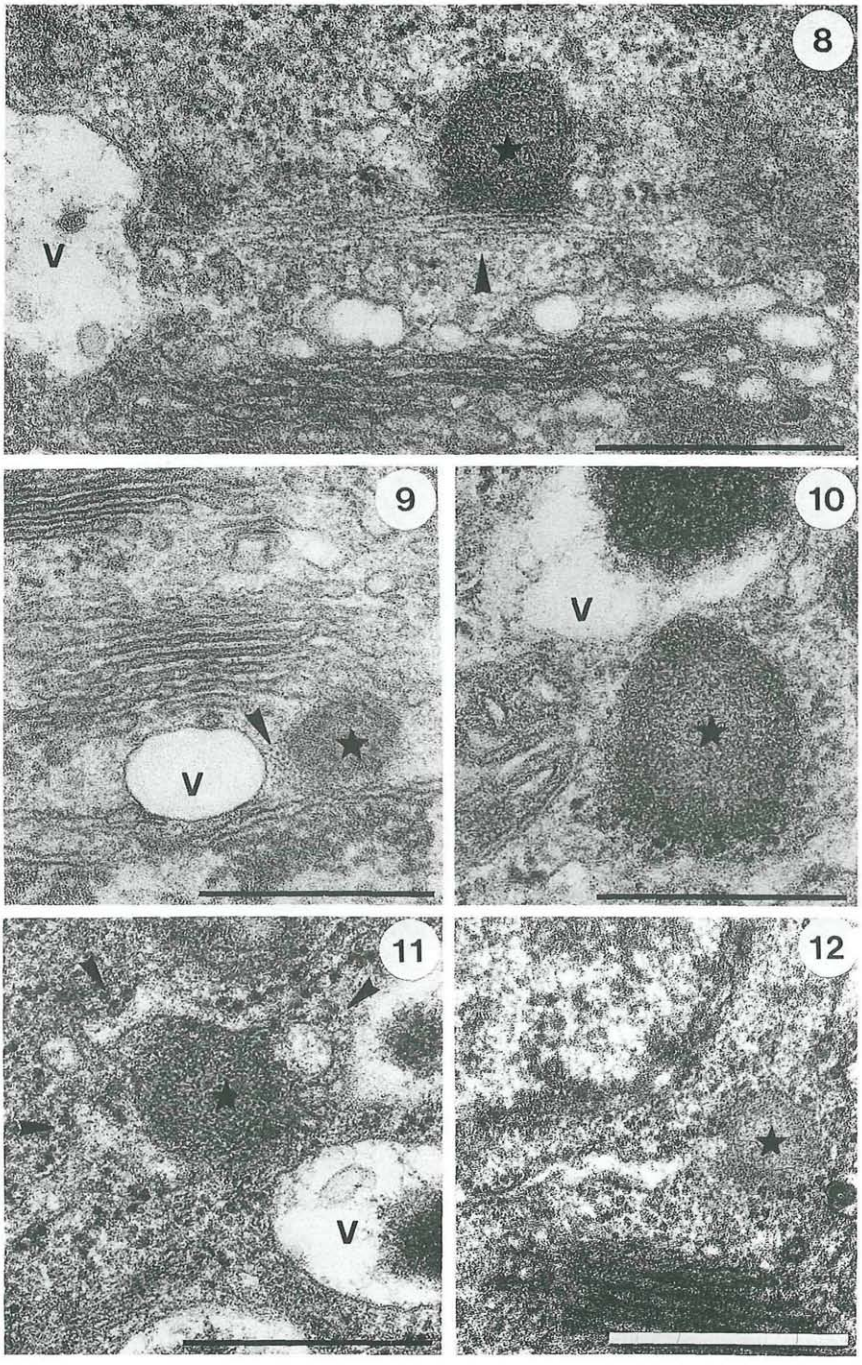


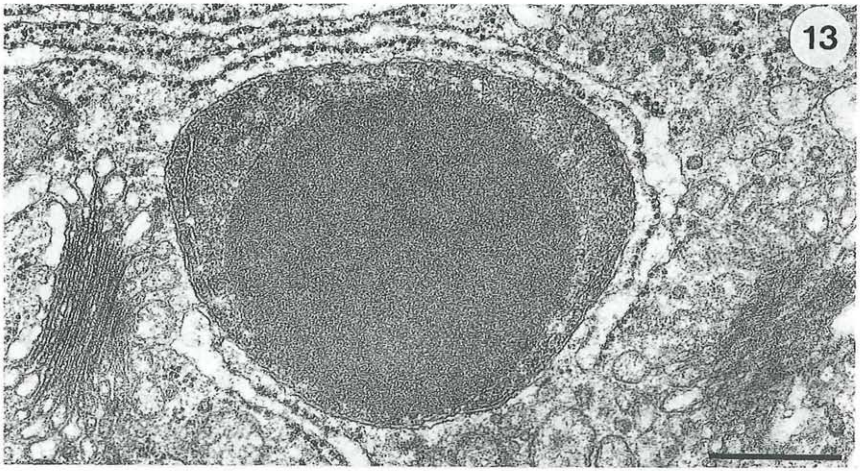


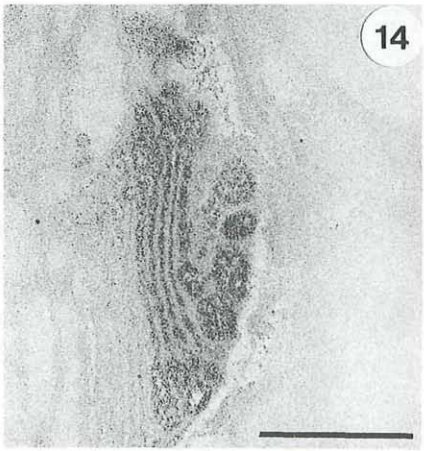


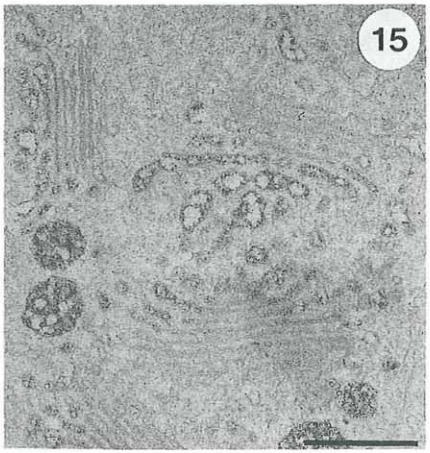


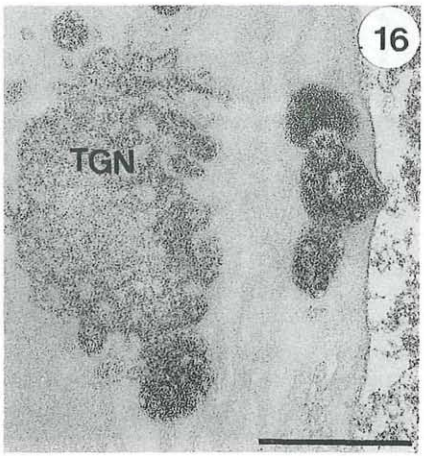


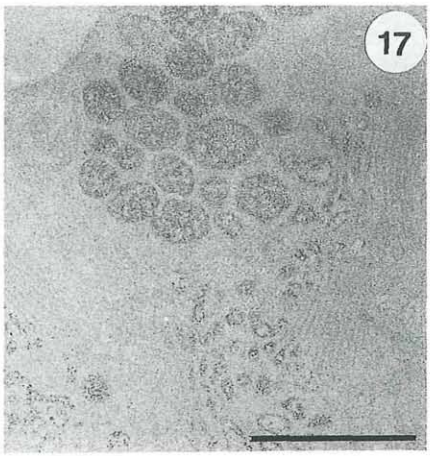












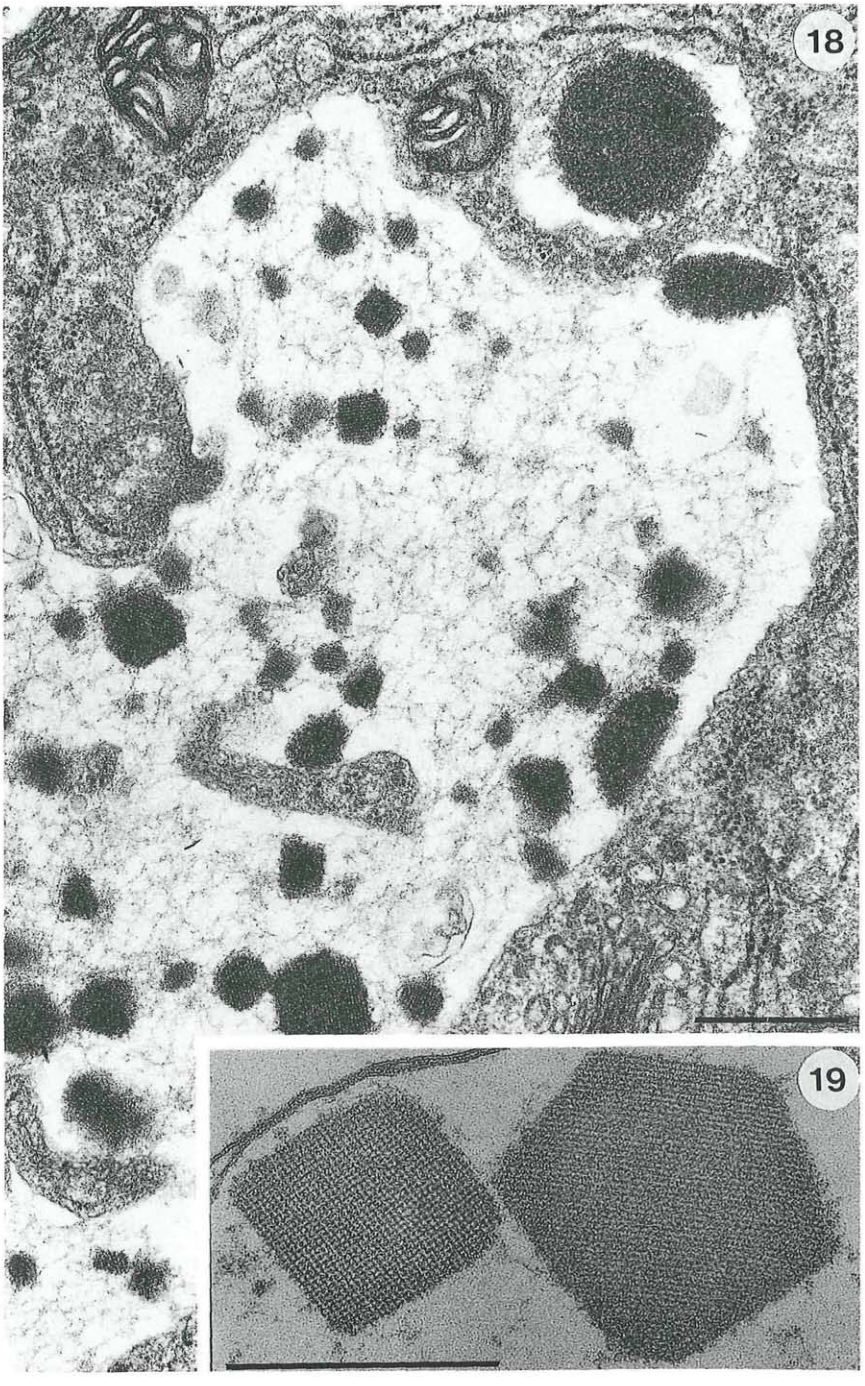


Figure legends

Fig. 1. Brightfield micrograph of a three celled glandular hair of *Zebrina pendula*. Head cell contains numerous leucoplasts. Bar = 50 µm.

Fig. 2. Protuberances (P) of the cell wall at the tip of the head cell of a glandular hair, leucoplast (L), vacuole (V), dictyosome (D), mitochondrium (M). Bar = 10 µm.

Figs. 3–19. Parts of secretory head cells. Bars = 500 nm.

Fig. 3. RER-dictyosome association, RER seems to bud vesicles (arrowheads), numerous vacuoles (V).

Fig. 4. Numerous transitory vesicles between RER and the cis-cisterna of a dictyosome (arrowheads).

Fig. 5. Close association of vesicles with the ER cisterna (arrowhead).

Fig. 6. Treatment with 50 µg/ml CB for 45 min; dictyosome with intercisternal elements, rims of cisternae are poorly dilated, the TGN is inflated (arrowhead).

Fig. 7. Dictyosome with a normally developed TGN (arrowhead).

Fig. 8. PB (asterisk) in close association with a filament bundle (arrowhead), vacuole (V).

Fig. 9. PB (asterisk) in close association with cross sectioned filaments (arrowhead) at a vacuole (V).

Fig. 10. PB (asterisk) in close association with numerous ribosomes, vacuole (V).

Fig. 11. PB (asterisk) surrounded by cisternae of the RER, vacuole (V).

Fig. 12. Treatment with 50 µg/ml CB for 60 min; PB (asterisk) buds on an inflated RER.

Fig. 13. Leucoplast, containing a MBB, is surrounded by a partly dilated RER.

Figs. 14–17. Detection of polysaccharides, PAT-Ag Prot-reaction.

Fig. 14. Positive reaction in five cisternae and in vesicles at the trans-side.

Fig. 15. After treatment with 50 µg/ml CB for 15 min; positive reaction only in 2 cisternae and in vesicles at the trans-side.

Fig. 16. Positive reaction in the TGN, in secretory vesicles close to the plasma membrane. The plasma membrane and mucilage in the extraplasmic space indicate also polysaccharides.

Fig. 17. After treatment with 50 µg/ml CB for 30 min; polysaccharides are indicated in numerous vesicles and few cisternae of the trans-side.

Fig. 18. Part of the vacuole containing amorphous and crystalline inclusions.

Fig. 19. Different patterns of crystalline inclusions in the vacuole.