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The Ultrastructure of the Digestive Glands in *Pinguicula vulgaris* L. (*Lentibulariaceae*) Relative to their Function. I. The Changes During Maturation

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ABSTRACT

The digestive glands of *Pinguicula vulgaris* become fully mature whilst still enclosed in the bud. All the gland cells remain intact on the fully expanded unstimulated leaves. As the secretory head cells mature, a special layer forms between the plasmalemma and the cell wall. This layer is shown to be different from the typical labyrinthine wall of transfer cells and serves for the storage of digestive enzymes. Ultrastructural analysis, including morphometry, indicates that the digestive enzymes are synthesized on the RER of the head cells and transferred into the cell wall, particularly into the slime layer, and vacuoles. This transfer is achieved firstly through continuity of the endoplasmic reticulum with vacuoles (static) and the periplasmic space (dynamic) and, secondly, into the latter through exocytosis of coated Golgi vesicles and of some vacuoles filled with enzymes.

Key words: *Pinguicula vulgaris* L., carnivorous plant, digestive glands, ultrastructure, protein synthesis, secretion.

INTRODUCTION

In recent years, our knowledge of carnivorous plants has expanded considerably, through the application of electron microscopy, cytochemistry and autoradiography. An important contribution has been made by Heslop-Harrison and co-authors (Heslop-Harrison and Knox, 1971; Heslop-Harrison, 1975, 1976*a*, *b*; Heslop-Harrison and Heslop-Harrison, 1980, 1981) who worked mainly with species of *Pinguicula*. From the results obtained, they concluded that, in this species, hydrolytic enzymes, e.g. acid proteases, acid phosphatase, esterase, etc., involved in the digestion of the prey, are synthesized prior to stimulation, namely, during the maturation of the sessile digestive glands, and are stored until the prey is captured. The major storage site appears to be a special pectin-rich compartment, the spongy layer of the secretory cells situated between the plasmalemma and the cell wall proper. This layer was compared by the authors with the labyrinthine walls characteristic of transfer cells. Hydrolases were also deposited, but to a lesser extent, in the vacuoles of the gland head cells. The enzymes were released onto the surface of the glands as digestive fluid only after contact with prey, captured by the slime secreted by the stalked glands.

Y. Heslop-Harrison was less conclusive on the localization of synthesis, e.g. whether on free or membrane-bound ribosomes, and on the mechanisms of the hydrolase transfer to the storage sites, e.g. whether holocrine, granulocrine or eccrine. Initially she (Heslop-Harrison, 1975, 1976*a*) suggested a holocrine secretion without the involvement of the Golgi apparatus; hydrolases were assumed to enter the periplasmic space (outside the plasmalemma) from the peripheral hyaloplasm as a result of the isolation of cytoplasmic protrusions, followed by the breakdown of their delimiting membrane. Vesicular secretion, or the enzyme release from the RER cisternae directly into the periplasmic space through anastomoses of their membranes with the plasmalemma were also not excluded. However, the latter pathway was inconsistent with the treatment of the spongy layer as a labyrinthine cell wall (Heslop-Harrison and Heslop-Harrison, 1981); such a structure is specialized for eccrine secretion across the plasmalemma. This led the authors to suggest that digestive enzymes synthesized on the free ribosomes (in the cytosol) diffuse in a monomolecular form through the plasmalemma, whose surface is amplified due to the wall ingrowth formation.

Based on the ultrastructural data obtained, the authors concluded that, during the final matura-

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tion of the head cells, the hydrolases are released by total autophagy. The cell membranes break down and hydrolases are discharged into the lumen. The gland head becomes thus transformed into a dead sac of enzymes, whereas the other cells of the gland, barrier (endodermal) and reservoir (basal), retain living protoplasts. These cells ensure detection of the stimulus and the release of water into non-living secretory cells resulting in the leakage of hydrolases onto the surface of the gland. Heslop-Harrison and Heslop-Harrison (1981) also think that these non-secretory cells of the digestive glands actively absorb the products of digestion.

Our studies of the ultrastructural changes of secretory tissues during their development in *P. vulgaris*, using quantitative parameters, are somewhat at variance with the above. Our results concern the nature of the so-called 'spongy layer', the site of the digestive enzyme synthesis, and the mode of its discharge, as well as the role of the head cells in the gland activity after stimulation. Some results in Russian have been reported elsewhere (Vassilyev and Muravnik, 1979; Muravnik, 1983, 1984; Muravnik and Vassilyev, 1983).

MATERIALS AND METHODS

The ultrastructure of the gland cells was studied during their development on the leaf primordia at bud burst (mid-April); on young leaves during their emergence from the buds (May–June) and also on the adult leaves. Plants were grown in their natural habitats near Leningrad. Fixation was for 4–5 h at room temperature in 3% glutaraldehyde or paraformaldehyde in phosphate buffer (pH 7,2), followed by post-fixation in 2% osmium tetroxide for 16 h at 4 °C. After dehydration through graded ethanol, the tissue was embedded in epoxy resins. Ultrathin sections were examined in JEM-7A and Hitachi-600 electron microscopes. Volume densities of the cell components were determined on median sections of 5–15 glands at each stage of development, using an automatic image analyser IBAS. Surface density of the free ribosomes was counted on hyaloplasm areas of 0·5 and 1 μm^2.

OBSERVATIONS

The general structure of the digestive glands and pattern of their development have been described by Heslop-Harrison and Heslop-Harrison (1981). The glands are initiated from one protodermal cell of the leaf primordia near the shoot apex and mature fully whilst still enclosed in the bud. The differentiation of the glands proceeds acropetally and the basal cell becomes fully differentiated when the head cells, which mature last, are still dividing.

The terminal cell and its derivatives retain the ultrastructural characteristics of the meristematic cells up to the completion of their divisions. They are poorly vacuolated, rich in the hyaloplasm (Table 1) which is electron dense and contain many free ribosomes (approx. 450 in 1 μm^2), the endoplasmic reticulum (ER) is sparse (Table 2) in the form of short granular cisternae.

The nucleus becomes lobed, characteristic of the secretory cells of the mature glands. Within the nucleoplasm, near the nucleolus, a small protein crystal appears which consists of thin parallel lamellae. At maturity of the secretory cell, it usually decreases in size or totally disappears. During this period the nucleus moves towards the basal part of the cell and enlarges, only to shrink again, in the mature glands. The nucleolus undergoes similar size changes. During the maturation stage, the granular and fibrillar components of the nucleolus are distributed in a mosaic, although most of the granules are located on the nucleolar periphery. Both nucleolar components are intergraded (Fig. 1A) and, in some sections, one or two light fibrillar centres are visible. At the end of the cell's maturation a distinct nucleolar segregation occurs and the granular component is extruded onto the nucleolar periphery in a ring, whereas the core of the nucleolus is occupied by only the fibrillar component (Fig. 1B). The surface density of the free ribosomes is maintained at a high level up to the point of leaf emergence from the bud, after which it substantially falls (approx. 300 in 1 μm^2).

Whilst the head cells are still dividing, the periplasmic space begins to appear in some parts of the cells due to the deposition firstly of thin fibrils (Fig. 2A) and later of small granules. These deposits are apparently the components of the slime. In the periplasmic space, various forms of small cytoplasmic evaginations with ribosomes are seen, as well as vesicles, tubules and membrane fragments. The rest of the plasmalemma acquires a slightly sinuous outline. This situation is different from *Dionaea*, where the labyrinthine wall is laid down late in the development of the digestive gland (Robins and Juniper, 1980*a*). In *P. vulgaris* the periplasmic space gradually enlarges near the outer, and especially inner, parts of the anticlinal walls. Finally, in the mature glands it occupies more than one-fourth of the cell volume (Table 1). The expansion of the periplasmic space is accompanied by a rise in the concentration of its fibrils and granules. The plasmalemma forms the irregular folds and the number of its evaginations increases. Within cytoplasmic evaginations ER

TABLE 1. *Changes in volume fraction of the cell components during the development of the digestive glands*

Developmental stage and cell type	Nucleus	Vacuome	Chondriome	Plastidome	ER	Golgi apparatus	Hyaloplasm	Slime layer
Formating glands								
1- to 2-celled								
Initial and terminal cells	21·5±2·19	12·4±6·99	6·1±0·73	3·5±0·67	0·8±0·14	2·3±1·60	49·8	3·6±0·88
Basal cell	19·6	50·6	4·0	1·2	1·2	0·4	23·0	—
3-celled								
Terminal cell	18·2±6·90	14·3±4·37	6·4±2·18	3·1±1·18	0·7±0·17	1·1±1·30	50·7	5·7±1·24
Barrier cell	20·7	12·3±6·85	5·1±2·0	4·2±1·52	2·0±0·76	2·5±1·52	53·2	—
4-celled								
Terminal cell	31·2±12·2	24·3±9·25	6·0±1·26	2·5±0·51	1·9±0·28	0·9±0·25	37·9	5·8±1·44
8- to 10-celled								
Secretory cells	25·3±2·02	23·6±2·76	5·7±0·25	1·7±0·30	1·3±0·26	1·3±0·32	35·8	5·3±0·59
Fully formed glands (Secretory cells)								
Initiation of specialization	24·5	33·0±6·87	7·1±1·29	2·3±0·69	2·4±0·82	2·0±1·30	14·4	14·3±9·16
Prematuration stage	28·9	38·8±6·13	8·0±2·30	3·9±1·68	2·1±0·46	1·0±0·71	3·0	14·3±3·87
Mature glands	23·9	24·4±6·02	8·6±1·04	6·6±1·53	1·3±0·18	0·6±0·20	8·5	26·1±2·19

TABLE 2. *Changes in volume fraction of the granular endoplasmic reticulum and Golgi apparatus (at a rate on the unit of hyaloplasm volume) during the development of the digestive glands*

Developmental stage	Cell type	ER	Golgi apparatus
	Formating glands		
3-celled	Terminal cell	3·8 ± 0·46	9·2 ± 3·35
4-celled	Terminal cell	5·7 ± 0·56	8·6 ± 1·78
8- to 10-celled	Secretory cells	8·7 ± 0·95	8·7 ± 1·35
	Fully formed glands		
Initiation of specialization	Secretory cells	6·6 ± 1·13	9·7 ± 1·65
Prematuration stage	Secretory cells	15·3 ± 3·62	14·1 ± 5·92
Mature glands	Secretory cells	8·0 ± 2·36	8·3 ± 1·89

elements, mitochondria and leucoplasts are occasionally observed, in addition to the ribosomes. The evaginations in section commonly appear as the isolated islets of the cytoplasm in a slime layer.

At the prematuration stage, irregular dense areas appear in the slime (Fig. 2B) and these are indistinguishable in structure from the primary cell walls. Some dense areas are continuous with the wall and with each other. They are slightly reminiscent of wall protuberances characteristic of the transfer cells. The plasmalemma is not contiguous with the 'protuberances' but is separated from them by a wide layer of the electron translucent material.

As the glands mature, additional irregular areas with flocculent or empty contents appear in the slime layer near the plasmalemma (Fig. 2C). The character of the slime layer varies greatly, even within the same gland. Sometimes it appears almost empty, with only the densities being distinguishable. Paraformaldehyde, as compared with glutaraldehyde, proved to be superior for the preservation of all components of the slime layer.

At maturity, the groups of the microtubules are invariably seen near the plasmalemma in the regions of the anticlinal walls. These usually lie obliquely in relation to the plasmalemma and with a criss-cross pattern (Fig. 1C). At the same time, in the regions of the outer wall, single peripheral microtubules are common. In addition, occasional microtubules can be seen in the vicinity of the dictyosomes, and lying freely within the hyaloplasm. As the glands mature, the microtubules are reduced in number and seem to disappear in the adult glands.

During maturation the secretory cells of the glands, especially their apical halves, vacuolate. Initially the number of small vacuoles increases. These appear to be formed as local distensions of the granular ER cisternae which lose their ribosomes. The flocculent contents of the small vacuoles is similar to that of the lighter areas of the slime layer. The young vacuoles may become isolated from the cisternae and fuse with one another. The flocculent content of larger vacuoles is still seen (Fig. 2C). In the apical half of some head cells, all the vacuoles may fuse into one large vacuole. The tonoplast of these vacuoles acquires the structure and thickness identical to that of the plasmalemma.

At maturity, the secretory cells are polarized with the nucleus and the main mass of the cytoplasm in the lower part of the cells, and the upper part occupied predominantly by the vacuoles. As the cells mature, the volume fraction of the vacuome increases to 35–40% but in the adult glands it falls again, with a parallel increase in the volume of the slime layer (see Table 1). These negatively-correlated volume changes may be explained, not only by the direct release of material into the periplasmic space, but also, apparently, by a discharge of vacuolar content into this space via exocytosis-like processes (Fig. 3A). This process is apparently initiated by the formation of local contacts between the plasmalemma and the tonoplast (Fig. 3B). Through exocytosis some vacuoles, which were previously isolated structures, become the regions of the slime layer forming its third component, the areas with translucent or flocculent contents adjacent to the plasmalemma. Since the anastomosis between the periplasmic space and vacuolar contents is limited in area, it is difficult to

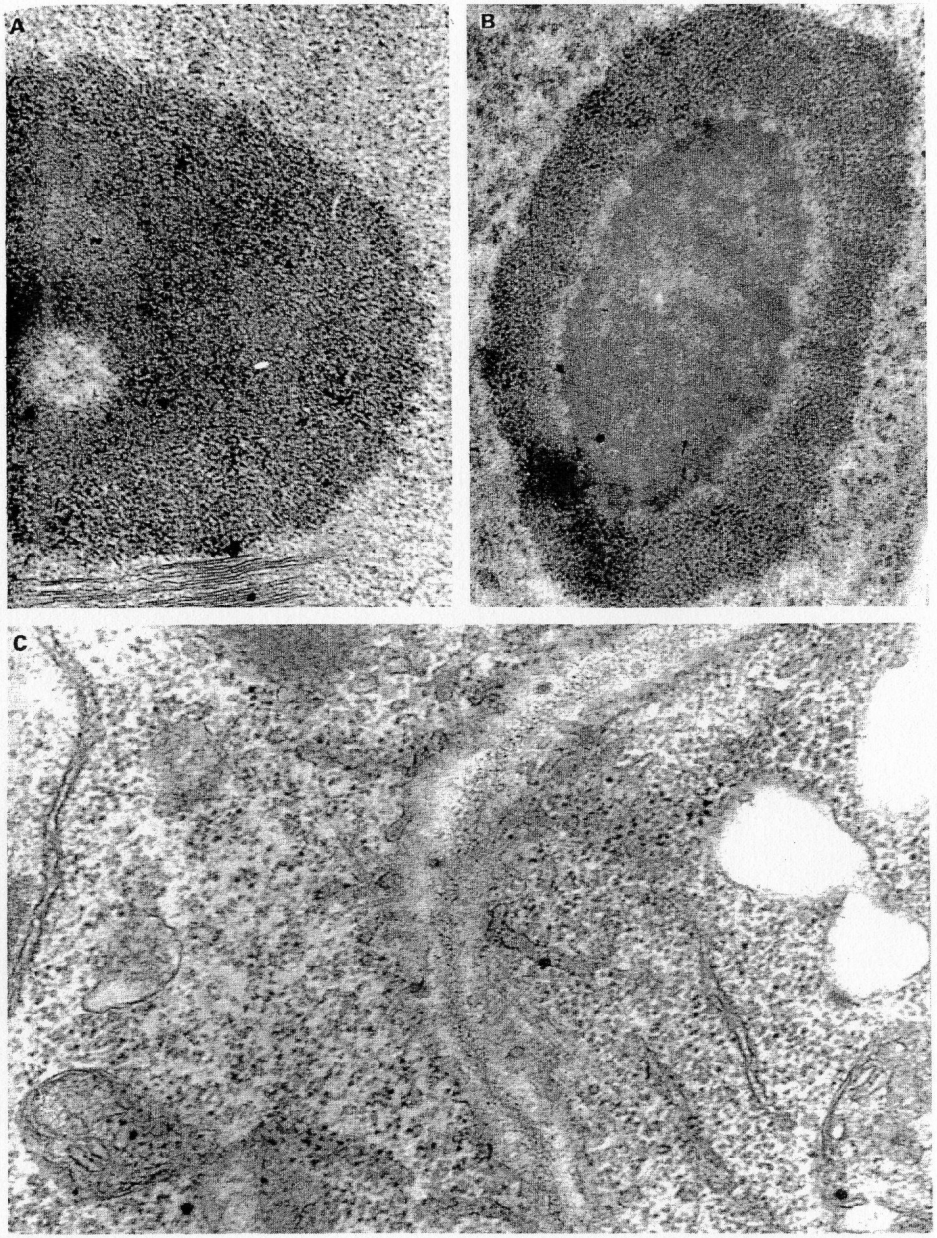

FIG. 1. A, A nucleolus in a secretory cell at maturation of the gland. Also shown is a portion of a lamellar protein crystal in the nucleoplasm (bottom left), ×42000; B, a segregated nucleolus in a secretory cell of the mature gland, ×41000; C, microtubules along the anticlinal wall of a secretory cell at maturation, ×58000.

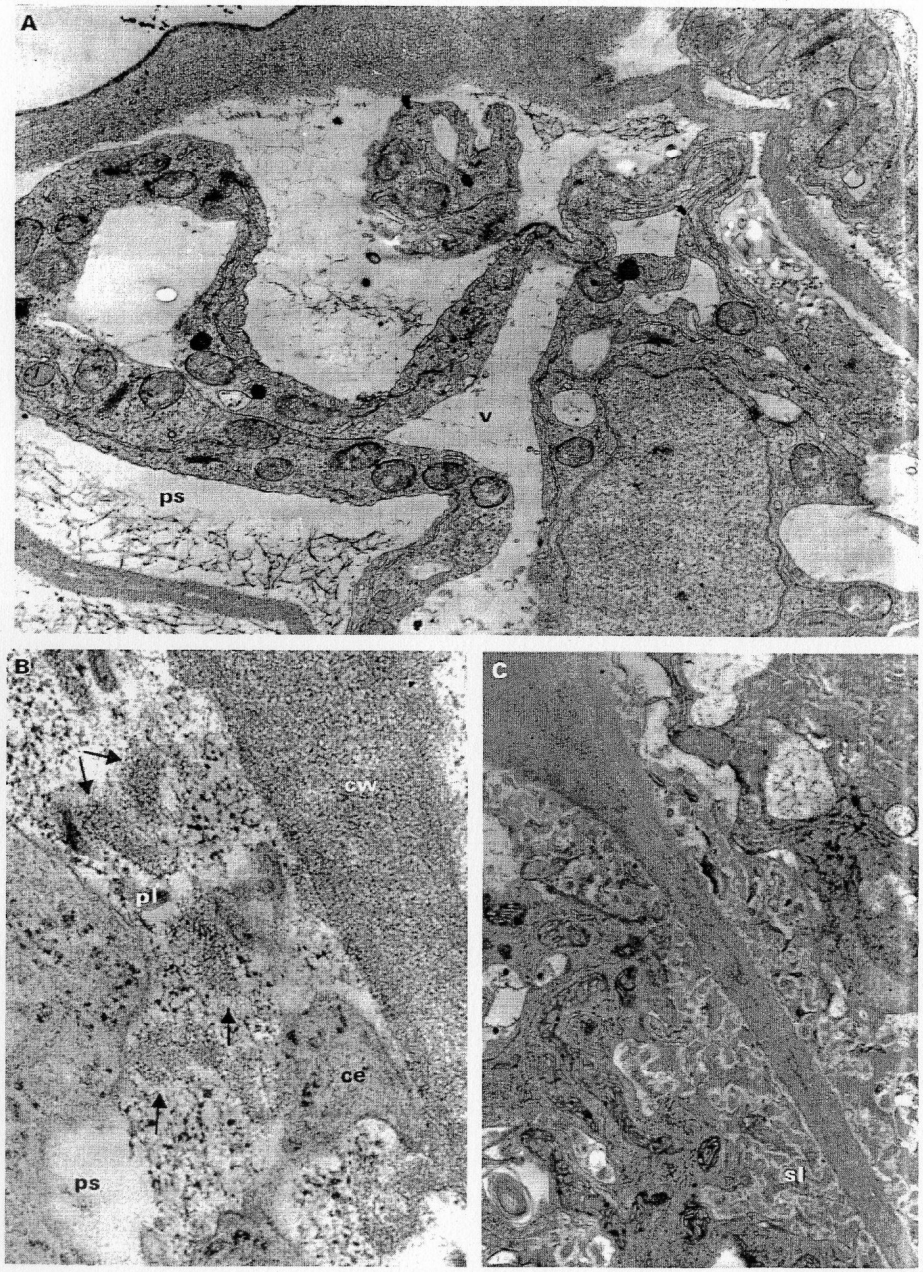

FIG. 2. A, A portion of a secretory cell at maturation. Note the irregular periplasmic space (ps) with fibrils as well as vacuoles (v) and a thin continuous cuticle, × 9000; B, the emergence of dense areas (arrows) inside the ps similar to cell wall (cw) material, note cytoplasmic evagination (ce) and fibrils and granules of the slime in ps, pl, plasmalemma, × 65000; C, typical slime layer in a mature gland, note the presence of the light component (top) similar to the content of the vacuoles, × 13000.

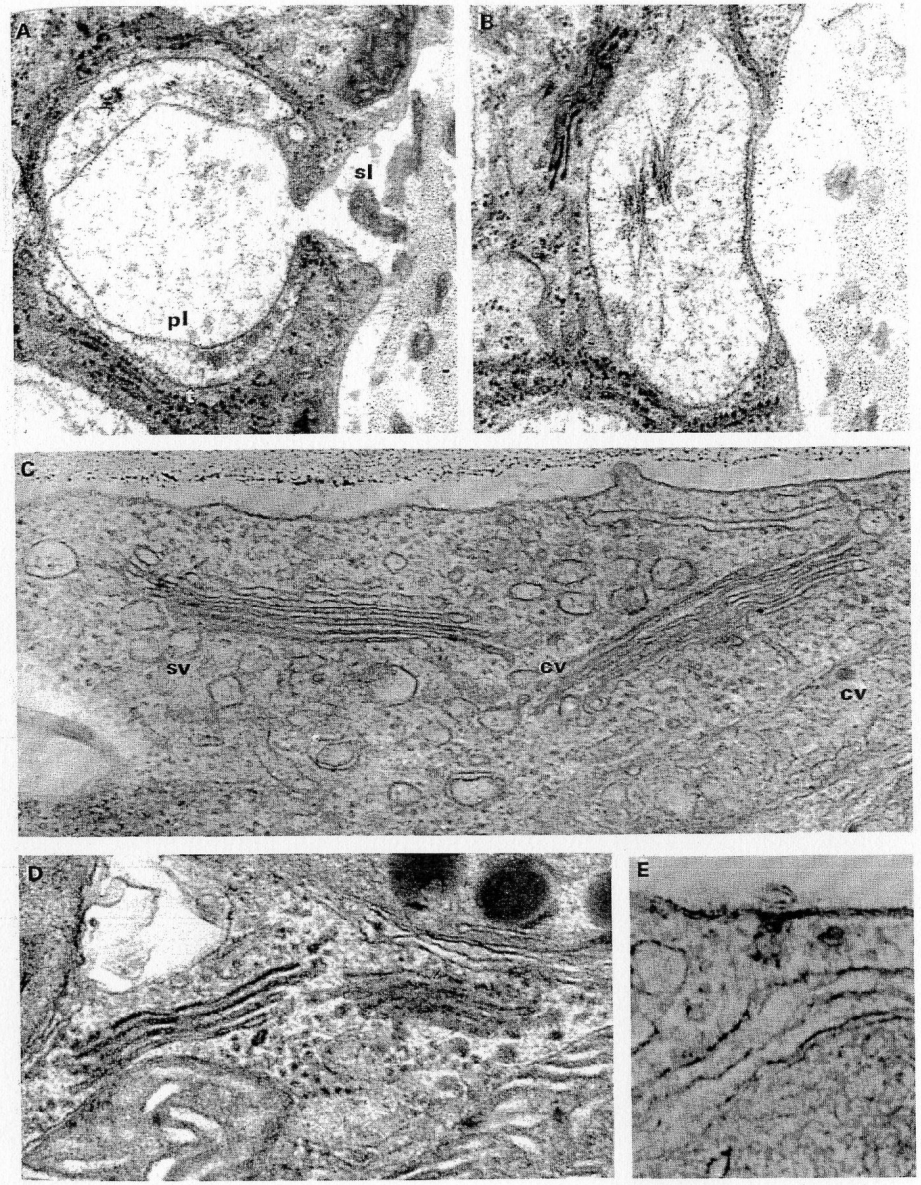

FIG. 3. A, Exocytosis of the contents of vacuoles into the slime layer (sl) at the prematuration stage of the gland, t, tonoplast; pl, plasmalemma, × 34000; B, establishment of contact between the tonoplast and plasmalemma prior to vacuole exocytosis, × 33000; C, active dictyosomes in the secretory cell at the onset of gland maturation; cv, coated vesicle; sv, slime vesicle, × 67000; D, inactive dictyosomes in a mature gland, × 76000; E, exocytosis of coated vesicles into the periplasmic space, × 110000.

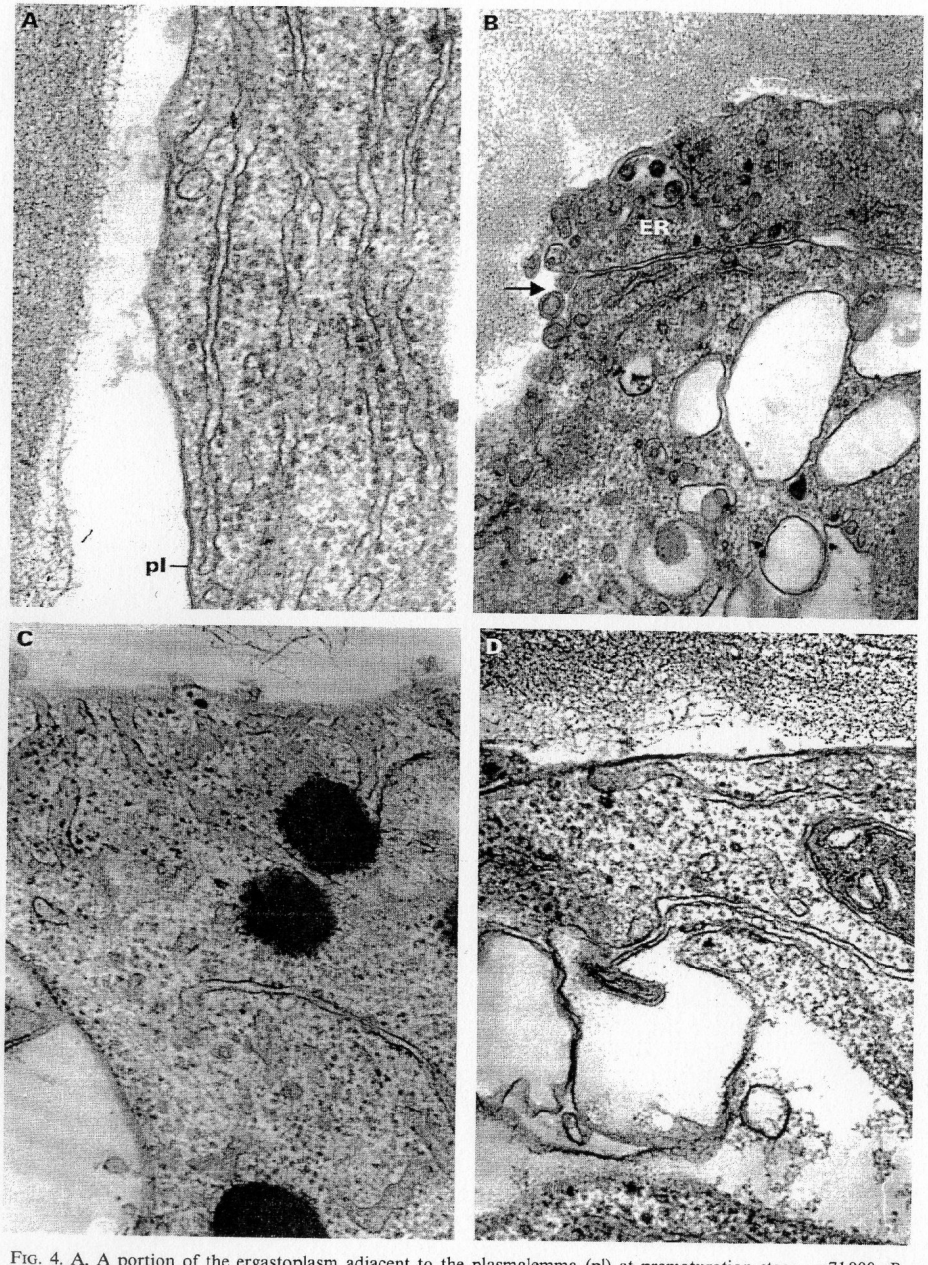

FIG. 4. A, A portion of the ergastoplasm adjacent to the plasmalemma (pl) at prematuration stage, ×71000; B, anastomosis of the endoplasmic reticulum (ER) with the plasmalemma (arrow), ×45000; C, an aggregation of the agranular tubules in contact to the plasmalemma, ×52000; D, the ER membranes continuous with the tonoplast of a vacuole, ×58000.

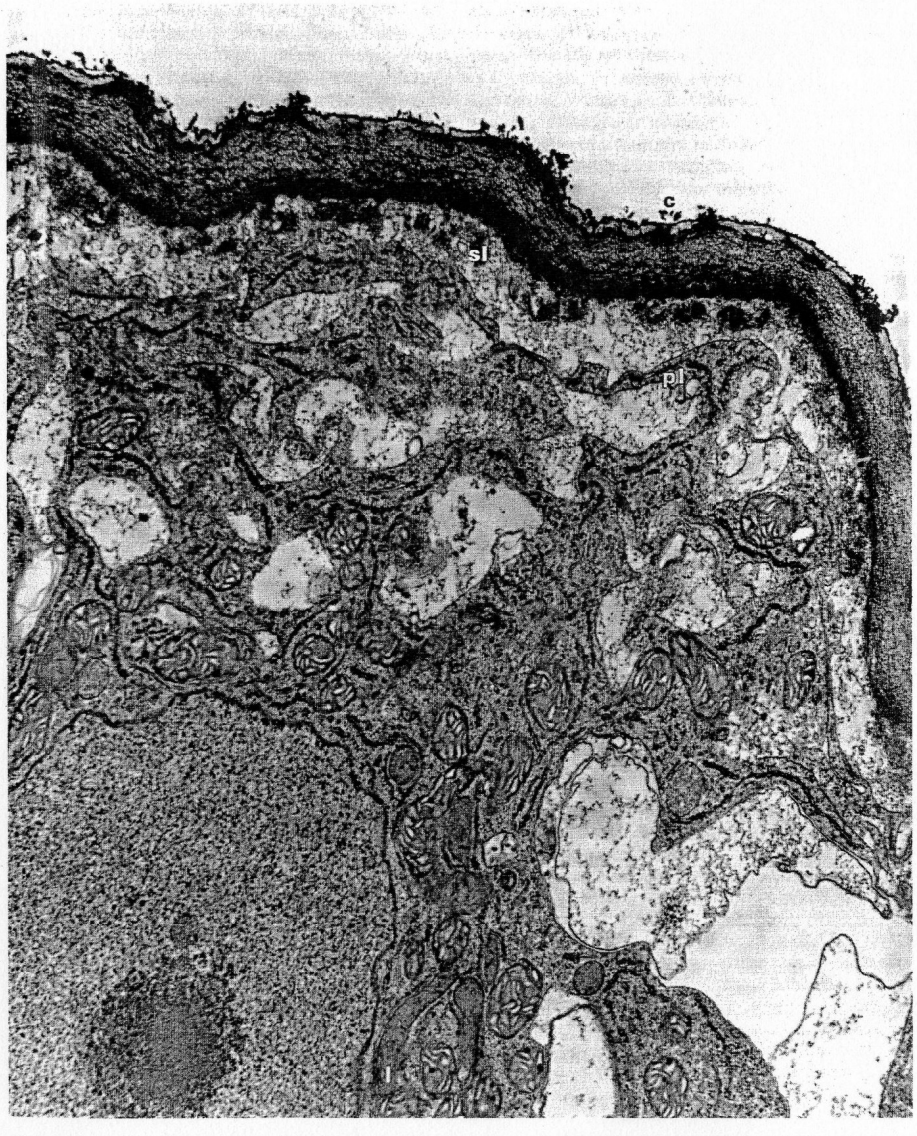

FIG. 5. A portion of a secretory cell in the mature digestive gland, pl, plasmalemma; l, leucoplast, sl, slime layer; c, cuticle with interruptions, ×18000.

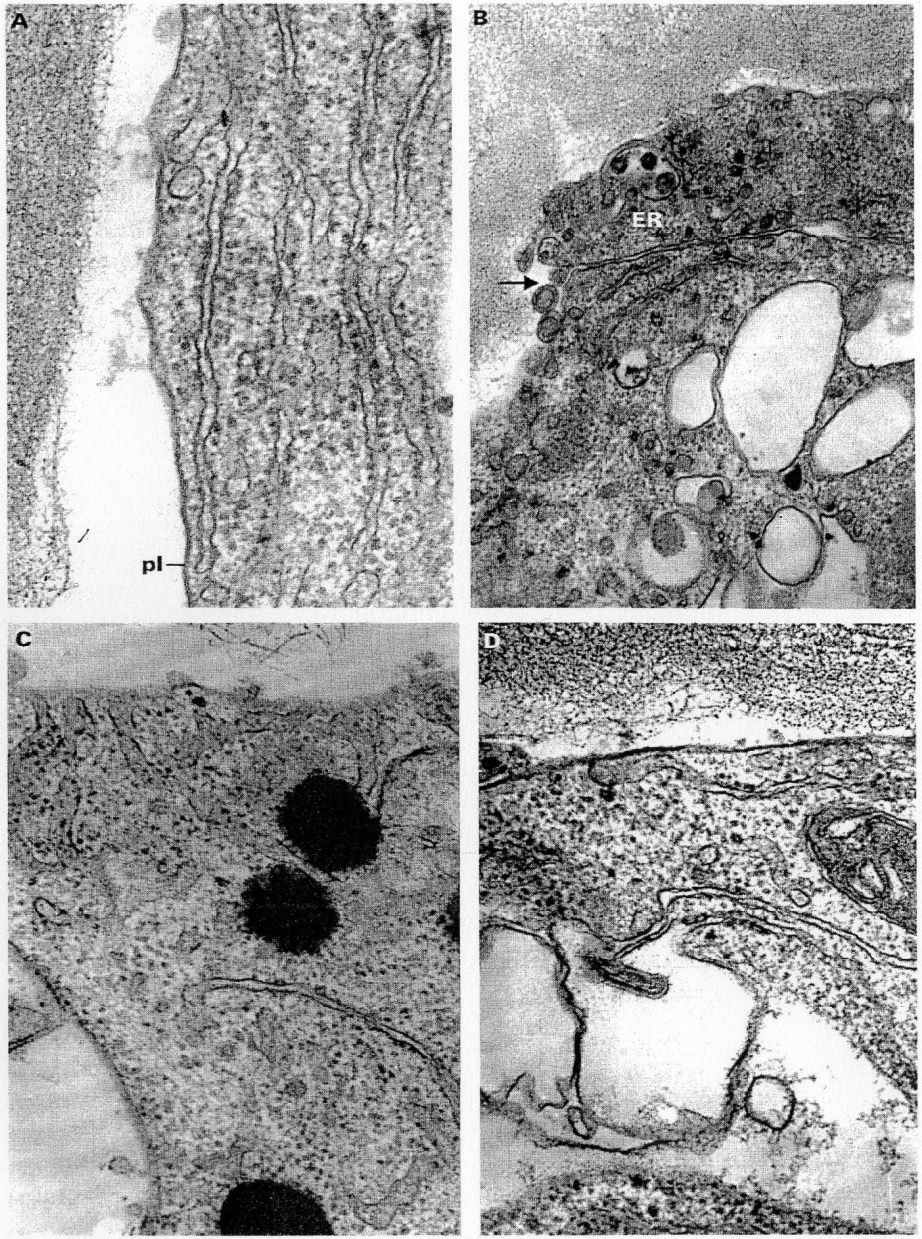

FIG. 4. A, A portion of the ergastoplasm adjacent to the plasmalemma (pl) at prematuration stage, ×71000; B, anastomosis of the endoplasmic reticulum (ER) with the plasmalemma (arrow), ×45000; C, an aggregation of the agranular tubules in contact to the plasmalemma, ×52000; D, the ER membranes continuous with the tonoplast of a vacuole, ×58000.

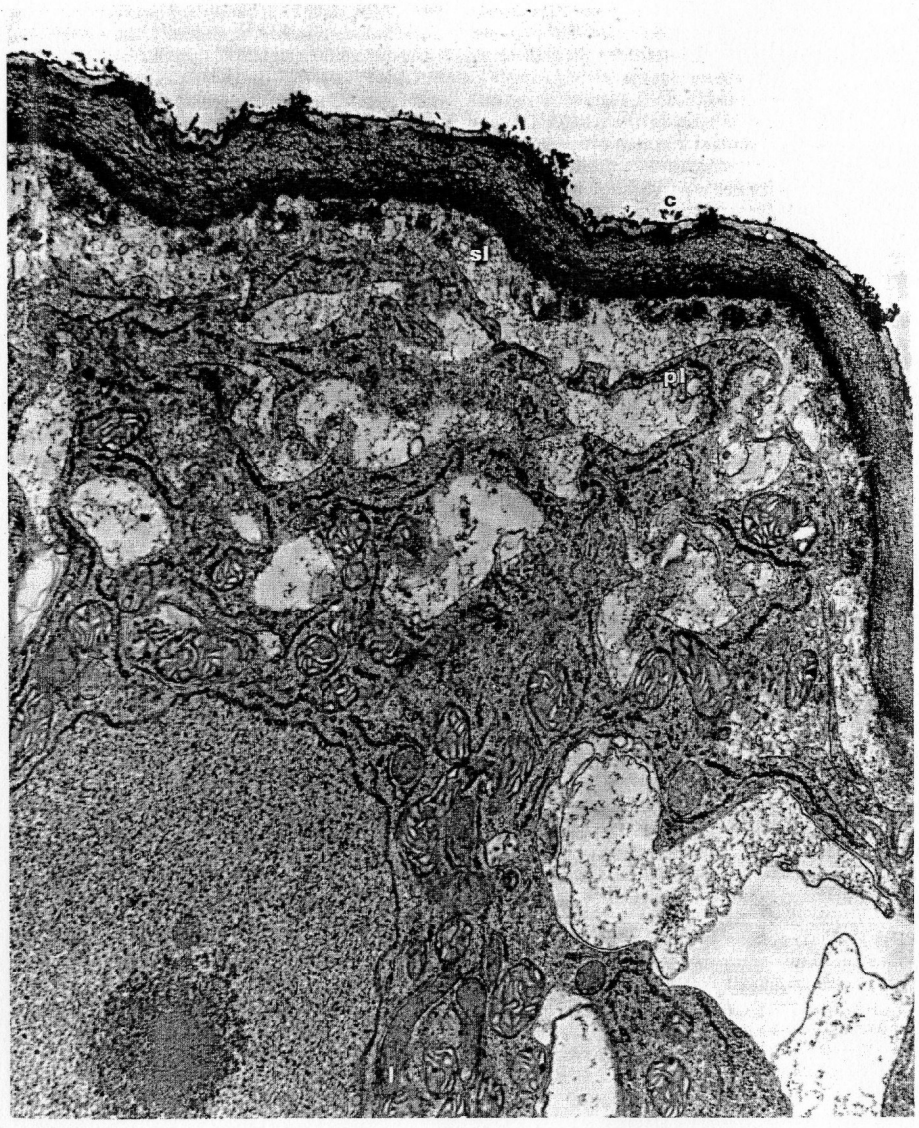

FIG. 5. A portion of a secretory cell in the mature digestive gland, pl, plasmalemma; l, leucoplast, sl, slime layer; c, cuticle with interruptions, ×18000.

distinguish the isolated vacuoles from those fused or fusing with the periplasmic space. Through the exocytosis of large vacuoles, the plasmalemma may acquire its irregular outline.

The Golgi apparatus becomes active at the cell division phase in the head of the glands. Dictyosomes become abundant and bud off small coated and large, smooth vesicles with fibrillar content (Fig. 3C). The former were also noted by Heslop-Harrison and Heslop-Harrison (1981) in the *P. grandiflora* gland head. During maturation of the secretory cells, the Golgi apparatus becomes even more active, both in number of dictyosomes and production of vesicles. The volume fraction of the Golgi apparatus reaches its maximum before maturation and prior to the emergence of the leaf from the bud. In the fully expanded leaf, Golgi activity falls to its initial level (see Table 2) the number of vesicles is drastically reduced and the fibrillar vesicles totally disappear (Fig. 3D).

During the maturation phase, both types of secretory vesicles are seen throughout the hyaloplasm. Evidence for their exocytosis into the slime layer can be seen in Fig. 3E. Direct continuity between the ER elements and dictyosome cisternae was not detected though both frequently lie near each other.

The change in the amount of the ER follows the same pattern (see Table 2). The volume fraction of the ER at prematuration and maximum secretory activity is four times as great as in the terminal cells in the three-celled glands. The stacks of parallel RER and the zones of the ergastoplasm appear at this stage (Fig. 4A). Some cisternae, here and there in the cell, form interrupted and parallel contacts with the plasmalemma. The membranes very occasionally show direct continuities (Fig. 4B). A mesh of tubules of SER appears and its density increases. Some tubules make contact with the plasma membrane (Fig. 4C) and closely follow its outline. Other tubules make contact with the cisternae of the granular ER.

During the whole of maturation, provacuoles continue to be formed from dilation of the ER elements. Sometimes a continuity can be observed between the ER membranes and the tonoplast of fairly large vacuoles (Fig. 4D). During cell division in the gland head, the number and length of the cristae in mitochondria increases. As the gland matures, the cristae become distended and parallel; and the mitochondrial matrix becomes dense. The volume fraction of mitochondria also gradually increases, reaching its maximum in the mature glands (see Table 1).

Little can be added to the description of the leucoplasts of the secretory cells given by Heslop-Harrison and Heslop-Harrison (1981). The formation of ER sheaths around the plastids starts when the cells are still dividing. Initially, the ER cisterna surrounds only a small portion of a plastid surface but finally becomes nearly continuous. In the fully mature cells, the sheath remains unchanged.

In conclusion, in the mature unstimulated secretory cells, all the components retain their normal ultrastructure. No sign of the degeneration mentioned by Heslop-Harrison and Heslop-Harrison (1981) was observed (Fig. 5).

DISCUSSION

As was first shown by Heslop-Harrison (1976*a*), and later confirmed by Muravnik (1983), the principal storage site of the acid hydrolases used to digest prey is, in this species of *Pinguicula*, the 'spongy layer' of the sessile glands of the leaves. This layer is filled with a fibrillar–granular material of various densities and includes cytoplasmic evaginations. Judged from developmental, ultrastructural and cytochemical evidence, it is not a typical labyrinthine wall characteristic of transfer cells, such as are found in the secretory cells of the digestive glands in *Drosophyllum* (Schnepf, 1961*a*), *Dionaea* (Schwab, Simmons and Scala, 1969; Robins and Juniper, 1980*a*) and *Genlisea* (Heslop-Harrison, 1976*a*). Firstly this layer in *Pinguicula* differs from a normal cell wall in chemical composition. As Heslop-Harrison and Heslop-Harrison (1981) demonstrated histochemically, this layer is filled with pectins and, unlike typical wall ingrowths (Gunning and Pate, 1974), does not contain cellulose. Further the true labyrinthine wall is initiated as the plasmalemma invests protrusions of the cell wall which gradually grow into the cell lumen and fuse with each other (Gunning and Pate, 1974). The densities reminiscent of wall protuberances appear, as we have shown, in the secretory cells only at the advanced stages of periplasmic space. They are formed with no connection with the plasmalemma, and often deeply inside of the periplasm. The overall electron density of the layer in section is invariably much lower than that of the cell wall. There is, therefore, no typical labyrinthine wall in *Pinguicula* which is a part of the cell wall, but a water-soluble pectic mucilage of semi-fluid consistency which is readily leaked out during a fixation procedure. Ultrastructurally the slime layer of the *Pinguicula* digestive gland is very similar to the pectic slime deposit in the periplasmic space of the slime-secreting glands of *Mimulus* (Schnepf and Busch, 1976), *Zebrina* (Bouchet, Audran and Bobichon, 1985) and *Timmiella* (Ligrone, 1986). Interestingly, Dexheimer (1976) who studied the glands in *Drosera* also showed the mucilaginous nature of deposits in the periplasmic space of the gland cells and referred to this as a slime layer. Although the major role of the slime layer in *Pinguicula* is enzyme storage, it may also function as a labyrinthine wall of transfer cells through the cytoplasmic evaginations which enlarge the area of the plasmalemma. However, as will be discussed in the second paper of the series (Vassilyev and Muravnik, 1988), these evaginations obviously play a role in the active efflux of ions from stimulated head cells and in the absorption of digestive products as opposed to enzyme secretion as Heslop-Harrison and Heslop-Harrison (1981) suggest. In the secretory cells of the *Nepenthes* digestive glands, wall protuberances became apparent when the secretion of digestive fluid into pitcher cavity is complete and the absorption of soluble products is in progress (Vassilyev, 1977). Also, in *Dionaea*, during the phase of enzyme release, the labyrinthine walls disappear from the secretory cells and the plasmalemma pulls away from the anticlinal walls (Schwab *et al.*, 1969). The wide (up to 5 μm) periplasmic space thus formed is filled with filamentous material and is similar to that of the slime layer in *Pinguicula*.

The secretory cells of the digestive glands in *P. vulgaris*, as indicated by their ultrastructure, reach the maximum of their activity in the leaf primordia shortly before the emergence from the bud. At this stage the cells contain a very active nucleolus, chondriome and Golgi apparatus, well developed granular and agranular ER and numerous leucoplasts, completely invested by RER.

In the non-secretory cells of the leaf, these organelles (except the plastids in mesophyll) are much less developed, and the plastids lack sheath. The above gland cell components somehow or other contribute to the synthesis and secretion of hydrolases and pectic material. These processes are also thought mainly to be confined to the prematuration stage of gland development, as Heslop-Harrison and Heslop-Harrison (1981) suggested. This conclusion is in agreement with the idea put forward by Vassilyev (1977) for *Nepenthes*, and later confirmed by Robins and Juniper (1980*b*) for *Dionaea*, that the synthesis of hydrolytic enzymes occurs predominantly in developing rather than in fully mature digestive glands.

In secretory cells of maturing sessile glands of *P. vulgaris* the most striking change is the proliferation of the cisternae of the RER. There is a fourfold increase in the volume fraction of the RER (Table 2) accompanied by the formation of the regions of the ergastoplasm typical of protein-secreting plant and animal cells (Vassilyev, 1977). There is, however, no rise in the surface density of free ribosomes at the onset of enzyme deposition and, moreover, their total amount decreases as the hyaloplasm volume is reduced. It appears, therefore, that the synthesis of secretory enzymes in *P. vulgaris* glands occurs on the membrane-bound ribosomes of the RER. This suggestion was first made by Schnepf (1961) who presented the first description of the ultrastructure of the *P. bakeriana* mature gland and found that, in this plant, the RER is well developed in both digestive and slime glands, particularly in the former.

A similar conclusion on the role of the RER has also been reached in the studies of *Dionaea* digestive glands (Robins & Juniper, 1980*b*; Juniper, Hawes and Horne, 1982) using EM autoradiography and morphometry. We disagree with Heslop-Harrison and Heslop-Harrison (1981) that, in the glands of the species of *Pinguicula* they studied, the synthesis of digestive enzymes occurs on the free ribosomes. The authors did not study the changes in the proportion of free and membrane-bound ribosomes during gland development and simply noted that the RER and free ribosomes were moderately abundant in the head cells.

We consider, below, the possible mechanisms of intracellular transport of digestive enzymes from the RER cisternae to the vacuoles and the cell wall (including the slime layer) of secretory cells in this species. Besides these two major depository sites, a certain proportion of hydrolases may be stored in the unstimulated mature secretory cells within the lumina of the RER cisternae themselves and within the SER tubules which are continuous with the cisternae (Heslop-Harrison and Heslop-Harrison, 1981; Muravnik, 1983). Robins and Juniper (1980*b*) concluded that in the unstimulated digestive glands of *Dionaea* the SER may be a major site for the storage of secretory proteins.

In *P. vulgaris* digestive enzymes may be transferred directly from the ER into vacuoles and slime layer through the continuity of the reticulum membranes with the tonoplast and plasmalemma. Such a mechanism seems to be the principal way of vacuole initiation and growth and is observed frequently in *P. vulgaris*. A similar mechanism has also been suggested by Heslop-Harrison (1975) for *P. grandiflora*. Also, in *Dionaea* no connections have been seen between the ER and vacuoles (Robins and Juniper, 1980*b*). However, the authors think that the smooth profiles of the ER may produce small vacuoles which they demonstrated to be an important intracellular store of proteins ready to be secreted.

We also showed connections between the ER membranes and the plasmalemma. Their low frequency may be explained by their dynamic (transient) character as well as by the difficulties in

their unequivocal demonstration in thin sections. Robins and Juniper (1980*a*, *b*) considered that in *Dionaea* the formation of the anastomoses is the only mode of hydrolase secretion outside the plasmalemma.

In *P. vulgaris*, certain enzymes appear to pass through the Golgi apparatus, though we obtained no ultrastructural evidence as to how proteins are transferred from the ER into dictyosomes. The involvement of the Golgi apparatus in secretion is supported by the increase in its activity parallel to the proliferation of the ER in maturing secretory cells and its decrease in the mature glands. The enzymes may be modified within the Golgi cisternae and then secreted via exocytosis of coated Golgi vesicles which were observed in association with both the dictyosomes and plasmalemma. Coated vesicles appear also to be involved in the export of proteins in the leaf glands of *Pharbitis nil* (Unzelman and Healey, 1974). In animal cells they are one of the components of the lysosomal system and may transfer hydrolytic enzymes from the Golgi apparatus to the cell exterior (Lemansky *et al.*, 1987). The other type of Golgi vesicles (smooth, with fibrils) found in the *P. vulgaris* glands appear to export pectin-rich material of the slime layer.

The leucoplasts, with their sheath of ER, may perform a regulatory function in the accumulation of hydrolases. A similar role was reported recently (Bajracharya *et al.*, 1987) for plastids of cotyledonary cells in the control of the development of peroxysomal enzymes during germination.

The cortical microtubules, characteristic of the secretory cells during the period of secretion, appear to control the directed transport of the exocytic vesicles into certain regions of the protoplast surface and their subsequent fusion to the plasmalemma. They also may play a role in drawing off the plasmalemma from the cell wall to form the periplasmic space.

Finally, the secretion of hydrolases into the cell wall appears to proceed by the exocytosis of some vacuoles. This mode of the vacuolar enzyme release is supported by the similarity in structure between the tonoplast and plasmalemma, between the vacuolar content and the third component of the slime layer and the negative correlation between the volume fraction of the vacuome and that of the slime layer (see Fig. 8E in Heslop-Harrison and Heslop-Harrison (1981) with a different interpretation). The exocytosis of vacuoles was also suggested by Figier (1969) for the protein-secreting leaf glands of *Mercurialis* and by Sevinate-Pinto (1982) for the hydrolase-secreting aleurone layer of *Avena*. However, demonstration of the stages of exocytosis, which are very fast, with ultrathin sections, is difficult. Moreover, chemical fixation damages membranes, making their identification even more difficult (Morgenstern, Neumann and Patscheke, 1987).

Heslop-Harrison and Heslop-Harrison (1981) believe that hydrolases are released by direct perfusion through the plasmalemma. This eccrine method seems unlikely. According to these authors, enzyme secretion in *Pinguicula* via exocytosis and luminal continuities, calls into question the significance of the transfer-cell type labyrinths in secretory cells. In our treatment of the periplasmic space as a slime layer there is no discrepancy with the above postulated secretory mechanism. In our scheme there is no need to protect the protoplast from its own potentially autophagic enzymes since they are always suitably packaged in the membrane-bound compartments and are never in a soluble state inside the protoplast.

The data presented here establish beyond doubt that the head cells in *P. vulgaris* remain intact in the glands of the mature leaves. Heslop-Harrison and Heslop-Harrison's conclusion (1981) that in *P. grandiflora* these cells undergo an autolysis during the final stage of their maturation, and on the mature leaves they are simply 'a sac of enzymes' is not applicable to our species. As it will be shown in detail in the second communication of this series, in *P. vulgaris* the gland cells remain active even some days after stimulation and begin to regress only at senescence of the whole leaf.

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