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The Digestive Glands of *Pinguicula*: Structure and Cytochemistry

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ABSTRACT

The digestive glands of the carnivorous genus *Pinguicula* have three functional compartments, (*a*) a basal reservoir cell, (*b*) an intervening cell of endodermal character and (*c*) a group of secretory head cells. The gland complex is derived from a single epidermal initial. The reservoir cell, which is rich in Cl^- ions, is highly turgid before discharge; it is linked by plasmodesmata to the surrounding epidermal cells, and is ensheathed by a pectin-rich inner wall layer. The endodermal cell is bounded by a Casparian strip to which the plasmalemma is tightly attached; it contains abundant storage lipid and numerous mitochondria. The head cells of the developing gland have labyrinthine radial walls of the transfer-cell type, the ingrowths being composed of pectic polysaccharides. The bounding cuticle is discontinuous, although lacking well-formed pores. Mitochondria are numerous, with well-developed cristae; the plastids are large and ramifying, and invested by ribosomal endoplasmic reticulum. Dictyosomes are sparse, and where they occur, are associated with coated vesicles. Ribosomal endoplasmic reticulum is moderately abundant in the head cells, and so also are free ribosomes. Optical and electron microscopic localization methods indicate that the digestive enzymes are synthesized in the head cells and transferred both into the vacuoles and into the walls. There is no evidence of a granulocrine mode of secretion, and the transfer seems to be initially by direct perfusion through the plasmalemma. During the final phase of maturation of the head cells they suffer a form of autolysis, vacuoles, cytoplasm and wall becoming confluent as all of the membranes of the cell undergo dissolution. The gland head is thus, in effect, simply a sac of enzymes at the time of the ultimate discharge.

Key words: *Pinguicula*, carnivorous plant, insectivorous plant, enzyme secretion, digestive gland.

INTRODUCTION

The structure of the digestive glands of the carnivorous plant genus *Pinguicula* was investigated first by Klein (1883) and later by Fenner (1904), who described the cellular organisation in some detail. The gland complex consists of three compartments, (*a*) an enlarged basal cell, (*b*) overlying this, a barrel-shaped cell bearing a cutinized peripheral wall (Haberlandt, 1914) and thus having something of the character of an endodermal cell and (*c*) a group of 4–32 secretory head cells. The basal cell is highly turgid in the unstimulated gland and is rich in Cl^- ions (Heslop-Harrison and Heslop-Harrison, 1980); because of its role in providing the main water source during the early phase of gland discharge, we will refer to it here as the reservoir cell. The enzyme-secreting glands of all carnivorous plants share this same type of organization, the differences lying in the numbers of secretory head cells and of cells contributing to the underlying endodermal layer, and in the character of the reservoir tissue, which in some genera – such, for example, as *Drosera* – may be tracheidal (Heslop-Harrison, 1975, 1976).

The first electron microscopic study of the digestive glands of *Pinguicula* was made by Vogel (1960), who recorded the fact that the head cells of the glands of *P. vulgaris* of the age he investigated contained large plastids of strikingly irregular form, closely invested by sheets of endoplasmic reticulum. The enveloping membranes were found to bear ribosomes on the outer face, but not on that apposed to the plastid surface. Further observations were made by Schnepf (1961, 1963) who found that in *P. bakeriana*

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the mucilage-secreting head cells of the stalked glands possessed a large population of dictyosomes in the 'active' configuration with numerous peripheral vesicles, while the sessile digestive glands had few dictyosomes, and these with only small numbers of associated vesicles. Schnepf also showed that certain walls of the head cells of the sessile glands bore ramifying ingrowths of the type that has since become associated with 'transfer cells' (Gunning and Pate, 1969).

The digestive glands of *Pinguicula* share with those of *Dionaea muscipula* and *Drosophyllum lusitanicum*, both of the Droseraceae, the special characteristic that they do not secrete until stimulated by the presence of prey; in this respect they differ from the corresponding glands of most other carnivorous plant genera, which secrete during part of all their active life without stimulation. In his remarkable work on *P. vulgaris*, Darwin (1888) found that a wide variety of nitrogen-containing substances promoted the release of secretion, and observed that a visible secretion pool appeared on the leaf within 40 min of the application of fragments of roast meat. We have found that individual glands of a fresh, turgid leaf may in fact begin to secrete within 10 min of stimulation, the flow continuing thereafter for up to 2 h. The digestive enzymes are present from the onset, indicating that they are present in a stored state in the unstimulated gland. This was confirmed by Heslop-Harrison and Knox (1971), who used optical-microscopic cytochemical methods to localize a number of enzymes in the unstimulated glands of *P. grandiflora* and to follow their release on stimulation. Acid phosphatase and non-specific esterase activity were found to be distributed throughout the head cells, with a heavy deposition of reaction products also associated with the spongy anticlinal walls. There was no indication of the association of activity with particulate structures in the intact cells, and the conclusion reached from the uniform distribution of reaction product in the upper part of each head cell was that the enzymes were likely to be held in vacuole-like inclusions.

These observations left certain questions unanswered. Clearly there is a progressive transfer of enzymes into the wall before the onset of bulk secretion, but the details of the process have been obscure hitherto. Schwab, Simmons and Scala (1969) have speculated that a granulocrine system involving the Golgi apparatus may operate during the secretion phase in *Dionaea muscipula*; yet this system can hardly be concerned with the transfer of proteins in the digestive glands of *Pinguicula*, since dictyosomes are few in number in the head cells during the main period of enzyme accumulation, and have limited associated vesicle populations (Schnepf, 1961). A further possibility is that products are transferred into the wall through continuities between the endoplasmic reticulum and the plasmalemma, in the manner suggested by Unzelman and Healey (1974) for the movement of the protein–carbohydrate secretion product of the leaf trichomes of *Pharbitis nil* (Convolvulaceae). However, the postulation of a granulocrine system, whether involving dictyosome vesicles or the endoplasmic reticulum, calls into question the significance of the transfer-cell type wall labyrinths in the *Pinguicula* gland head cells. The effect of the development of the wall ingrowths is to increase the area of the plasmalemma for a given volume of cell content, amplifying the symplast/apoplast interface. Pate and Gunning and their collaborators (e.g. Pate and Gunning, 1972; Gunning and Pate, 1974; Browning and Gunning, 1979) have pointed out that where the passage of water and solutes is limited by the transporting capacity of the plasmalemma, the development of wall ingrowths could increase the potential rate by enlarging the effective area of the membrane, and that this would be true were the movement across the membrane to be by diffusion, by mass flow as a consequence of a pressure gradient, or by the action of 'pumps' in the membrane itself. It might be argued that such an adaptation would be redundant if the principal method of mass transport were some form of granulocrine secretion, since the transporting capacity of the plasmalemma would not then be a major factor.

In the case of the enzyme-secreting head cells of the *Pinguicula* gland, a related problem concerns which compartment of the cell holds the secretion product *before* the passage into the wall. A granulocrine mode of transfer would imply that the enzymes are accumulated initially in the lumina of the endoplasmic reticulum–Golgi system, the generally accepted process in enzyme-secreting animal cells (Palade, Siekevitz and Caro, 1962). To postulate an eccrine mode of secretion would, on the other hand, mean accepting that the enzymes exist, at least transitorily, in the cytosol. Such an hypothesis may seem less than attractive, since it is widely supposed that the protection of a cell from its own potentially autophagic enzymes requires that they should be suitably packaged (Matile and Wiemken, 1976). An eccrine mode of secretion seems, nevertheless, to be established for one type of enzyme secretion in plants: the transfer of α-amylase and 1,3-glucanase from the aleurone cells of the barley caryopsis (Jones, 1969, 1972). These enzymes are present in soluble state in the cell, and their release appears not to be associated with any vesicular activity. Indeed, the attack on wall glucans during the secretion phase occurs immediately on the outer face of the plasmalemma, suggesting a direct mode of release.

In the present study of the digestive glands of *Pinguicula*, we have followed in some detail the final stage of development leading up to the point where the head cells are set to discharge upon stimulation by the prey. We find that none of the models hitherto proposed for protein secretion by plant cells precisely covers the events during this period. While the vacuoles seem indeed to constitute a 'lytic compartment' in the sense of Matile (1974) and probably receive enzymes from the lumen of the endoplasmic reticulum system, there appears also to be a synthesis in the cytosol and a direct transfer into the labyrinthine walls. Although in the nature of the case it is scarcely possible to assert that a granulocrine system *never* operates in the passage of products into the wall, we have been unable to trace evidence of exclusion by a vesicular system or by continuities between the plasmalemma and the membranes of the endoplasmic reticulum, and conclude that neither of these is likely to be the main means of transfer.

However, it is the events during the final maturation of the gland that provide the most unusual features. At this time the head-cell contents undergo a kind of autophagic process, during which the membrane systems are degraded. The thickenings of the labyrinthine wall progressively become confluent with the vacuolar system of the cell, ultimately forming a single enzyme-containing compartment. This is the state of the cells of the gland head at the time when the leaf surface is prepared for the capture of prey, but *before* the stimulation that leads to the discharge of the gland contents. The secretion is thus of an 'interrupted' holocrine type.

In the present paper we do not deal with the discharge and resorption phases of the cycle of gland function; an account of these will be given in a second paper.

MATERIALS AND METHODS

Plant material

The observations were made on *Pinguicula grandiflora* Lamck., *P. ionantha* Godf. and *P. moranensis* Kunth, growth in the greenhouse or in small growth chambers.

Optical microscopy

For the observation of living glands, fragments of unstimulated leaves were mounted in water, or water–glycerine mixtures, and viewed directly. Material for sectioning was fixed as for electron microscopy (details below) and embedded in JB4 resin (Polysciences Inc.). Sections were cut with glass knives at thicknesses of 0·5–2·0 μm.

For the cytochemical characterization of wall components, both fresh, intact and

fixed, sectioned glands were used. The standard periodic acid-Schiff (PAS) procedure was used for the general localization of polysaccharides with 1,2-glycol groups (Pearse, 1968). Wall polysaccharides were also stained with the fluorescent whitener Calcofluor white MR2 (Polysciences Inc.), used at a concentration of 0·05 or 0·1 per cent in aqueous solution. Chemical evidence indicates that this stain has affinity with 1,3- and 1,4-linked glucans (Maeda and Ishida, 1976; Takeuchi and Komamine, 1978). We do not find that it reveals meiocyte or pollen tube callose, but it stains strongly wall components that are seen, electron-microscopically, to have a microfibrillar component after the extraction of pectins and hemicelluloses (J. Heslop-Harrison, 1979). From this evidence it seems reasonable to assume that the wall component fluorescing after calcofluor white staining is indeed likely to be cellulose.

Pectic substances were localized by alcian blue and ruthenium red staining. Alcian blue 8GX (R. A. Lamb) was made up at 1 per cent in 3 per cent acetic acid, giving a final pH of 2·5. At this pH the dye is considered to react mainly with uronic acid groups (Pearse, 1972). Ruthenium red was used in 0·02 per cent aqueous solution (Gurr, 1965). This dye has long been used for the detection of pectins in plant tissues, and more recently has been adopted for the electron-microscopic localization of cell surface polysaccharides in animal tissues (Luft, 1964).

Callose was sought by its fluorescence in decolourized aniline blue (Eschrich and Currier, 1964). The justification for accepting that this procedure detects a distinct structural wall-glucan rich in 1,3-linkages has been discussed elsewhere (J. Heslop-Harrison, 1979). The dye (BDH) was used at 0·05 per cent at pH 11.

For localization of cutin and suberin in both whole glands and sections, the fluorescent stain auramine 0 was used at 0·01 per cent in tris–HCl buffer at pH 7·2 (Heslop-Harrison, 1977). Lipids were detected in sectioned material with scarlet R in saturated solution in 70 per cent ethanol (Gurr, 1965).

Protein was localized in resin sections with Coomassie blue (0·25 per cent in water: Methanol:acetic acid, 87:10:3 v/v), and with Nobel stain (Coomassie blue, azocarmine and amido-black all at 0·25 per cent in water: ethanol:acetic acid, 60:30:10 v/v).

Electron microscopy

The sessile glands illustrated in Fig. 1A were observed in a Cambridge Stereoscan S600 scanning electron microscope in the fresh state without fixation or coating (Heslop Harrison, 1970).

Material for transmission electron microscopy was fixed (*a*) 1–4 h in 1·5 per cent glutaraldehyde in 0·05 M phosphate buffer, pH 7·2 at room temperature, followed by post-fixation in 1 per cent osmium tetroxide for up to 2 h at *c.* 4 °C, or (*b*) 2 h in 1 per cent potassium permanganate. The fixed material was washed, dehydrated through an ethanol series and then embedded in Araldite by standard procedures.

Sections from the potassium permanganate-fixed material were viewed without further treatment. Those from the glutaraldehyde–osmium tetroxide fixed leaves were post-stained in uranyl acetate (saturated solution in 50 per cent ethanol) and observed immediately or after further staining in lead citrate. The staining times were adjusted to produce the contrast of wall materials and cell contents required. In general, 20 min staining in uranyl acetate at 37 °C produced an adequate definition of wall microfibrils and membranes, and lead citrate staining of 5 or 10 min provided satisfactory contrast for ribosomes.

For the examination of wall-membrane associations, sections were stained with phosphotungstic acid (PTA), 1 per cent in 10 per cent HCl, for 5–20 min. This procedure has been said to add electron opacity, especially to the plasmalemma (Roland, 1978). The effect on the contrast of the membranes was indeterminate in *Pinguicula* gland preparations, but the stain was found to reveal components of the wall that were indistinguishable, or less conspicuous, following other staining procedures. Comparison with optical-microscopic preparations suggest that these might be areas of concentrated pectic materials.

The Thiery procedure (Roland, 1978) was adopted for the electron-microscopic localization of polysaccharide. Sections mounted on gold grids were oxidized for 20 min in 1 per cent periodic acid, washed, exposed overnight to 0·2 per cent thiosemicarbazide in 20 per cent acetic acid, transferred through an acetic acid series to water, and then exposed for 20 min to 1 per cent aqueous silver proteinate (TAAB) in the dark.

Enzyme localization

Acid phosphatase and non-specific esterase were adopted as markers for the group of lysosome-type hydrolases and transferases known to be present in the secretions of the digestive glands (Heslop-Harrison, 1975, 1976). Acid phosphatase was detected with naphthol AS-BI phosphate as a substrate in a coupling reaction with hexazotized pararosanilin (Barka and Anderson, 1962). For the localization of non-specific esterase, α-naphthyl acetate was used as a substrate and tetrazotized o-anisidine as the coupling reagent (Pearse, 1972). Controls were run with the full reaction mixture less substrate.

In the earlier study (Heslop-Harrison and Knox, 1971), the procedures used for localizing enzyme activity in the gland head cells gave good resolution, but certain ambiguities remained. An association with the walls of head cells was found in both intact and freeze-sectioned glands, but the possibility that some transfer might have taken place in consequence of the handling procedure, or indeed in response to stimulation by the substrate itself, was not entirely excluded. To eliminate this as a source of artefact, carefully excised samples of fresh, unstimulated leaves were dried to complete flaccidity over desiccant at 37 °C, and the localization procedure then applied. This treatment should prevent rapid passage of water through the gland head and so preclude any induced movements of the enzymes. The wall localization obtained was identical with that observed in fresh leaves.

Acid phosphatase activity was localized electron-microscopically using the method of Knox and J. Heslop-Harrison (1971), which is based upon that of Hanker *et al.* (1964). The substrate was 2-naphthyl thiol phosphate with fast blue BBN as a coupling agent, and electron opacity was given to the reaction product by exposure to osmium tetroxide vapour at 50 °C. After this treatment, leaf samples bearing glands were dehydrated through an ethanol series and embedded by standard methods. As in application to pollen (Knox and J. Heslop-Harrison, 1971), the procedure gave satisfactory localization of acid phosphatase activity in wall sites. However, the method produced disappointing results with intracellular structures because of the severe distortion of the cytoplasm caused by the combination of treatments. Nevertheless, it proved possible to identify some of the sites of the reaction product.

OBSERVATIONS

General organization

Mature glands of *P. grandiflora* are illustrated in the scanning electron micrograph of Fig. 1A. Figure 2 shows in diagrammatic form the structure of the individual gland as seen in vertical section, and indicates the relationship of the three functional compartments, (*a*) the basal reservoir cell, (*b*) the endodermal cell and (*c*) the head (or secretory) cells, to each other and neighbouring epidermal and mesophyll cells.

The whole gland complex is derived from a single epidermal cell through the sequence of divisions seen in Fig. 3A–E. The epidermal cell becomes papillate and then divides in

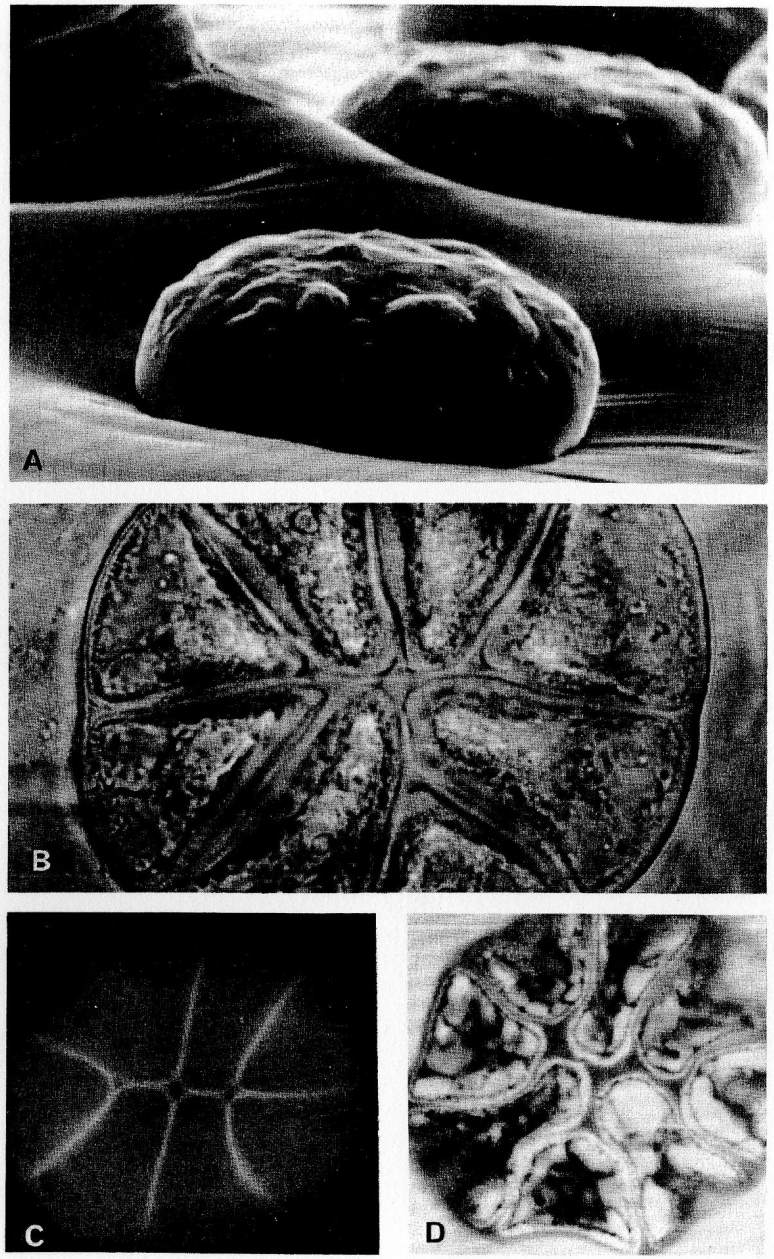

FIG. 1.

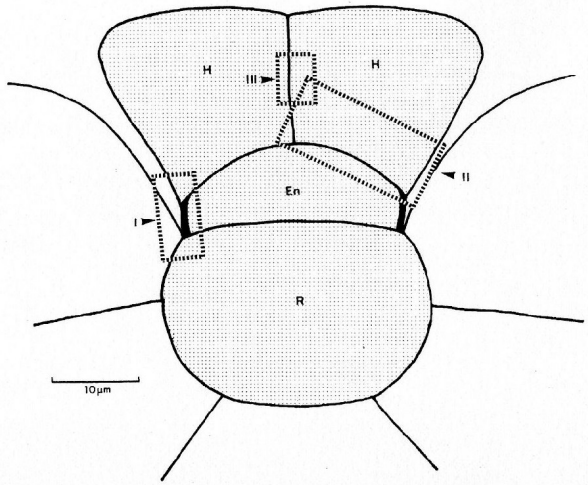

FIG. 2. Diagram of a median longitudinal section of a sessile gland from the upper leaf surface of *Pinguicula* spp. The gland (shaded) is partially sunken into the epidermis and surrounding mesophyll cells. It consists of three functional components, (a) a single reservoir or communicatory cell (R) (Heslop-Harrison, 1975, 1976; Heslop-Harrison and Heslop-Harrison, 1980) named by Goebel (1893) the basal cell, (b) a single endodermal cell (En) (Heslop-Harrison, 1975, 1976), variously named as the columellar cell (Klein, 1883), middle cell (Goebel, 1893) or endodermis or barrier cell (Schnepf, 1962, 1974) and (c) a small group of head or secretory cells (H). Dotted outlines mark the approximate positions of certain of the accompanying electron micrographs: I, Fig. 4A, II, Fig. 6A, and III, Fig. 8D.

the plane of the leaf to give two daughter cells. The lower of the pair maintains its interfaces with adjoining epidermal cells, and differentiates as the reservoir cell. The upper divides again in the paradermal plane to cut off the endodermal cell, which begins immediately to form the peripheral Casparian strip. Further divisions of the upper cell in the anticlinal plane then produce the head cells.

Cell numbers, gland head and endodermal cell dimensions, and frequency per unit area of mature leaf are given in Table 1 for three species of *Pinguicula*.

FIG. 1. A. Scanning electron micrograph of living, uncoated sessile glands of *Pinguicula grandiflora*. ×*c.* 1600. B. A living, undischarged gland of *P. ionantha*; optical micrograph, focus at the level of the labyrinthine radial walls. The distinction between the inner primary (cellulosic) wall and the wall ingrowths (probably pectic) is readily seen. At this stage of development the nuclei of the head cells lie towards the base and towards the periphery (cf. Fig. 3E). ×*c.* 2200. C. Fluorescence micrograph of an unfixed gland head of *P. ionantha*, calcofluor white staining. This staining reveals the microfibrillar, presumably cellulosic, part of the wall. ×*c.* 1250. D. Optical micrograph of a 1–1·5 μm section of resin-embedded gland of *P. grandiflora*, Nobel staining. The accumulation of protein in the apoplast is readily seen. ×*c.* 1300.

Key to abbreviations in Figs 1–9: C, cuticle; Co, core of wall ingrowths; Cs, casparian strip; Cv, coated vesicle; D, dictyosome; E, endodermal cell; Ep, epidermal cell; H, gland head cell; L, lipid droplet; Lw, labyrinthine wall; MW, microfibrillar wall; N, nucleolus; Ne, nuclear envelope; P, plastid; Pa, plasmalemma; Pf, pitfield; Pl, protein lamellae; Ps, plastid stroma; R, reservoir cell; T, tonoplast; V, vacuole.

TABLE 1. *Dimensions of gland heads and endodermal cells and cell number in the gland head, in three species of* Pinguicula

Species	Gland head		Endodermal cell diameter (μm)	Gland no. per mm^2
	Diameter (μm)	Cell no.		
P. grandiflora	37·75 ± 0·52	7·86 ± 0·06	25·44 ± 0·41	120
P. vulgaris	46·55 ± 0·72	8·00	25·68 ± 0·60	112
P. ionantha	36·10 ± 0·74	5·17 ± 0·11	23·21 ± 0·56	88

The reservoir cell

The reservoir cell, the first to be defined during the development of the gland (Fig. 3B), is the only one of the gland complex to maintain its interfaces with the adjoining epidermal cells. In *P. grandiflora* it retains contact with four to eight cells, which converge in upon it in a characteristic manner (Fig. 3F) recalling Haberlandt's (1914) 'collecting cell' configuration, presumed to facilitate translocation (Gunning, 1976). The intervening walls are perforated with pit fields, each traversed by 20 or more plasmodesmata (Fig. 4A). The reservoir cell, which is always turgid in the mature, undischarged gland complex, is also linked below to mesophyll cells.

The wall of the reservoir cell is lined by an inner coating, electron-transparent with normal staining procedures (Fig. 4A, C), but gaining electron opacity after PTA staining. What is presumed to be the same layer is visible with the optical microscope as a continuous ruthenium red-staining sheath, often with striations oriented at right angles to the plane of the leaf. A similar layer, but thinner and much less conspicuous, is occasionally observed in the adjoining 'collecting cells'.

The reservoir cell is linked with the overlying endodermal cell by numerous plasmodesmata, which appear invariably to have desmotubules (Fig. 4C, D).

The endodermal cell

In the mature gland complex, the endodermal cell has a plano-convex or concavo-convex form, the head cells adjoining the highly domed upper surface, with the reservoir cell closely apposed to the lower (Figs 3E and 4A). The cell is bounded laterally by the Casparian strip (Fig. 4A), the material of which has the staining properties attributed to cutin (Juniper and Martin, 1970). The strip is continuous with the cuticles of the

FIG. 3. A–E. Optical micrographs showing the sequence of divisions through which the gland complex is formed in *P. grandiflora*. A single epidermal cell (A) divides to set off the reservoir cell (B), which remains in plasmodesmatal connexion with neighbouring epidermal cells (F). The upper cell then divides to give the endodermal cell (C), which proceeds immediately to form the Casparian strip. Further divisions in the other cell then give the cells of the gland head (D and E). × *c.* 550. F. Optical micrograph of a 1–1·4 μm section of a resin-embedded leaf of *P. grandiflora* in the paradermal plane at the level of the top of the reservoir cell. The neighbouring cells of the epidermis radiate out from the reservoir cell in a manner reminiscent of Haberlandt's 'collecting cells'. × *c.* 300. G. Optical micrograph of the localization of esterase activity in the gland head of *P. ionantha*, α-naphthyl acetate substrate, tetrazotized o-anisidine coupling agent. The focal plane corresponds to that of Fig. 1B, C. The activity in the apoplast is clearly defined, and also the association of activity with the radial walls. The more diffusely distributed reaction product is associated with the vacuoles. × *c.* 1350. H. As G, focal plane at the surface of the gland head. Some reaction product is present on the surface, especially over the zones where the radial walls join the outer wall of the gland; this presumably indicates slow leakage from the head walls through the discontinuous cuticle in these sites. × *c.* 1350. I. Control for G and H; full reaction mix less substrate. × *c.* 1400.

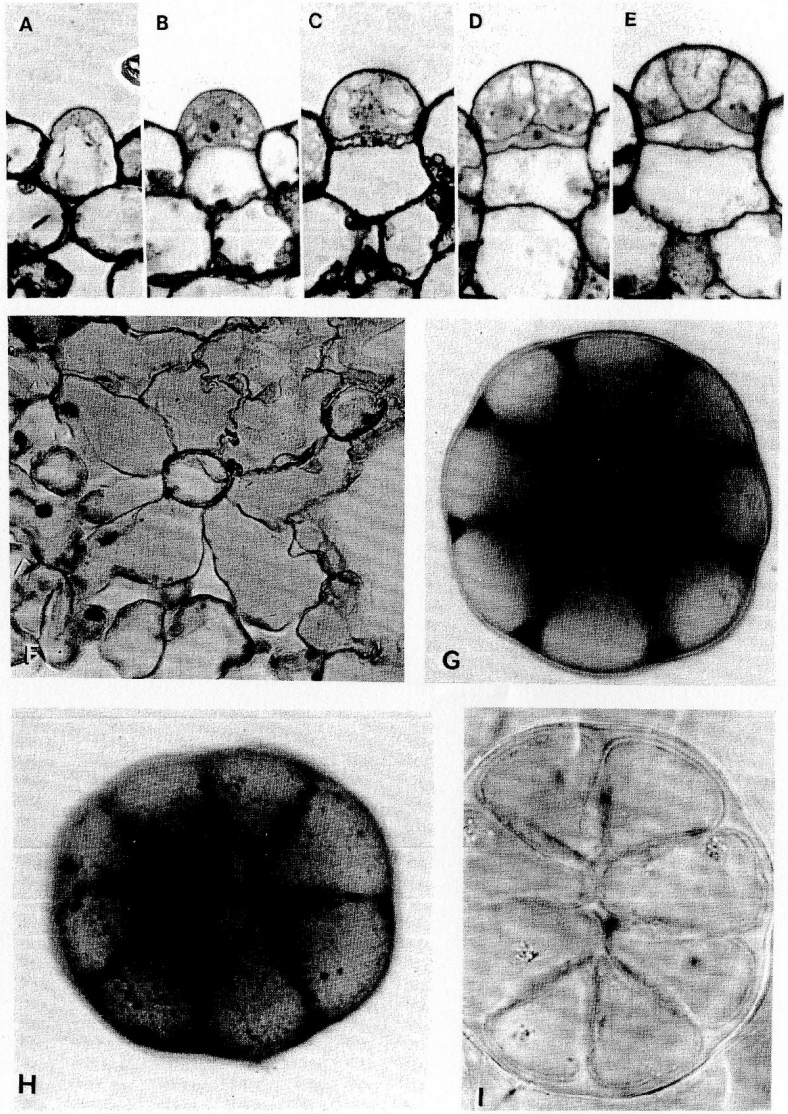

FIG. 3.

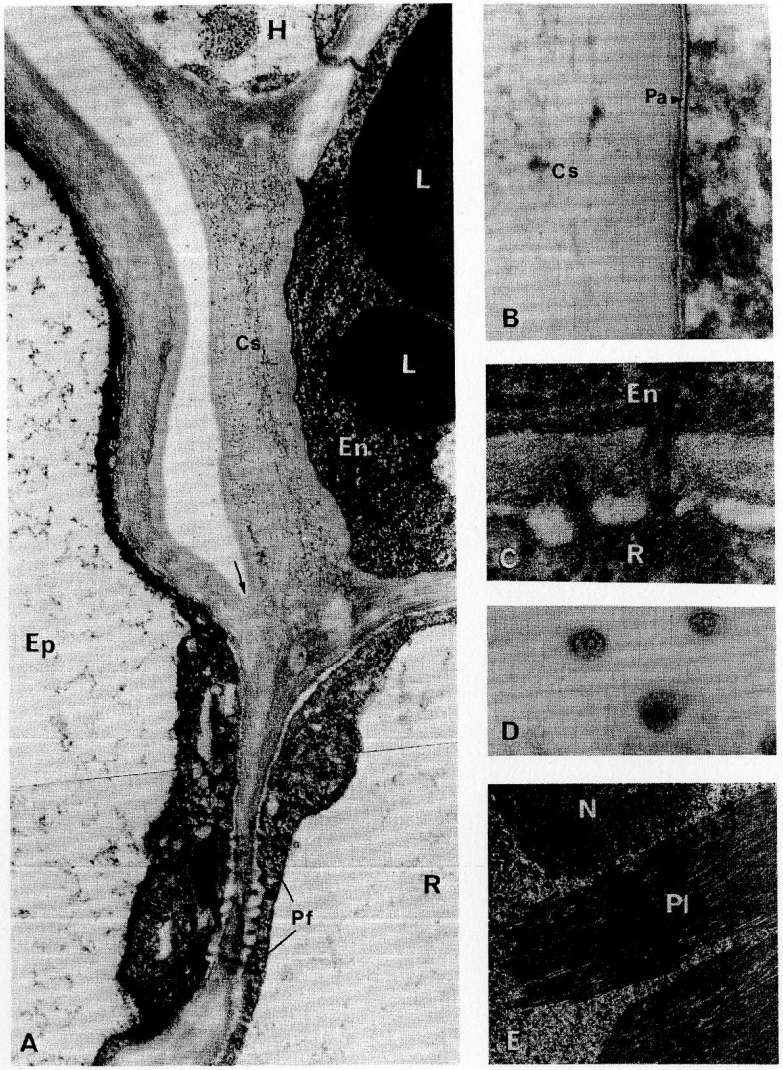

FIG. 4.

adjoining epidermal cells and with the less well-defined cuticles of the head cells. The plasmalemma of the endodermal cell makes intimate contact with the cutinized wall around the periphery (Fig. 4B), forming a 'tight junction' of the type familar in the root endodermis (Clarkson and Robards, 1975) and known in other glandular trichomes (Fahn, 1979).

The endodermal cell contains numerous mitochondria, smaller than those of the head cells, and usually one or more large lipid droplets (Fig. 4A). The plastids are inconspicuous, and without stored starch. The nucleus usually lies towards one side, and invariably contains quasi-crystalline aggregates, which may occupy as much as half of its volume (Heslop-Harrison, 1975; Fig. 4E). These inclusions were first noted in the cells of *Pinguicula* by Klein (1883), and they were investigated electron-microscopically by Schnepf (1960*a*) in various cells of the leaves of *P. bakeriana*. Thomas and Gouranton (1979) have recently studied the corresponding structures in *P. lusitanica* further, and have concluded that they are proteinaceous in nature and formed of stacked lamellae. Their function remains obscure.

The endodermal cell is linked to each of the head cells of the gland complex by plasmodesmata, again with desmotubules (Fig. 6A).

The head cells

The autophagic process which ultimately degrades all of the membranes of the head cells begins in the upper, vacuolate part and continues until all parts have been affected. The different cells of a single gland head do not behave synchronously, so that neighbouring cells may show the membrane systems in different stages of dissolution (Fig. 8B). Although the process is a continuous one, it is convenient for the purpose of description to consider first the state of the cells before the dispersion of the membranes, and then the events of the final phase of maturation during which the membranes are eliminated and vacuoles, cytoplasm and walls become confluent.

The walls

In the optical micrograph of a living gland of *P. ionantha* of Fig. 1B, the radial walls of the gland head clearly show the ramifying ingrowths of the transfer cell type, the primary walls forming a 'core' to the thickenings on either face. The staining reactions of the walls are summarized in Table 2. The primary wall stains heavily both with the PAS procedure and with calcofluor white, but the ingrowths show little affinity for the former and none at all for the latter. They do, however, stain lightly with alcian blue at pH 3·5 and with ruthenium red, and show metachromatic staining with toluidine blue at pH 5·6. These reactions indicate that the primary wall has a cellulosic component, but the ingrowths of the wall labyrinth are clearly not cellulosic. The stainability of the

FIG. 4. Electron micrographs of digestive glands of *P. grandiflora*. A. Longitudinal section of the central part of a digestive gland in the site indicated in Fig. 1. The three components of the gland complex – head cell, endodermal cell and reservoir cell – appear, and also a neighbouring epidermal cell. The reservoir cell is linked to the epidermal cell by plasmodesmata in the pit field, and also shows the inner electron-transparent wall layer, presumed to be that accepting ruthenium red staining. The Casparian strip of the endodermal cell is continuous above with the interrupted cuticle of the gland head cells, and below (arrow) with the cuticle of the adjacent epidermal cell. The wall between the endodermal cell and the head cells is highly domed (cf. Fig. 2), and traversed by numerous plasmodesmata. The cell itself is rich in stored lipid. × *c.* 28000. B–D. Plasmodesmata linking the endodermal and reservoir cells (B), in longitudinal (C) and transverse (D) sections, showing desmotubules. In C, the electron-transparent inner wall on the reservoir cell side is evident. C, × *c.* 115000; D, × *c.* 100000. E. Nucleolus and protein lamellae in the nucleus of an endodermal cell. × *c.* 27000.

TABLE 2. *Staining properties of the walls of the different components of the digestive gland complex in* Pinguicula

Stain	Head cells			Endodermal cell		Reservoir cell	
	Primary wall	Ingrowths	Cuticle	Primary wall	Casparian strip	Primary wall	Inner wall layer*
PAS	+++	(+)†	0	+++	0	+++	—
Calcofluor white	+++	0	0	++	0	+++	—
Alcian blue	+++	+	0	++	0	++	—
Ruthenium red	+++	+	0	+	0	++	++‡
Decolourized aniline blue	0	0	0	0	0	0	—
Toluidine blue	+++ (purple/red)	+ (pink)	+ (blue)	++ (purple/red)	0	++ (purple/red)	++‡ (pink)
Coomassie blue	+§	—‖	0	0	0	0	—
Nobel stain	+§	—‖	0	0	0	0	—
Auramine	?¶	0	+	0	++	0	—
Scarlet R	0	0	+	0	+++	0	—
Osmium tetroxide	0	0	+	0	++	0	—

0, No appreciable staining, +, light staining; ++, moderate staining; +++, heavy staining; —, ambiguous, or could not be resolved.

* In most instances this layer, conspicuous in electron micrographs, could not be distinguished with the optical microscope.

† PAS staining only apparent in thick sections.

‡ With conspicuous striations oriented at right angles to the plane of the leaf when observed in tangential section. It is an assumption that this layer corresponds with that seen in electron micrographs of this wall.

§ Localized in the region of the middle lamellae and at the cell corners; compare Fig. 1D.

‖ Ambiguous because of the interdigitation of wall and protoplast.

¶ Slight yellowish fluorescence, perhaps indicating dispersed lipid.

ingrowths is comparable with that of the middle lamella, and suggestive of a pectin polysaccharide composition.

The primary walls of the head cells show a microfibrillar structure with a high degree of order following standard uranyl acetate and lead citrate poststaining (Fig. 5B). The associated ingrowths are more electron-transparent, and microfibrils, when evident at all, are loosely dispersed. In favourable sections, the ingrowths show a slightly denser, non-fibrillar core (Fig. 5A). This core is increased in electron opacity following phospho-tungstic acid-HCl (PTA) staining (Fig. 5C). Polysaccharides reacting with the Thiery procedure are present in all parts of the wall (Fig. 5D). The silver depositions follow the orientation of the microfibrils in the primary wall, and are denser over the central, PTA-staining cores of the wall ingrowths.

The outer surfaces of the head cells are cutinized, but the cuticle is thin and

FIG. 5. Electron micrographs of the walls of gland head cells of *P. grandiflora* in the pre-maturation phase. A. Wall ingrowths arising from the microfibrillar radial wall, normal uranyl acetate-lead citrate staining. The plasmalemma is well-defined, and is seen to ensheath the ingrowths completely. The staining reveals occasional darker centres in the ingrowths, but no well defined cores. ×*c.* 85000. B. As A, extended uranyl acetate staining. The microfibrillar part of the wall is more clearly defined, and also some content in the ingrowths. ×*c.* 80000. C. A neighbouring section to that of A, PTA staining. The cores of the wall ingrowths are now very well defined, but the plasmalemma is less heavily stained. ×*c.* 73000. D. As A, Thiery procedure for the localization of polysaccharides. In the primary wall (Mw), the silver deposits follow the orientation of the microfibrils. The central PTA-staining cores of the ingrowths are reactive, and the plasmalemma (Pa) is also defined. ×*c.* 70000.

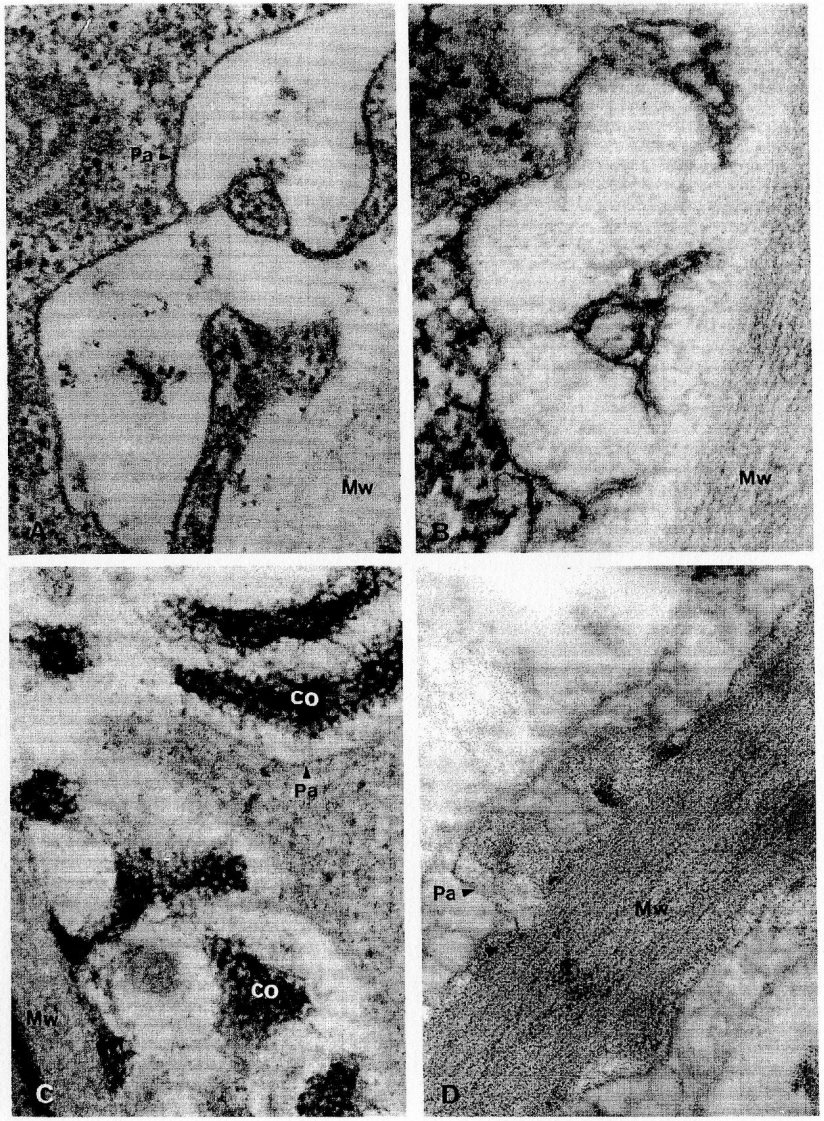

FIG. 5.

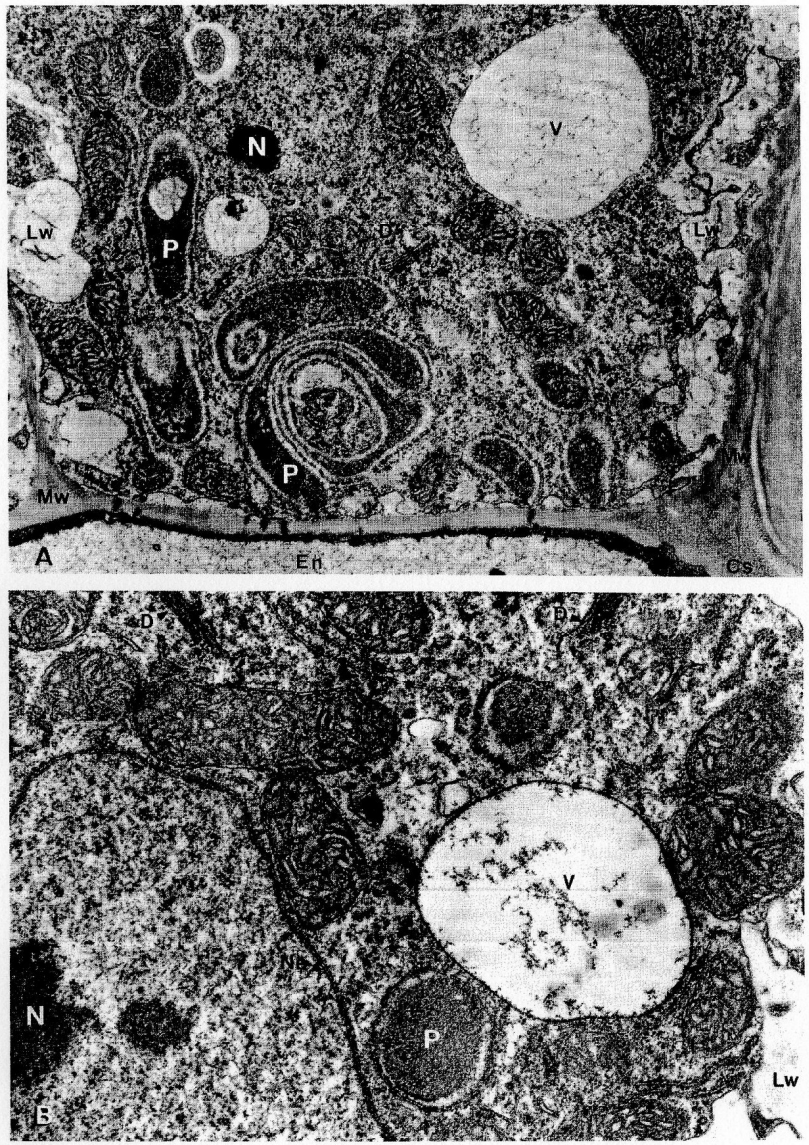

FIG. 6. Electron micrographs of gland head cells of *P. grandiflora*, pre-maturation stage, standard uranyl acetate-lead citrate staining. A. Corner of a head cell, in the plane indicated in Fig. 2. A portion of the endodermal cell is seen below, and the cuticle of the neighbouring epidermal cell to the right. The section shows the complex convoluted form of the plastids and also stretches of the labyrinthine ingrowths of the radial walls. × *c.* 14500. B. Portion of the cytoplasm showing the principal organelles and a small vacuole. × *c.* 24000.

discontinuous. There are no pores such as are present in the cuticles of the gland head cells of *Drosophyllum* and *Drosera* (Heslop-Harrison, 1975, 1976), but the discontinuities are often moderately well-defined, notably over the zones where the anticlinal walls separating the head cells abut the outer wall (Fig. 8B).

The nuclei

After the final divisions in the gland head, the nuclei move into the lower parts of the head cells (Fig. 3D, E), and remain there throughout their further development. They possess conspicuous nucleoli (Fig. 6B), and occasionally, although not invariably, lamellar aggregates similar to those seen in the endodermal cell nucleus illustrated in Fig 3E.

Organelles and membranes

Head cells of the glands of *P. grandiflora* contain mitochondria, mostly clustered in the lower half (Fig. 6A, B). They are ovoid, with a maximum dimension of up to 2·5 μm, and have prominent cristae. Vogel (1960) noted in the earliest fine-structural description of the glands of *Pinguicula* that the plastids of the head cells are extremely large, and assume complex shapes. Because of their ramifying form (Figs 6A and 8A), it is scarcely possible to estimate the numbers per cell. The maximum dimension measured from an intercept in an electron micrograph for *P. grandiflora* was 4·5 μm.

As Vogel (1960) recorded, the plastids are consistently associated with sheets of endoplasmic reticulum during the later stages of development of the gland. Aspects of the association are seen in Figs. 7A–C and 8A. The membranes applied to the surface of the plastids have ribosomes on the outer face but not the inner (Fig. 7A), and glancing sections show that these are in polysome configurations (Fig. 7B). The endoplasmic reticulum follows the convolutions of the plastids faithfully, but in the invaginations the membranes are without ribosomes (Fig. 7C). The separation of the inner membrane of the reticulum from the outer membrane of the plastid envelope is never less than 30 nm, and in no instance has continuity between the two been observed; the intervening space is, however, bridged by tenuous filaments (Fig. 7A, C).

In addition to the membranes associated with the plastids, single profiles of ribosomal endoplasmic reticulum occur in the cytoplasm, and also occasional stacked profiles (Fig. 7D), Elsewhere, smooth-surfaced membranes are present, usually in the form of ramifying tubules.

Dictyosomes are present in the head cells, but in relatively small numbers. They are associated with coated vesicles (Fig. 7D), but lack the large cisternae found in conjunction with the dictyosomes of the mucilage-secreting stalked glands.

In the light of the suggestion by Unzelman and Healey (1974), that the transfer into the wall of the protein–carbohydrate secretion product of the leaf trichomes of *Pharbitis nil* might take place through continuities between the endoplasmic reticulum and the plasmalemma, we have studied the relationships of the two membrane systems in the *Pinguicula* leaf glands in detail. Some scores of preparations from glands in the pre-maturation stage were scanned, and in each head cell the plasmalemma was traced around as much as possible of the periphery. The interface between cytoplasm and the wall is highly convoluted in the neighbourhood of the labyrinthine thickenings so that the plasmalemma can never be seen in profile throughout its length in any one section, and this does introduce ambiguity. However, in no single instance was an unequivocal junction between cytoplasmic membranes and the plasmalemma observed. Either such junctions do not occur at all during the development of the head cells, or they are formed so infrequently – or so transiently – that the chance of encountering them in fixed and sectioned cells is very low.

In younger glands, continuities between the endoplasmic reticulum and the tonoplasts of developing vacuoles appear frequently. These are eliminated as the vacuoles enlarge, although membrane profiles often persist in close proximity to the surfaces of the vacuoles.

Free ribosomes occur abundantly throughout the cytoplasm (Figs. 6B and 7D), occasionally in polysome conformations, notably in the cytoplasm clothing the wall ingrowths.

The maturation phase

The sequence of events during the final maturation of the gland has been pieced together from the comparison of glands of different ages, and of neighbouring head cells of the same gland in different stages of development. Comparisons of the second kind are particularly valuable since they offer an in-built control: any suspicion that images of degrading membranes in a cell may simply reflect an artefact of fixation or staining can be dismissed when it can be seen that adjacent cells possess a normal membrane complement (Fig. 8D). The sequence is as follows.

(i) The tonoplasts undergo progressive dissolution, beginning in the upper part of the gland (Fig. 8C). As this proceeds, the vacuoles become confluent.

(ii) The ingrowths of the radial walls of the gland head enlarge (Fig. 8D). Concurrently the stainability as observed by optical microscopy is lost. Ultimately the investing plasmalemma is disrupted, and wall and cytoplasm become confluent.

(iii) The expanding wall ingrowths reach the boundaries of the vacuoles, and vacuole and wall become confluent (Fig. 8E).

(iv) The membranes of the endoplasmic reticulum, including those investing the plastids, are disrupted and the plastids themselves lose part or all of their bounding envelopes, although they retain their form. All of the membranes of the mitochondria undergo dissolution, passing through a distinctive alveolate stage, illustrated in Fig. 8A. The nuclear envelope is dispersed during the same interval.

In consequence of these changes, the compartmentation of the head cells of the gland is destroyed. Nevertheless, some structural organization is preserved. The nucleus remains distinguishable, and polysome configurations persist, often in close association with the tenuous limits of the wall ramifications, or embedded in the dispersing wall (Fig. 9B). The microfibrillar components of the head cell walls and the basal wall separating them from the endodermal cell retain their structure throughout (Fig. 9B).

Enzyme localization

The distribution of non-specific esterase activity in an intact gland of *P. ionantha* as revealed by the optical-microscopic localization method is seen in Fig. 3G, H (control, Fig. 3I). The sites of activity can be placed more accurately by comparison with (*a*) the disposition of the walls in the micrograph of a living gland in Fig. 1B, (*b*) the location of calcofluor white – and presumably cellulosic – parts of the walls in Fig. 1C and (*c*) the location of wall proteins in the paradermal section of a resin-embedded gland in Fig. 1D.

FIG. 7. Electron micrographs of membranes and organelles in gland head cells of *P. grandiflora*, pre-maturation stage, standard uranyl acetate–lead citrate staining. A. Segment of a convoluted plastid, showing the dense stroma and the periplastidial endoplasmic reticulum, with ribosomes on the outer membrane. ×*c.* 62000. B. As A, glancing view of the plastid surface, showing ribosomes in polysome configurations. ×*c.* 66000. C. As A, invagination of a plastid enclosing a single plate of endoplasmic reticulum with ribosomes on neither membrane. Faint fibrillar connections can be discerned between the membranes of the endoplasmic reticulum and the outer membranes of the plastid envelope. ×*c.* 100000. D. Dictyosome with associated coated vesicles. The vacuole towards the right possesses a normal tonoplast showing a triple-layer 'unit membrane' profile. Three plates of ribosomal endoplasmic reticulum follow the contour of the vacuole. ×*c.* 85000.

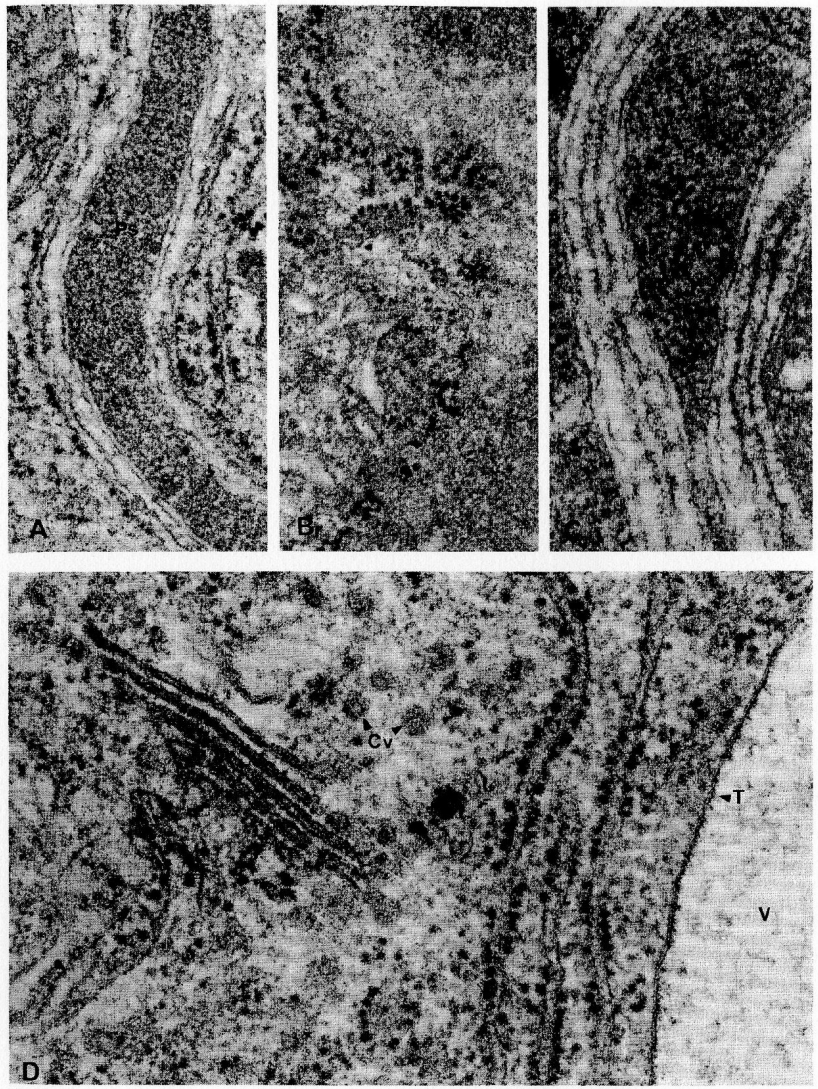

FIG. 7.

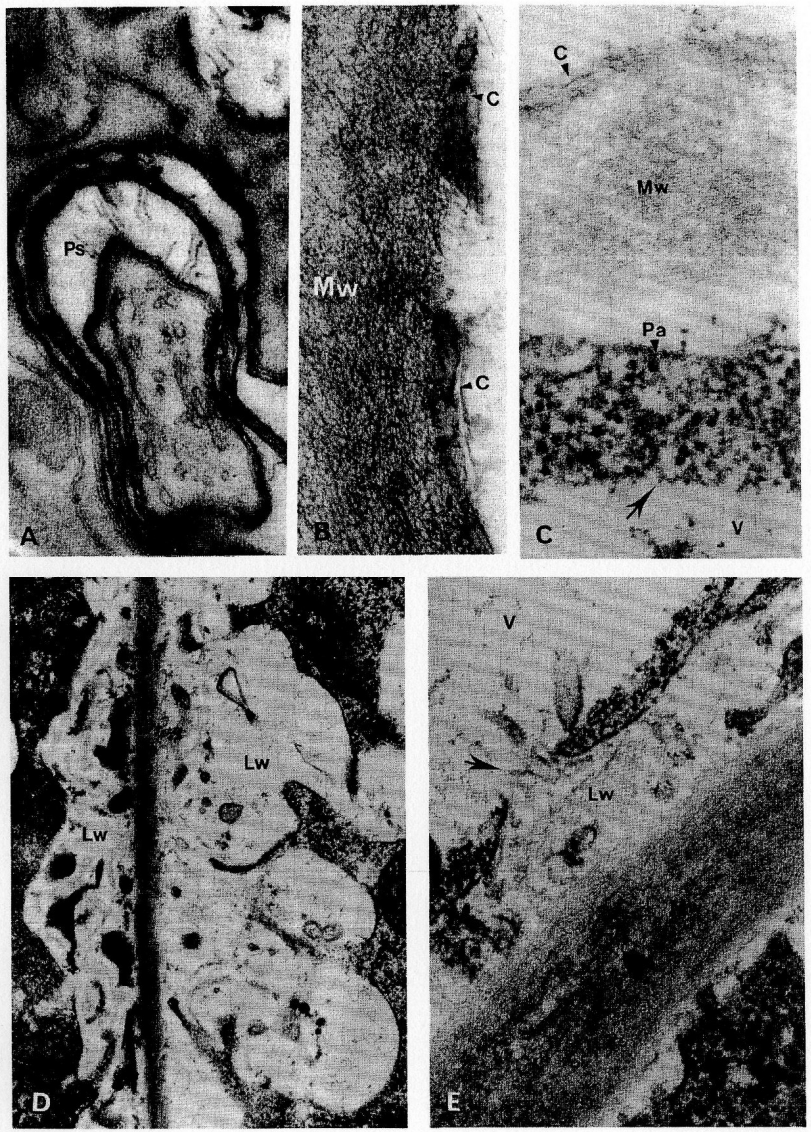

FIG. 8.

The distribution of acid phosphatase activity closely matches that of esterase. The enzymes are evidently present in the apoplast, especially at the junctions of the head cell walls. In these regions the activity is greatest where the calcofluor white staining is least, but where Nobel staining reveals a considerable concentration of protein. Enzyme activity is also associated with the labyrinthine walls, but the resolution offered by the optical-microscopic technique is inadequate to indicate whether it is in the wall ingrowths or in the intervening cytoplasmic enclaves.

The presence of enzyme activity in the wall is amply confirmed by the electron-microscopic technique for acid phosphatase. The reaction product appears throughout the outer walls of the gland head, and is seen especially in the cuticular discontinuities (Fig. 9 C).

Figure 9 D illustrates an example of the intracellular localization of acid phosphatase. Notwithstanding the distortion of the cytoplasm, membranes are distinguishable in this gland, indicating that it was in the pre-maturation stage at the time of treatment. The reaction product is dispersed thinly throughout the cytoplasm, but the main concentrations are in the vacuoles, forming a lining to the tonoplasts, and in enclaves in the cytoplasm probably to be interpreted as cisternae of the endoplasmic reticulum. In the vicinity of the wall ingrowths the reaction product appears at the plasmalemma, but there is no indication of a concentration in the ingrowths.

Figure 9 E illustrates a profile of the wall from another gland, seemingly at a later stage of development, corresponding to that of Fig. 9 B. Reaction product is seen throughout the wall.

DISCUSSION

Table 3 provides a comparison of certain fine-structural features of the enzyme-secreting cells of *Pinguicula*, *Dionaea* and *Drosera*. Although all three of these genera are leaf trappers, the strategies they illustrate are different, and certain cellular characteristics reflect this fact. *Drosera* and *Pinguicula* capture prey by a 'fly-paper' mechanism, through adhesion to a viscid mucilage held on gland heads; but in *Drosera* the capturing 'tentacles' also secrete the digestive enzymes, while in *Pinguicula* capture is effected by the stalked glands and digestion principally by the sessile glands, which do not secrete until stimulated. The sessile glands of *Pinguicula* are therefore more akin in function to the digestive glands of *Dionaea*, which are similarly specialized for enzyme secretion and do not discharge until the prey is captured, in this case by the closing of the leaf. Notwithstanding this similarity, *Dionaea* and *Pinguicula* glands differ significantly in another

FIG. 8. Electron micrographs of gland head cells of *P. grandiflora*. A, potassium permanganate fixation; B–E, glutaraldehyde–osmium tetroxide fixation. A. Profile of a convoluted plastid. With permanganate fixation the material of the stroma is lost, but a system of tubuli, mostly oriented at right angles to the plastid envelope, is revealed. This micrograph shows the close association of the endoplasmic reticulum with the plastid envelope. × *c.* 33000. B. Outer wall of gland head, extended uranyl acetate staining. The microfibrillar part of the wall is bounded by the cuticle, but cutinization is discontinuous, leaving considerable interruptions, especially over the zones adjoining the radial walls of the head cells (cf. Fig. 3 H). × *c.* 62000. C. Outer wall and adjacent protoplast at the time of the earliest dissolution of the membranes, staining as in Fig. 5 A. The plasmalemma is still intact, but by this stage the tonoplast is disrupted, and there is no barrier between the cytoplasm and the vacuole. A discontinuity is seen in the cuticle overlying the microfibrillar wall. × *c.* 90000. D. Two adjacent head cells during the final maturation period. The dissolution of the membranes is further advanced in that to the right, and the enlarging wall ingrowths are beginning to reach the vacuoles. The gelation and dispersal of the wall ingrowths does not affect the microfibrillar primary wall separating the head cells. × *c.* 23000. E. Detail of a cell in the state of that to the right in D, showing the confluence of a vacuole and the wall ingrowths. The tonoplast has disappeared, but stretches of the plasmalemma remain. × *c.* 47000.

respect: those of *Dionaea* recover their capacity for enzyme synthesis and secretion after a cycle of discharge and digest resorption, whereas those of *Pinguicula* function but once, and are incapable of recovery after discharge.

All three genera share in common the possession of labyrinthine wall thickenings in the secretory cells, a characteristic also found in the glands of another leaf-trapper of the Droseraceae, *Drosophyllum lusitanicum* (Schnepf, 1960*b*, 1963, 1974), and, among other Lentibulariceae, in *Genlisea* (Heslop-Harrison, 1976). The cytochemical evidence by Schnepf (1963) for *Drosophyllum* and by Dexheimer (1976) for *Drosera* suggests that the wall ingrowths in these genera have a composition similar to those of *Pinguicula*, the staining properties showing that they are rich in pectic polysaccharides, a supposition supported by Dexheimer's demonstration for *Drosera* that the reactivity of the wall ingrowths in the Thiery procedure is eliminated by prior incubation with fungal pectinase. Scala, Schwab and Simmons (1968) have speculated that the corresponding ingrowths in the gland cells of *Dionaea* might be callosic, but the cytochemical evidence does not support this in *Pinguicula* or *Drosera*, and we have been unable to detect callose in any sites in the gland head cells of *Dionaea* itself.

The functional difference between the glands of *Drosera* and those of *Dionaea* and *Pinguicula* – the former secreting both polysaccharides and proteins and the latter pair principally proteins – is reflected in the different pattern of dictyosome behaviour. The timing of dictyosome activity is conspicuously different. Dexheimer (1972, 1976) found that during the production of the mucilage droplet held on the gland head of *Drosera* numerous dictyosomes are present in the cytoplasm of the secretory cells; these show the 'active' configuration, associated with a considerable population of large vesicles. The vesicles have a content matching that of the secretion in staining properties, and Dexheimer's electron micrographs convincingly illustrate fusions both between the vesicles and the plasmalemma. Schnepf (1960*b*, 1961, 1963) had earlier shown that the secretion of mucilage by the stalked capturing glands of *Pinguicula* and *Drosophyllum* is accompanied by a similar type of dictyosome activity. In the *digestive* glands of *Pinguicula*, large dictyosome vesicles appear only during the interval of wall thickening; in the ensuing period of enzyme accumulation, the dictyosomes are associated with the smaller coated vesicles. A parallel is found in *Dionaea*, where Schwab *et al.* (1969) noted that large vesicles appear only during that phase of the secretion–resorption–regeneration cycle when the wall ingrowths are being re-formed. In the resting gland before secretion, the dictyosomes are again associated with the smaller coated vesicles. From the accounts of Ciobanu and Tăcină (1973) and Dexheimer (1972, 1976) it is apparent that in *Drosera*, also, only the smaller type of dictyosome vesicle is produced after the conclusion of the phase of mucilage secretion.

FIG. 9. A, B. Electron micrographs of head cells of a gland of *P. grandiflora* during the final maturation stage. Standard uranyl acetate–lead citrate staining. A. Dissolution of the mitochondrial membranes. All of the membranes, including those of the cristae, are disrupted at this time, giving a characteristic alveolate appearance. ×*c.* 60000. B. Interface of the cytoplasm and the extending wall ingrowths. The plasmalemma is now entirely dispersed. The cores of the wall ingrowths can be distinguished, and numerous polysomes remain structurally unaffected in the cytoplasm. ×*c.* 92000. C–E. Electron micrographs of gland head cells of *P. grandiflora*, acid phosphatase localization. C. Discontinuity in the cuticle over the outer wall of a gland head, showing the accumulation of reaction product. ×*c.* 84000. D. Cytoplasm in pre-maturation stage. Reaction product forms a lining to the tonoplasts of the vacuoles, and is seen in the cisternae of the endoplasmic reticulum. A part of a wall ingrowth is seen towards the bottom left. The body in the left centre may be a plastid; but the resolution given with the method does not permit a certain identification. ×*c.* 80000. E. Cytoplasm–wall interface late in the maturation period. The reaction product is present in the cytoplasm, more sparsely in the more tenuous parts of the wall ingrowths, but abundantly in the inner part. ×*c.* 75000.

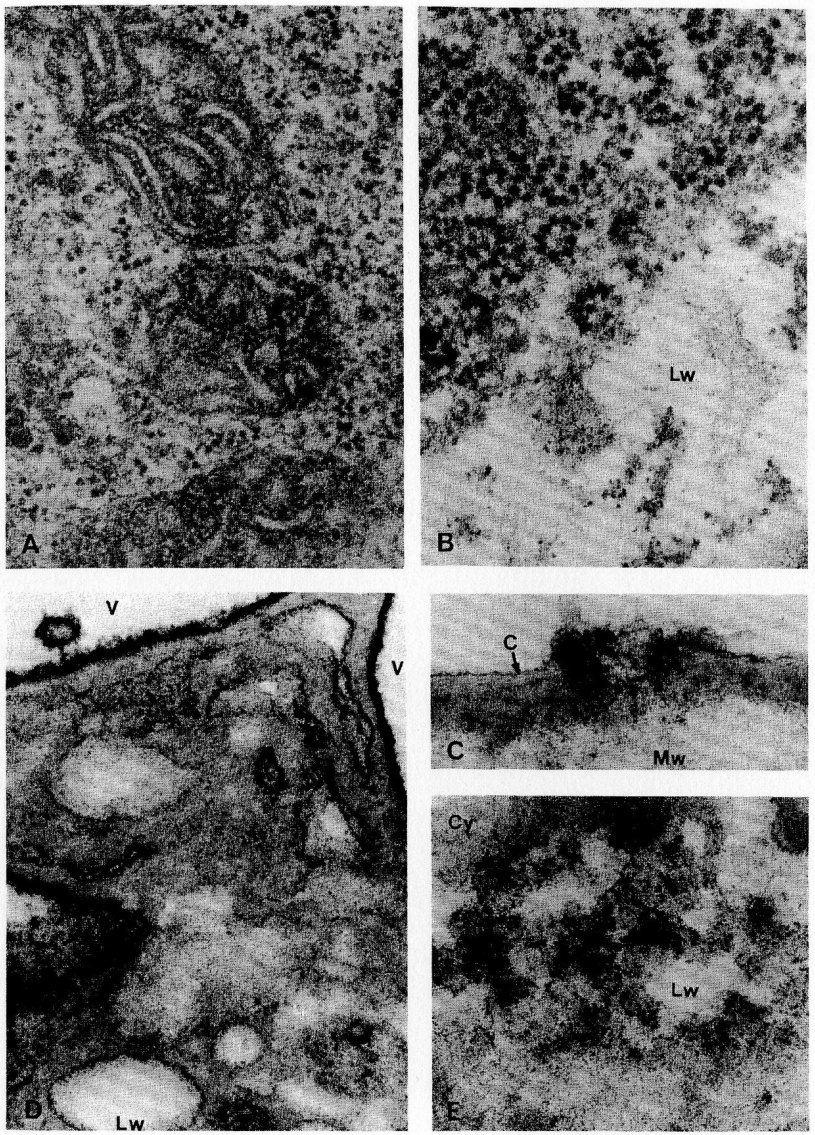

FIG. 9.

TABLE 3. *A comparison of some fine-structural features of the gland-head cells of three carnivorous genera*

	Dionaea	*Drosera*	*Pinguicula*
Plastids	Ovoid-elongate; dense stroma; several thylakoids; osmiophilic globuli and plastid centre. Traversed by tubuli. No special association with the endoplasmic reticulum.	Ovoid-elongate or irregular; stroma of medium density; 2–5 lamellae. No plastid centre or association with endoplasmic reticulum	Complex convoluted form in the mature gland; dense stroma; few lamellae and no plastid centre. Traversed by tubuli and closely invested by elements of the endoplasmic reticulum.
Mitochondria	Numerous; spherical to ovoid, well-defined cristae.	As in *Dionaea*	As in *Dionaea*.
Dictyosomes	Associated with coated vesicles during the pre-discharge period.	Associated with numerous dilated, polysaccharide-containing vesicles during mucilage secretion; later with coated vesicles.	As in *Dionaea*
Endoplasmic reticulum	Elongated, ribosomal elements, often associated with wall ingrowths.	Numerous ribosomal elements, often following profiles of the plasmalemma and tonoplast.	Numerous ribosomal elements, sometimes stacked; often following profiles of plasmalemma and tonoplast.
Free ribosomes	Numerous	Numerous, often in polysome configurations	*As in Drosera*
Vacuoles	With very dense osmiophilic content, presumed to be lipid-protein in character.	With less abundant osmiophilic content, dispersed or associated with tonoplast.	With osmiophilic content, dispersed in younger glands, associated with the tonoplast in older glands.

The lateral, and to some extent the outer walls, of the gland head cells of all three genera bear complex ingrowths of the transfer-cell type.
With some staining procedures, these are seen to have a dense core and an electron-transparent outer zone bounded by the plasmalemma.

The secretory cells of *Dionaea*, *Drosera* and *Pinguicula* all possess numerous large mitochondria, with well-formed cristae (Table 3), a characteristic shared with other plant secretory tissues (Fahn, 1979). The plastids of the gland head cells have certain features in common, notably the possession of a dense stroma, reminiscent of that of protein-storing plastids described from root tips and other organs (e.g. Newcomb, 1967). The plastids of *Dionaea* and *Drosera* appear to retain rather more lamellae than those of *Pinguicula*, while *Pinguicula* shares with *Dionaea* an enigmatic system of tubuli (Fig. 8 A), although seemingly lacking the reticulate plastid centre of that genus. The plastids of *Pinguicula* are considerably larger than those of *Dionaea* and *Drosera*, and possess an elaborate convoluted form; they also show a consistent and intimate association with the endoplasmic reticulum not seen in those genera. This 'periplastidial' endoplasmic reticulum, noted first by Vogel (1960), has its counterpart in other secretory cells, such as, for example, the resin cells of *Pinus* (Benayoun and Fahn, 1979). Characteristically, the periplastidial endoplasmic reticulum in *Pinguicula* is ribosomal on the outer membrane but not on the inner, which is linked to the plastid envelope by fine fibrils. Elsewhere in the secretory cells the endoplasmic reticulum occurs in single profiles or stacks of a few lamellae, and is invariably ribosomal, as is the case in the other genera.

Judged from the distribution of acid phosphatase and esterase activity, enzymes are progressively transferred into the apoplast even before the final period of development in the gland head cells of *Pinguicula* – that is to say, while the membranes still remain intact. At the time of transfer, enzyme activity is detectable in the vacuoles, and, as Fig. 9 D shows, also in cisternae of the endoplasmic reticulum. Profiles of endoplasmic reticulum are often to be seen in close association with the plasmalemma bounding the wall ingrowths, ramifying, indeed, in the interstices. Yet, as already indicated, continuities between the membranes of the endoplasmic reticulum and the plasmalemma have not been observed, notwithstanding a thorough search for just such junctions. Equally, we have found no evidence of a vesiculate mode of release. Accordingly, we conclude that it is improbable that protein transfer into the apoplast in *Pinguicula* glands depends upon a granulocrine mechanism. Gunning and Pate (1969), in an early review of transfer-cell structure and function, commented on the absence of junctions between the internal membrane systems of the cell and the plasmalemma overlying wall ingrowths in other tissues with the transfer-cell type of adaptation, and in work subsequent to their paper few unarguable instances have been described. One is seemingly provided by the sugar secreting nectaries of *Lonicera*, in which Fahn and Rachmilevitz (1970) have illustrated various examples of endoplasmic reticulum cisternae in continuity with wall protuberances. Lüttge and Schnepf (1976) appear not to be convinced that the evidence of these two authors does indicate a granulocrine mode of secretion of the nectar; nevertheless, the electron micrographs of *Lonicera* glandular tissue can perhaps be taken as indicating the minimum that might have been expected in the secretory cells of *Pinguicula* were the transfer of enzymes into the apoplastic space to depend on fusions between the internal cell membrane system and the plasmalemma.

If the granulocrine mode is excluded as the principal means of transfer, the passage into the wall must be by perfusion through the plasmalemma. Direct evidence for such an eccrine mode of secretion is not available for *Pinguicula*, and indeed it is not obvious at present what form this might take. However, there are various lines of indirect evidence. The cytoplasm contains an abundance of free ribosomes, often in polysome configurations, revealing the presence of a massive protein-synthesizing system which clearly cannot transfer the product directly into the cisternal space of the endoplasmic reticulum. Furthermore, the electron-microscopic localization of acid phosphatase shows that activity is present in the cytoplasm, and notably, also, at the plasmalemma bounding the wall ingrowths. As we have noted in the introductory paragraphs, the presence of the labyrinthine wall ingrowths itself provides a pertinent argument, for

the most reasonable interpretation of the function of this type of adaptation is that it facilitates membrane perfusion by increasing the area of the interface between the symplast and the apoplast.

If enzymes are transferred into the wall of the *Pinguicula* gland by direct passage through the plasmalemma over the wall ingrowths, then they must have been held in the cytosol before transfer, and not sequestered in the lytic compartments of the cell. How, then, is the cell guarded from premature autolysis? In discussing enzyme secretion from animal cells, Rothman (1975) has considered this point in detail, arguing that even in the cells of the pancreas – the tissue above all associated with the classical model for granulocrine enzyme secretion – a major part of the enzyme synthesis is in the cytoplasmic compartment, and that exclusion is by direct transfer across the cell membrane. Rothman notes that the accepted theory includes several assumptions, including the idea that membranes of the endoplasmic reticulum are protected from destruction by the digestive enzymes while other components of the cell are not. However, digestive enzymes *do* appear in the cytoplasmic fractions of pancreas preparations, and according to Rothman these cannot be accounted for entirely by the rupture of membrane-bounded enclaves. Among the reasons advanced by Rothman for supposing that the passage of enzymes out of the pancreatic cell must at least in part be by transport through the membranes is that the transfer is known in some cases to be selective, an unlikely circumstance were all of the diverse spectrum of enzymes synthesized by the cells to be released in vesicular capsules.

The evidence given in the present paper shows that in the final phase of development of the *Pinguicula* gland the compartmentation of the secretory cells is in fact finally destroyed. The steps are, (*a*) fusion of the vacuoles and the progressive elimination of the tonoplasts, (*b*) the dissolution of the plasmalemma, initially in the vicinity of the wall ingrowths, which progressively enlarge until they fuse with the vacuoles and (*c*) the disruption of the other membranes of the cell, including those of the mitochondria, which pass through a distinctive alveolate phase. This process, perhaps to be looked upon as one of controlled autolysis, leaves the head cells as enzyme-filled sacs, their state preceding the ultimate ('holocrine') discharge. Such a total loss of cell organization as that shown by the gland head cells of *Pinguicula* clearly cannot occur in *Dionaea*, where the secretory cells are capable of regenerating after the completion of the secretion and resorption cycle. However, there are aspects of the fine-structural changes accompanying final maturation of the gland head cells of *Drosera* which may indicate a behaviour similar to that seen in *Pinguicula* (Heslop-Harrison, 1976).

The present observations offer no clue as to the fate of the coated dictyosome vesicles produced during the period of enzyme synthesis and accumulation in the secretory cells of *Pinguicula*, nor do they cast any further light on the significance of the association between the ramifying plastids and the endoplasmic reticulum. It may be surmised that the plastids are concerned with enzyme synthesis, fulfilling a function akin to the 'plastolysomes' described by Gärtner and Nagl (1980). The association with the endoplasmic reticulum may then be related to some aspect of transfer, but at present this can be no more than speculation.

The principal structural characteristics of the endodermal cell subtending the secretory cells are the Casparian strip and the close attachment to it of the peripheral plasmalemma. We have noted in earlier papers (Heslop-Harrison and Knox, 1971; Heslop-Harrison, 1975, 1976) the likely function of these adaptations in blocking the apoplastic route from the mesophyll into the gland head cells and enforcing symplastic flow into the gland head cells. The corresponding layer in *Drosera* is composed of several cells each with a bounding Casparian strip, and Juniper and Gilchrist (1976) have given direct evidence that here the wall incrustations do indeed preclude apoplastic movement between the secretory cells and the tracheidal core of the gland head.

The reservoir cell underlying the endodermal cell is comparable in most structural features with the adjoining epidermal cells, with which it has plasmodesmatal contacts. The wall is unusual in having the inner electron-transparent layer, presumed to correspond to the striated sheath observable with the optical microscope after ruthenium red staining. The most distinctive feature of the cell is the accumulation of Cl^- ions in the vacuole (Heslop-Harrison and Heslop-Harrison, 1980). The endodermal and reservoir cells are not affected by the autolytic events that mark the last stage of development in the secretory head cells, retaining their organization intact until the discharge phase that follows upon the capture of prey. The dramatic changes in all cells of the gland complex accompanying the discharge itself and the subsequent events associated with the recovery of the digestion products will be described in a second paper.

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