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Nectar Secretion in *Abutilon:* a New Model

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Summary

Nectary trichomes of *Abutilon striatum* secrete copious amounts of sucrose, fructose and glucose. The nectar emerges from transient pores in the cuticle overlying the trichome tip cells. Calculations of the required transmembrane fluxes, either across the tip cell plasmalemma or across the cell membrane of the whole trichome, give very high rates compared with those obtained from other situations in plants and, therefore, cast doubt on the possibility that nectar secretion in *Abutilon* is an eccrine process. Quantitative evaluation of the possibility of granulocrine secretion, by successive fusion of vesicles with the cell membrane, suggests that this is an even less probable mechanism of secretion. Rapid freezing followed by freeze-substitution or freeze-fracture replication reveals that an extensive "secretory reticulum" (SR) is present within the hair cells. As similar micrographs are obtained from conventional, chemical fixation it is argued that the secretory reticulum is a relatively stable endomembrane system. Freeze-fracture and freeze-substitution micrographs show that this internal membrane system is closely associated with the plasmalemma. Taken together with other structural information, as well as physiological data, it is concluded that prenectar is actively loaded into the secretory reticulum of all trichome cells. Increase in hydrostatic pressure within this compartment leads to the opening of "sphincters" which connect the cisternal space of the SR to the outside of the plasmalemma. Thus a pulse of nectar is forcibly expelled into an apoplastic compartment sealed to the outside by the impermeable cuticle and on the inside by the plasmalemma. As this apoplastic compartment is also sealed at the stalk cell, the only route for pressure release is via the transient pores which overlay the tip cell. Distension renders these patent so that, again, pulsed secretion is observed. This hypothesis overcomes the necessity for envisaging excessively high transmembrane fluxes or rates of vesicle fusion. It would imply the need for a continuing supply of prenectar to the hair cells accompanied by active loading into the SR. This loading process may well be supported by the hydrolysis of sucrose to glucose and fructose and is probably the site where ions and other low molecular weight solutes are filtered from the nectar.

Keywords: *Abutilon*; Freeze-fracture replication; Freeze-substitution; Nectary; Secretory reticulum; Sugar secretion; Symplastic transport; Trichomes.

1. Introduction

Recent work on nectary secretion in *Abutilon* has largely left open the question of the mechanism of the secretory process itself (ROBARDS 1984, ROBARDS and OATES 1984, KRONESTEDT *et al.* 1986). The classical alternative processes of secretion involve either transmembrane fluxes mediated by carrier proteins (eccrine secretion) or fusion of vesicles carrying the secretory product with the plasmalemma (granulocrine secretion) (see LÜTTGE and SCHNEPF 1976). Previous structural studies of nectaries and other glands have often attempted to deduce functional mechanisms from ultrastructural observations. Thus, in some instances it has been concluded that eccrine secretion must occur while, in others, a granulocrine mode of operation has been postulated (see FAHN 1979). Only rarely has physiological data been incorporated with quantitative structural information so that the relative merits of alternative mechanisms can be adequately explored. The purpose of the work reported here is to attempt to clarify the secretory pathway and unloading mechanism in a specific nectary.

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The most comprehensively studied sugar secreting gland in plants is the nectary of *Abutilon* (see Kronestedt *et al.* 1986 for earlier references). Nevertheless, even here the mechanism of secretion remains unresolved (Robards 1984). The "per trichome" volume secretion rate has previously been determined by a number of authors and is usually within the range 1.0 to 100 $\mu m^3\ s^{-1}$ (Findlay *et al.* 1971, Gunning and Hughes 1976, Robards 1984). We have used a mid-range figure of 50 $\mu m^3\ s^{-1}$ in our calculations (= approx. 1.0×10^{-11} sugar s^{-1}). To secrete this amount of nectar would require a transmembrane flux of 85×10^{-6} mol $s^{-1}\ m^{-2}$ if the whole tip cell membrane only were involved or $3\text{–}4 \times 10^{-6}$ mol $s^{-1}\ m^{-2}$ if the total plasmalemma surface area of the hair were available for unloading.These rates of eccrine secretion appear high compared with other transmembrane sugar fluxes found in plants although less so when compared with some ion fluxes (Kronestedt and Robards 1987).

Turning to the possibility of granulocrine secretion, calculation of the required rate of vesicle fusion to sustain the necessary volume secretion rate reveals that, for 50 nm diameter vesicles, no less than 750,000 would need to fuse with the tip cell membrane every second. If the vesicles were 500 nm in diameter, the fusion rate would still be about 750 s^{-1}. In either case, cell membrane extension and the consequential need for membrane recycling would appear to impose impossible demands on the cell. Even if vesicle fusion were to occur in all the cells of the hair, the relevant rates would only be reduced by a factor of about 20. These calculations, derived from existing physiological data, do not, therefore, provide an unambiguous explanation of the processes of secretion in *Abutilon*. To proceed further, it is necessary to obtain more reliable structural information.

In order to make meaningful structural observations, it is necessary to consider the processes whereby cells can be chemically or physically immobilised. The value of chemical fixation to study rapid intracellular processes has to be called into question. It has been shown (Mersey and McCully 1978) that glutaraldehyde/formaldehyde fixatives penetrate plant cells at a rate of about 140 $\mu m\ min^{-1}$. Consequently, even in a small tissue slice, we can expect it to take at least 10 minutes to immobilise the cytoplasmic activities of a trichome tip cell (Robards 1984). For this reason, we decided to explore the possibilities of rapidly freezing the trichomes in preparation for electron microscopical examination. Indeed, one of the original reasons for choosing *Abutilon* as a model system for cellular transport processes in plants was because the trichomes should be relatively good specimens to freeze: they are superficial; they are cylindrical; and they contain up to 20% sugar solution which will confer some cryoprotective properties. Calculations predict that the whole of the distal parts of the *Abutilon* hairs should freeze within 1.0 ms of rapid immersion into liquid propane (Robards 1984, Robards and Sleytr 1985). Of the various methods available for processing frozen specimens, we decided that freeze-substitution should provide information about the ultrastructure of hair cells captured during the actual process of secretion, while freeze-fracture replicas would allow us to make the necessary assessment of membrane fracture faces. Both of these methods involve substantial technical difficulties when applied to plant tissues. However, when these problems are overcome, the quality of information provided is such that it allows more confident interpretation of intracellular activities than has previously been possible in such highly active secretory systems.

2. Materials and Methods

2.1. Plant Material

Clonal stock of *Abutilon striatum* var. Thompsonii was grown in a greenhouse with continuous light and a relatively constant temperature around 20 °C. Under these conditions, flowers were always available.

The glandular trichomes are found on the internal (adaxial) face of the fused sepals (Kronestedt *et al.* 1986).

2.2. Freezing

For freeze-substitution, small slices of the gland were attached to a fragment of filter paper using a thin film of 0.4 M sucrose as a "glue". The filter paper was held in fine forceps and plunged into a 1:3 mixture of isopentane: propane held in liquid nitrogen at < -180 °C (93 K). Optimisation of cooling efficiency, including use of a plunge velocity of approximately 6.6 $m\ s^{-1}$, was as described by Robards and Crosby (1983). The frozen pieces of tissue were removed from the cryogen and stored under liquid nitrogen.

For freeze-fracture, small slices of sepellary tissue were inserted into opposed pairs of hollow rivets as used in the standard specimen holder of a Leybold-Heraeus Bioetch 2005 freeze-etching unit (Sleytr and Umrath 1974, Robards and Sleytr 1985). Because of the hydrophobicity of the hairs, a 0.4 M solution of sucrose containing a trace of Triton X-100 was usually applied to the adaxial surface of the sepal prior to insertion into the rivets. This caused the entire surface of the hairs to "wet" and avoided air bubbles around the trichomes, so allowing the easier retention of large, intact replicas (Robards 1985). Freezing was by manual insertion of the rivets, held in special forceps (Sleytr and Umrath 1974, Robards and Sleytr 1985), into the isopentane: propane mixture described above or into subcooled liquid nitrogen at −210 °C (63 K). Frozen specimen/rivet assemblies were stored under liquid nitrogen.

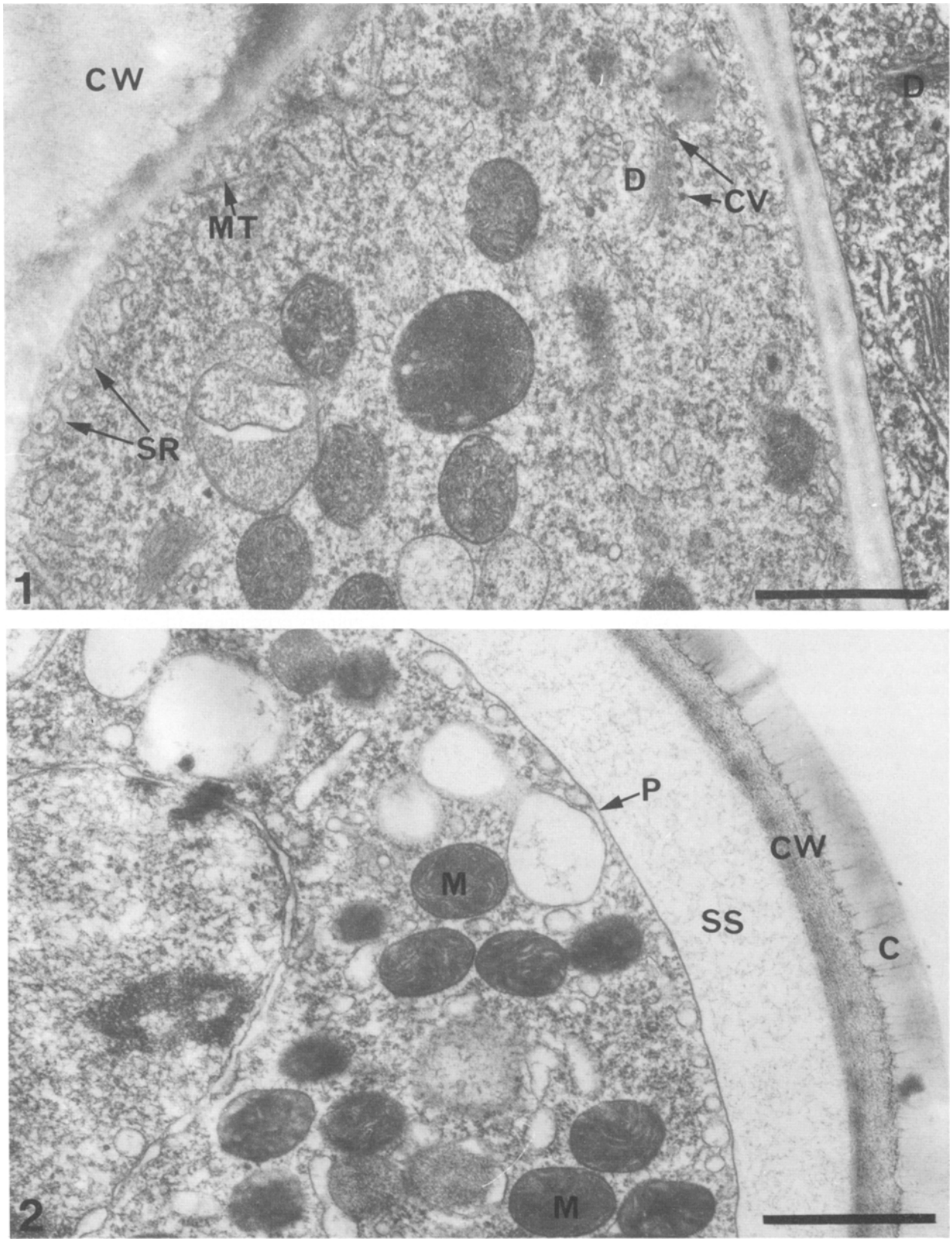


Fig. 1. Freeze-substituted hair cell of a secreting *Abutilon* gland. There is little, if any, evidence of disruption from ice crystals formed during freezing or during the substitution process. The section has cut through the cell wall (*CW*) rather obliquely, so revealing underlying microtubules (*MT*) and anastomosing cisternae of secretory reticulum (*SR*). Deeper within the cell, dictyosome stacks are seen (*D*), some having conspicuous coated vesicles (*CV*). (Scale marks on all micrographs correspond to 1.0 μm.)

Fig. 2. Transverse section through the periphery of a freeze-substituted secreting hair. The plasmalemma (*P*) is smooth and clearly defined. Between the plasmalemma and cell wall (*CW*) is a cavity (*SS*) which was presumably filled with nectar at the moment of freezing. Overlying the cell wall proper, and of about the same thickness, is the cuticle (*C*). The numerous mitochondria (*M*) have a condensed appearance

The rationale for the different freezing methods described above was that the naked hairs are capable of benefitting from the highest surface heat transfer that can be attained experimentally. The relatively larger freeze-fracture specimens, with the tissue necessarily enclosed within the thin metal wall of the brass rivets, will in any case cool much more slowly and, therefore, subcooled nitrogen is an adequate coolant to achieve the highest cooling rate as limited by the specimen itself.

2.3. *Freeze-substitution*

After preliminary experimentation (see Robards 1985) a routinely satisfactory freeze-substitution method was devised. The frozen specimens were transferred from liquid nitrogen into a plastic vial containing the substitution medium of 0.5% uranyl acetate, 1% osmium tetroxide, and 3% glutaraldehyde in pure, dry methanol (based on the work of Humbel *et al.* 1983 and Müller 1984; see also Robards and Sleytr 1985). While still under liquid nitrogen, the specimen pieces were thoroughly broken up by gently squashing them with a cold metal rod. The effect of this was to break off many hairs and also to break some hairs into two or more separate pieces. The substitution vials were then placed on a rotator in a low temperature deep-freeze at −80 °C. The warm-up/embedding schedule was variable but typically as follows: −80 °C, 8 days; 10 °C increments daily up to +10 °C, then change into fresh dry methanol. Specimens were centrifuged at each change of solvent. From methanol the material was transferred into acetone prior to the very gradual addition of 2 drops of 1:1 acetone: Quetol resin every half hour (Kushida 1974). This critical early resin infiltration stage (see Robards 1985; also McCully and Canny 1985) progressed over two to three days, the specimens being left in a refrigerator overnight. After about three days, the vials were left, without tops, in a desiccator at room temperature. The following morning the specimens were again centrifuged and the resin mixture was replaced with 100% fresh resin. After two further changes into fresh resin, the centrifuged specimens were polymerised at 60 °C in tapered plastic Eppendorf tubes.
Ultra-thin sections were cut in the normal way and stained with uranyl acetate in 50% ethanol followed by lead citrate prior to observation with a Hitachi HU-12 A electron microscope.

2.4. *Freeze-fracture Replication*

The pairs of rivets, together with the enclosed specimen, were loaded into the specimen holder of the Leybold-Heraeus Bioetch 2005 freeze-etching unit prior to insertion into the machine. Freeze-fracture runs were performed fully automatically, the rivets being pulled apart by an electromagnetically triggered spring mechanism at the appropriate point in the cycle (see Sleytr and Umrath 1976 and Robards and Sleytr 1985 for a full description of the operation of the Bioetch 2005). Most specimens were fractured and immediately replicated with platinum/carbon at −120 °C; a few were etched by fracturing and subliming ice at −100 °C for 1–2 minutes.
When the specimens and attached replicas were removed from the freeze-etching unit, the rivets were immediately placed onto the surface of frozen methanol cooled by liquid nitrogen. The liquid nitrogen was allowed to evaporate and, subsequently, the methanol to warm up so that it eventually melted (at about −94 °C) and the specimens gently sank into the cold solvent. In this way, the tissue essentially becomes freeze-substituted and loses its osmotic sensitivity. Once the methanol had reached room temperature, the rivets were picked up and transferred to 50% chromic acid via water/Triton and then water alone. By using the methanol thawing technique, much larger pieces of replica could be retained than would normally be possible (De Maziere *et al.* 1985, Robards and Sleytr 1985). After further changes of chromic acid solution, the replicas were thoroughly washed in distilled water prior to mounting on formvar coated grids for viewing in the electron microscope.

2.5. *Stereology*

Stereological determinations were carried out as described in Kronestedt *et al.* (1986).

3. Results

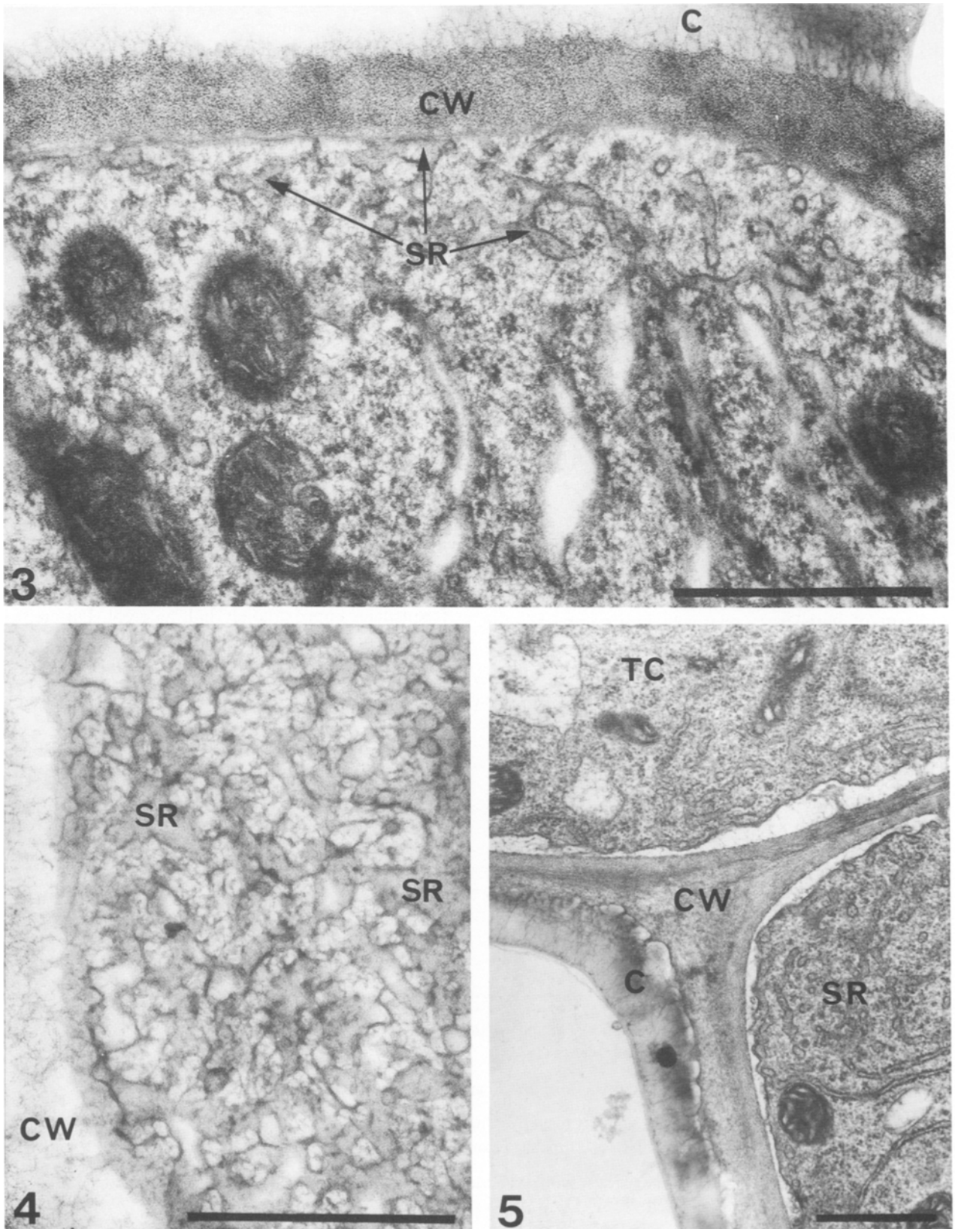
3.1. *Freeze-substitution*

After preliminary experiments (described in Robards 1985), it was confirmed that the nectary trichomes of *Abutilon* could be freeze-substituted to provide sections showing little, if any, discernable evidence of ice crystal damage (Fig. 1). Inevitably, many pieces of tissue could be found which were less well frozen than others; there was also clear evidence of ice crystal growth in cells that had not been fully substituted by the time that the warm-up sequence began.
A number of important features relating to transport processes emerge from the freeze-substitution study. Firstly, the extremely high cooling rate that must have occurred in well-frozen cells means that the cell ultrastructure can be considered to be very close to what it would have been in its dynamic *in vivo* state. Secondly, although the plasmalemma of most hairs cells was rather smooth and regular, in a way that appears typical of many freeze-substituted cells, it was often seen to be separated from the cell wall, so leaving a substantial

Fig. 3. Higher magnification micrograph illustrating the secretory reticulum (*SR*) in a freeze-substituted cell of a functional trichome. Connections of the peripheral secretory reticulum with large cisternal spaces deeper within the cell are clearly seen. Although the secretory reticulum has numerous extensions that approach the plasmalemma, it has been impossible to resolve a clear, open, continuity

Fig. 4. A glancing section through the secretory reticulum underlying the plasmalemma of a freeze-substituted cell from a secreting hair. The abundance of the secretory reticulum is clearly demonstrated in such views

Fig. 5. Conventionally fixed (glutaraldehyde/formaldehyde—Kronestedt *et al.* 1986) secreting trichome. The edge of the tip cell (*TC*) and the next most basal hair cell is shown. The plasmalemma has an undulating appearance and is not in close contact with the wall; nor is there a clearly defined extracellular space (cf. Fig. 2). However, large amounts of secretory reticulum are seen and the general cytoplasmic appearance is similar to that recorded from freeze-substituted material



Figs. 3–5

extracellular gap (Fig. 2). This gap was not continuous, the plasmalemma in some places being close to the overlying wall (*e.g.* Figs. 1, 3) and in other places being well separated from it (Fig. 2). Thirdly, cells of presecretory or actively secreting hairs were often characterised by a profuse peripheral membrane system (Figs. 3, 4). The membranes of this complex appeared tubular or cisternal and were commonly orientated normal to, or at an oblique angle to, the plasmalemma. Similar membranes are also seen in conventionally fixed hairs (Fig. 5). Stereological determinations of the surface density of this membrane system within the peripheral cytoplasm, which we refer to as the secretory reticulum (SR), provide an estimate of $1.3 \pm 0.14\,\mu m^2\,\mu m^{-3}$ (cytoplasm).

Dictyosomes were frequently seen in the peripheral cytoplasm of hair cells, often with numerous associated coated vesicles (Fig. 1).

3.2. *Freeze-fracture Replication*

Using the methods described, relatively large areas of replica could be retained (Figs. 6–11). Because of the hydrophobic cuticle and the requirement to fracture hairs longitudinally, freeze-fracture views of the cytoplasm of hairs were relatively rare compared, for example, with the abundant material obtained from the underlying sepal. However, in the course of numerous runs, sufficient replicas of fractured hairs were obtained to provide significant information. As described above, the freezing technique for the hairs provides lower cooling rates than those used for the freeze-substituted specimens. This is reflected in a generally larger ice crystal size. Nevertheless, immobilisation will still have taken place within a few milliseconds in the majority of hair cells and we can assume that the structures seen are closely representative of the *in vivo* situation.

Freeze-fracture replicas provided good pictures of the important interfaces between adjacent hair cells as well as of the numerous plasmodesmata (Figs. 6, 7, 9) although these looked little different from those described in freeze-fracture studies of other plant tissues (*e.g.* WILLISON 1976, ROBARDS and CLARKSON 1984). The two-layered structure of the cell wall and cuticle is clearly revealed by this technique. The outer surface of the cuticle appears generally smooth and featureless apart from evidence of lamellations which may represent extra-cuticular wax (Fig. 8).

Once again, it was possible to demonstrate a clear extracellular space, between the plasmalemma and cell wall, in many cells (Fig. 10). There were also numerous membrane-bound compartments in very close juxtaposition to the plasmalemma (Figs. 10, 11). When cross-fractures of some intracellular compartments were seen, it was noted that the ice crystal size was of a similar magnitude to that in the extracytoplasmic space (Figs. 10, 11). Although some of the membrane fracture faces are highly populated with intramembrane particles (Figs. 7, 9), the actual mean frequencies were not notably higher than found in other active plant membranes. P-faces of both sepellary and hair cells had frequencies of around 2,000 IMPs μm^{-2} with E-face frequencies approximately half as great. Different intracellular compartments could be distinguished both from each other as well as from the plasmalemma on the basis of differences in particle frequency and size.

4. Discussion

The structural information presented here has purposefully been limited to new observations on frozen specimens. Other aspects arising from the low temperature methods will be recorded separately. To interpret this adequately it must be considered alongside the considerable mass of previous information already published on the *Abutilon* nectary. The seminal papers of FINDLAY and MERCER (1971 a, b), FINDLAY *et al.*

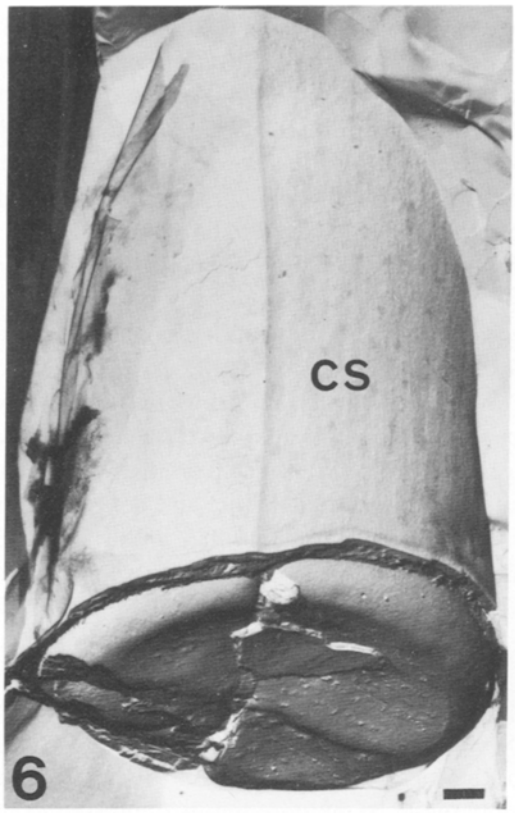
Figs. 6–9. Micrographs from a freeze-fracture replica of a secreting nectary hair. The fracture has passed along the outer surface of the cuticle and then transversely between two adjacent hair cells

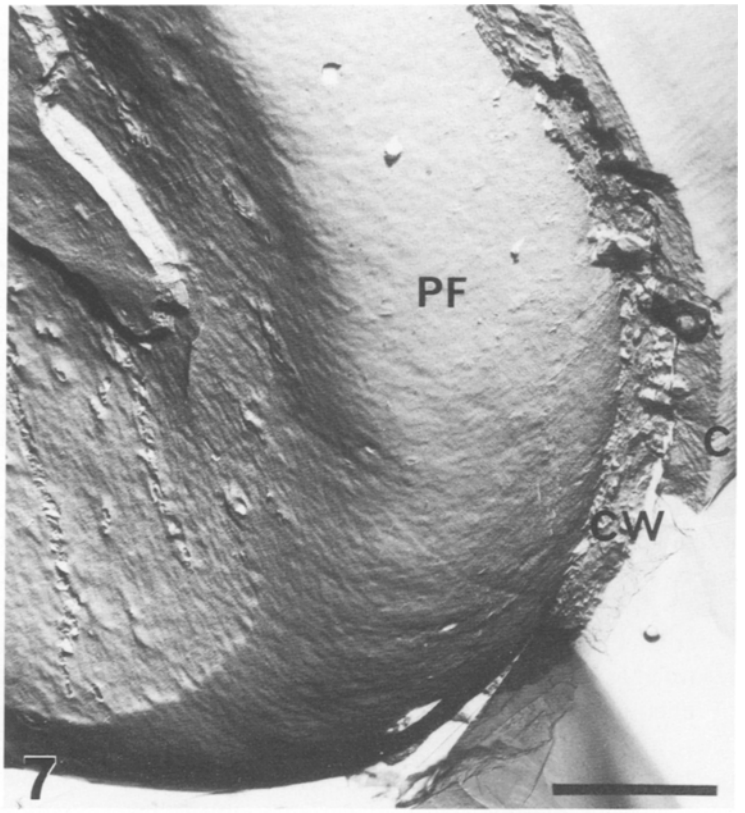
Fig. 6. Low magnification view showing the cuticular surface (*CS*) and the transverse partition between two hair cells

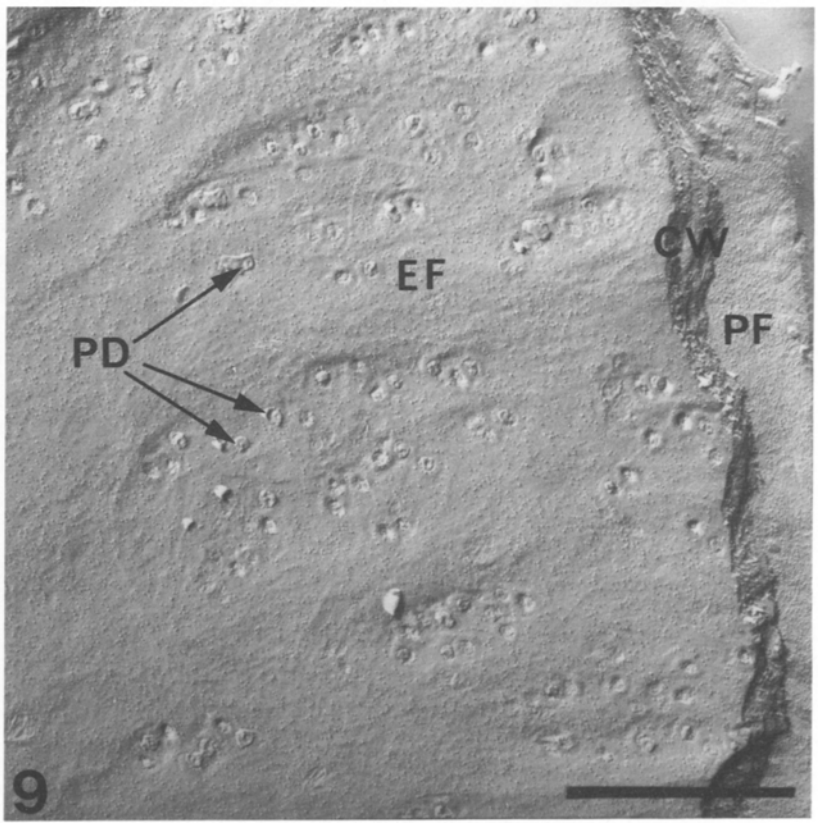
Fig. 7. Higher magnification from Fig. 6 showing the P-face (*PF*) of a hair cell. The cell wall (*CW*) and overlying cuticle (*C*) are clearly demarcated and easily separated from each other and from the plasmalemma (lower part of micrograph)

Fig. 8. High magnification illustration of the cuticular surface of a trichome showing the lamellate structure which may indicate an overlying waxy layer

Fig. 9. High magnification illustration of part of Fig. 6 showing the E-face (*EF*) of one cell together with the cell wall and the P-face of the adjacent cell. Both P- and E-faces have high intramembrane particle frequencies and numerous plasmodesmata (*PD*), mainly arranged into pit-fields, are seen







Figs. 6–9

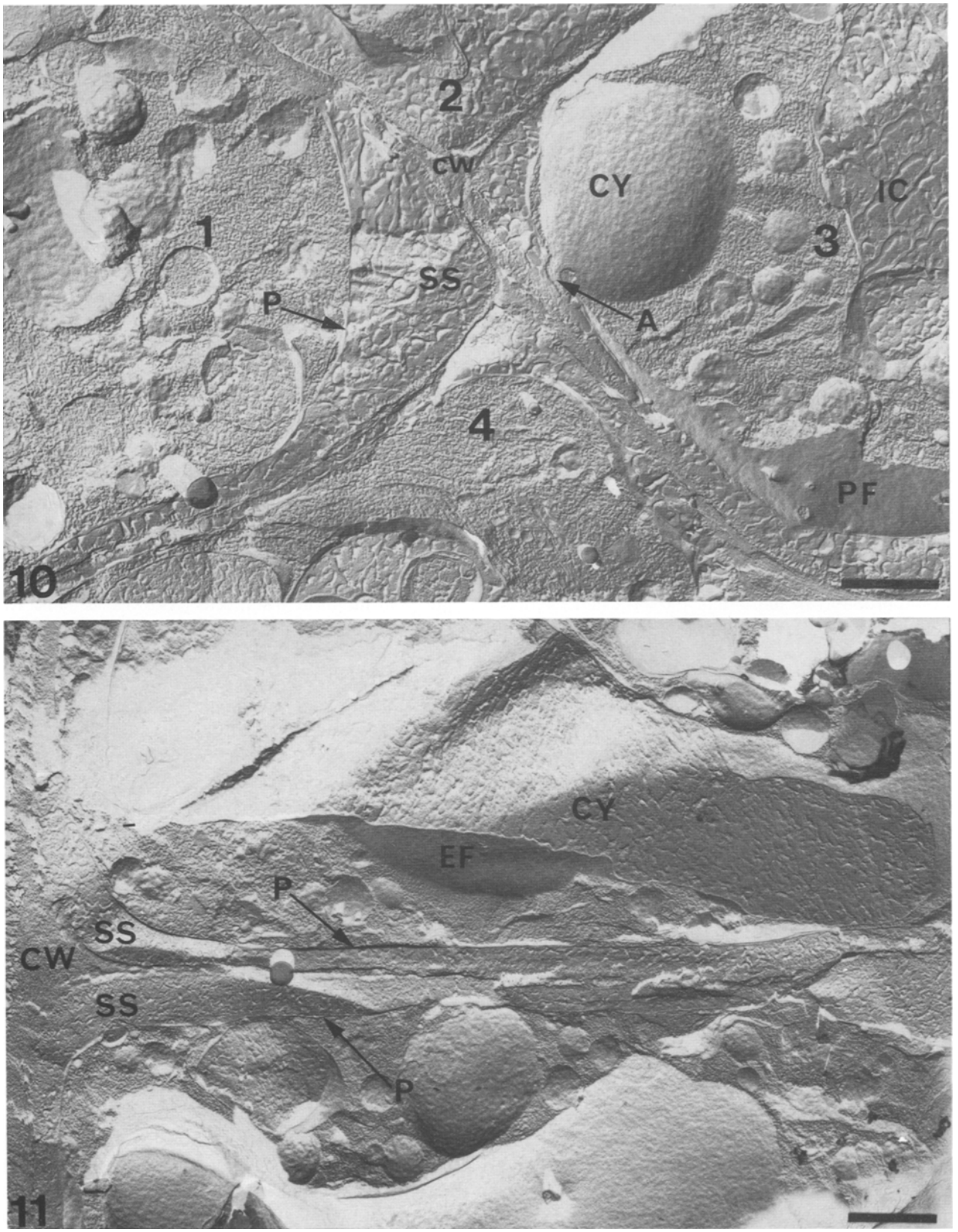
(1971) and REED *et al.* (1971) themselves followed a substantial number of papers that had already specifically, or in part, dealt with *Abutilon*. Subsequently, GUNNING and HUGHES (1976) made a quantitative assessment of symplastic transport into the trichomes, while our own laboratory has recently been associated with publications on the development of the nectary hairs (KRONESTEDT *et al.* 1986), freezing methods applied to this system (ROBARDS 1984, 1985), and ion distribution in the hairs and nectar (ROBARDS and OATES 1986). The present work records, for the first time to our knowledge, results from rapidly frozen, chemically untreated, nectary hairs. Because conventional chemical fixation is such a slow and deleterious process, any results accruing from such studies must be viewed with caution, particularly when applied to a highly dynamic system such as that operating here where the nectar flow is sufficient to fill and empty each hair cell more rapidly than once every minute. It is for this reason that the rapidly frozen material is regarded as so important in allowing the construction of functional hypotheses.

There are two especially interesting observations from the low temperature work. Firstly, it appears that an *open* extracytoplasmic space, of variable dimensions, can undoubtedly exist external to all cells of the hair. This differs from an apoplastic space within the cell wall itself which would be far more restrictive of the very high apoplastic volume flows that must occur. It has long been known that the nectar accumulates in a space between the cell membrane and the cuticle of the tip cell prior to its pulsed release. However, it is not possible to demonstrate unequivocally by light microscopy that such an open external space exists along the rest of the hair whereas our micrographs of freeze-substituted material demonstrate the space at all levels along the hairs. Conventionally fixed cells undoubtedly undergo severe osmotic changes during processing for electron microscopy. Thus, freeze-substitution has resolved this particular question. This is supported by the freeze-fracture work which also demonstrates the extracytoplasmic compartment. It might be argued here that the slower cooling rate would allow withdrawal of water across the cell membrane, so forming the observed space (Figs. 2, 10). However, this is highly improbable. Firstly, the cuticle is impermeable (as readily demonstrated by the non-penetration of externally applied fluorescent dyes) and, therefore, the plasmalemma of the hair cells is not directly in contact with the external solution, so no osmotic gradient can occur. Secondly, the ice crystal size in the extracytoplasmic compartment is similar to that within the presumptive secretory reticulum, thus leading to the conclusion that the (sugar) solution within each of these two compartments is of approximately the same concentration. Thirdly, it is again improbable that, even if the first two considerations did not apply, there would be enough time before freezing took place for the substantial volume of liquid to flow across the plasmalemma. It must be concluded, therefore, that the extracytoplasmic space is real and not artefactual. As might be expected, this space is not uniform over the whole of the trichome, some parts of the cell wall and cuticle apparently being more tightly appressed to the plasmalemma than in other areas.

The second important observation from the rapidly material is the demonstration of the secretory reticulum by both freeze-substitution and freeze-fracture replication. However, this endomembrane system is also clearly evident in our own micrographs of conventionally fixed cells (Fig. 5) and was demonstrated in the chemically fixed micrographs of HUGHES (1977). It cannot, therefore, be considered particularly ephemeral or labile. The SR is very abundant, with a surface area of 1.3 μm^2 per μm^3 of cytoplasm (excluding the nucleus and vacuoles). As the nuclei occupy about 15% of the cell volume, and the vacuoles about 20% (at this stage of development), this means that the *actual* surface

Fig. 10. Micrograph of a transversely freeze-fractured hair. At the level of the fracture (towards the base of the trichome) there were four cells, part of each being shown here (*1, 2, 3, 4*). A large extracellular space (*SS*) between the plasmalemma (*P*) and the cell wall (*CW*) is particularly clearly seen in cell *1*. In cell *3* an extending arm (*A*) from a large cytoplasmic membrane-bound cavity (*CY*) is seen to be in very close proximity to the P-face (*PF*) of the plasmalemma. The IMP frequencies of these two membranes is quite different (although not observable at this magnification) and plasmodesmata are only seen on the membrane identified as plasmalemma. Ice crystals (*IC*) in a cross-factured cytoplasmic compartment are of a similar size to those seen in the extracytoplasmic space (*SS*)

Fig. 11. Transverse fracture through a secreting nectary hair. The cells illustrated here appear to have been frozen more rapidly than those shown in Fig. 10 as the ice crystal size is smaller. However, the presence of a large cytoplasmic compartment (*CY*) containing a solution that has frozen to give ice crystals of about the same size as those in an extracytoplasmic space (*SS*) is again seen here. Such compartments seen in freeze-fractured illustrations are interpreted as being equivalent to the secretory reticulum which is demonstrated more clearly by freeze-substitution (*e.g.* Figs. 3, 4)



Figs. 10 and 11

density of the SR derived from our freeze-substitution measurements would be about 0.85 μm^2 μm^{-3}. This is lower than our own earlier estimate from conventionally fixed material (1.25 to 3.0 μm^2 μm^{-3}—KRONESTEDT *et al.* 1986) which was itself in close agreement with the work of HUGHES (1977). However, the rather weakly contrasted tubular cisternae seen in the freeze-substituted material are almost certainly under-estimated compared with conventionally processed specimens. Further, such cisternal structures are in any case substantially underscored by the type of stereological technique used here (LOUD 1967, WILLIAMS 1977). Hence, it must be expected that the surface density of SR that we have measured is right at the bottom of the range that actually exists within the living cell. Using the surface density obtained from the freeze-substitution work would give a total surface area of about 23×10^3 μm^2 of SR in a single trichome—about 50% of that reported by Hughes. Correction factors in the literature range up to a factor of x2 (WILLIAMS 1977) and it can safely be supposed that the *actual* surface area of SR within a single trichome is *at least* 50,000 μm^2. This would mean that the *total* surface area of secretory reticulum in the hairs of a mature gland would probably be more than 100×100 mm. We have not explored the ontogeny of the SR in this study although it is *possible* that it is *either* a cytoplasmic membrane *or* a highly convoluted form of the plasmalemma, in which case it would represent a special form of "wall inpushing" characteristic of transfer cells. We do not favour the view that the SR is an inpushing of the plasmalemma on the grounds that: i) such an ontogeny has not been described in the literature for this membrane system; and, ii) the SR shows more similarities with the ER (*e.g.* membrane thickness) than it does with the plasmalemma of the same cell. Further, and as opposed to the presumptive function of transfer cells, the model proposed below envisages the release of nectar from the SR into the peri-trichome space to be a sphinctered process and not simply an eccrine transmembrane transport.

If the proposed model is a valid one, then it might have been expected that fusion sites of SR with the cell membrane would have been clearly demonstrated more frequently by freeze-substitution and, particularly, by freeze-fracture replication after rapid freezing. The fact that this was not so is probably accounted for by: i) the low probability of sectioning through the actual fusion site, following freeze-substitution; ii) the low yield of useable replicas from actively secreting hairs; and, iii) the higher proportion of fractures along the *cross-wall* of hairs, rather than along the longitudinal walls where most of the fusion activity is likely to occur. This question will only be resolved by a thorough, and lengthy, comparative study of freeze-fractured material from both secreting and non-secreting hairs.

Armed with these facts, we can now explore more confidently the possible pathway and mechanism of secretion through the *Abutilon* hairs. It is difficult to conclude that the secretory reticulum is present for any other function than as a transit compartment for the passage of the presecretory sugars. We have no evidence from *Abutilon* that the sugars are transported from cell-to-cell along the hair through the *desmotubule* of plasmodesmata. Structural (GUNNING and HUGHES 1977) and microinjection (TERRY and ROBARDS 1987) studies suggest that there is relatively free intercytoplasmic continuity via the cytoplasmic annulus of plasmodesmata along the hair. If, as we suppose, sugars are unloaded from each and every hair cell, then there would be a reducing demand for cytoplasmic continuity towards the tip of the hair and it may therefore be significant that there is, indeed, a reduction in plasmodesmatal frequency from about 12.0 plasmodesmata μm^{-2} in the stalk cell to about 4.0 plasmodesmata μm^{-2} in the more distal cells (KRONESTEDT and ROBARDS 1986).

GUNNING and HUGHES (1977) have already demonstrated that entry of the experimentally observed quantities of both sugar and water into the hair could be readily accommodated by the plasmodesmata in the transverse walls of the stalk cell (the apoplastic route being closed by the wall-impregnation at this level). As the prenectar moves through the hairs, we suggest that it is sequestered into the secretory reticulum. This is a crucial step in the secretory pathway. It is known that the sugar transported in the phloem is predominantly sucrose (*e.g.* FINDLAY and MERCER 1971, LÜTTGE 1964, 1977, THORNE 1985) and that it is accompanied by ions, including potassium. However, the secreted nectar comprises sucrose, glucose and fructose and has very much lower levels of potassium (LÜTTGE 1964, 1977, ROBARDS and OATES 1986). At some stage between the phloem and final unloading, therefore, a number of important processes occur: sucrase (invertase) activity hydrolyses some of the sucrose to its constitutent monosaccharides; potassium, and probably other ions and amino acids, are filtered out; the nectar becomes more concentrated; and there must be an active pump to unload the secretory product. Further, this must all be achieved within a relatively restricted energy budget of only 1–5 high energy phosphate equiv-

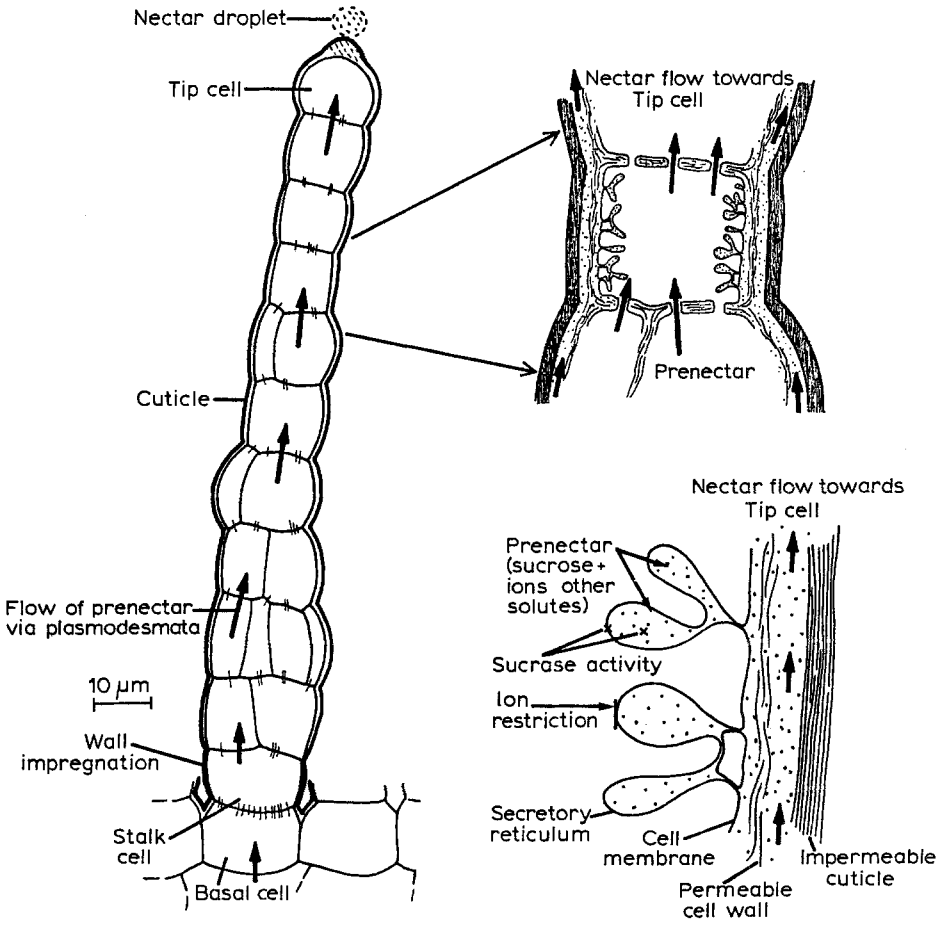


Fig. 12. Diagram illustrating the model for secretion based on the evidence from freeze-substitution, freeze-fracture and physiological experiments. It is envisaged that prenectar moves into the symplast of the hair via the numerous plasmodesmata in the transverse walls of the stalk cell. In each of the hair cells some of the prenectar is loaded from the cytoplasm into the secretory reticulum. It is at this stage that a filtration effect takes place, so defining the chemical composition of the secreted product. The sucrose is partially hydrolysed to glucose and fructose but evidence is not yet available as to whether this takes place at the membrane or within the cisternal cavity of the secretory reticulum. As loading into the secretory reticulum continues, a hydrostatic pressure builds up until, ultimately, a minute pulse of nectar is forced into the freely permeable apoplastic space bounded by the plasmalemma to the interior and the cuticle to the exterior. The continuing build-up of pressure within this compartment ultimately reaches the level where pores in the cuticle over the tip cell become patent and release a pulse of nectar to the exterior. Such a model takes account of all the currently available information concerning secretion in *Abutilon* but does not predict excessively high rates of either eccrine or (conventional) granulocrine secretion. It also overcomes the osmotic difficulties that would otherwise arise from the high concentrations of solutes that are transported in this system

alents per sugar molecule secreted (FINDLAY, REED and MERCER 1971).

These requirements can all be met if it is envisaged that the prenectar is loaded across the large area of membrane presented by the secretory reticulum. The transmembrane flux required would only be 6.0×10^{-7} mol m^{-2} s^{-1} which is not particularly high compared with many other transmembrane sugar fluxes (KRONESTEDT and ROBARDS 1987) and, therefore, should pose no conceptual difficulties. It can also be envisaged that a filtering process occurs in loading the compartment of the secretory reticulum, so retaining potassium and other solutes while loading the sugars. An active sugar carrier (or carriers) in the membrane of the SR is a clear necessity although we have no information concerning this. Further, it is possible that the sucrase could be associated with the membrane of the SR or contained within its cisternal space. While we have demonstrated (unpublished data), using cytochemical methods, the presence of an active sucrase in the nectary hairs we have not, as yet, been able to localise it to a specific subcellular site and, indeed, the possibility has not been eliminated that inversion of the sucrose takes place even before the prenectar enters the hairs. We prefer the hypothesis that the sucrase is membrane bound because, otherwise, it would presumably be se-

creted along with the nectar and we have no evidence from our preliminary results that this occurs. The hydrolysis of the sucrose would lead to a substantial increase in osmotic potential within the SR, so drawing in water and creating a hydrostatic pressure. We conjecture that the SR is either in permanent continuity with the plasmalemma, releasing pulses of nectar through sphincteres junctions, or that it intermittently fuses to form temporary connections to the external space. In either case the hypothesis overcomes any need for assuming extremely high transmembrane fluxes or membrane recycling processes. As each tiny burst of nectar is forced out of the SR under pressure, it would contribute to an increase in total pressure within the extracellular compartment bounded by the plasmalemma, the cuticle and the apoplastic barrier around the stalk cell (Fig. 12). The only exit is through the latent pores in the cuticle over the tip cell but even these remain tightly sealed until the pressure has risen sufficiently to distend the cuticle to the point at which they become patent, thus finally releasing a small pulse of nectar to the exterior.

The advantages of this model are that it accounts for all the presently available facts and does not predict any improbable activity on the part of the plant. Further, it is supported by the best information on the *in vivo* structure of the gland that we currently have to hand. Hughes (1977) in her Master's thesis proposed variations on this same general theme and, indeed, earlier authors had also envisaged the participation of rough "endoplasmic reticulum" in secretory processes from trichomes (*e.g.* Unzelman and Healey 1974). However, previous workers have not had access to rapidly frozen material. What has been proposed here is, necessarily, only an hypothesis and it now remains to adduce further supporting evidence, including an investigation of the specific subcellular localisation of the sucrase as well as a more detailed study of the nature of the association of the secretory reticulum with the plasmalemma. It will also prove both intesting and informative to compare the model for *Abutilon* with the secretory mechanism of other nectaries, including those of *Strelitzia reginae* (Kronestedt and Robards 1987) and *Fritillaria* (in preparation) which, in each case, appear to have arrived at different structural solutions to similar physiological problems.

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