

# Immunocytochemical localization of water-soluble glycoproteins, including Group 1 allergen, in pollen of ryegrass, *Lolium perenne*, using ferritin-labelled antibody

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## Summary

The cellular sites of the glycoproteins Group 1 allergen (glycoprotein 1) and Antigen A (glycoprotein 2) in mature ryegrass pollen have been investigated by immunoelectron microscopy. Radioimmunoassays confirm previous findings of cross-reactivity between the purified glycoprotein antigens at the high immunoglobulin G (IgG) concentrations used for localization.

Freeze-drying of anthers followed by anhydrous processing has been employed because of the water solubility and mobility of the glycoproteins. A double-embedding technique has been developed. This involves, first, embedding anthers in the water-soluble plastic resin JB-4, sectioning and incubating in ferritin-labelled antisera by the indirect method. The sections are then embedded in Spurr's resin for ultra-thin sectioning. Both glycoproteins are found in the following sites: (1) exine and intine wall layers; (2) pollen cytoplasm; (3) the orbicules and anther loculus; and (4) the anther cuticle. In the exine arcades and surface and in the anther loculus, the ferritin label is bound to pollenkit. The finding that the glycoproteins are in similar sites is predictable in view of the cross-specificity of the antisera. The extent of antibody penetration of the plastic sections has been examined; labelling is confined to cut grains and absent from intact grains.

## Introduction

One property of plant cells which distinguishes them from animal cells is their possession of a cell wall. The wall of the pollen grain, specialized plant cells which contains the male gametes or their progenitor cell, has two principal layers (Fig. 1): the

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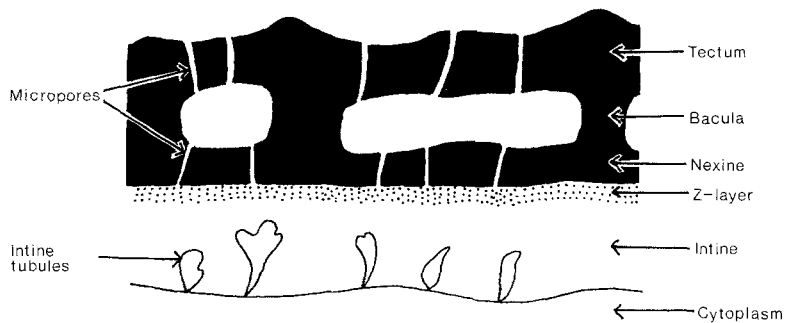


Fig. 1. Diagram showing a radial section of a ryegrass pollen grain wall.

inner intine, and the outer exine, which differ in chemical composition, morphology and ontogeny. The intine comprises a polysaccharide matrix with frequent tubular structures, apparently evaginations of the plasma membrane. In grasses, an additional layer of pectic polysaccharide, the Z-layer (Zwischenkörper), covers the intine and is especially thickened at the single germinal aperture (Heslop-Harrison & Heslop-Harrison, 1980). The exine is composed of sporopollenin, and is intricately sculptured: the various strata include the floor layer or nexine, and rods or bacula which support the roof or tectum (Fig. 1). In grasses, both tectum and nexine contain micropores which connect with the arcades of the exine (see review by Heslop-Harrison, 1979).

In several pollen types, these wall layers have been shown to contain a diverse range of macromolecules, including proteins and glycoproteins (Tsinger & Petrovskaya-Baronova, 1961; Knox & Heslop-Harrison, 1969, 1970; Knox *et al.*, 1975). Only three well-characterized glycoproteins have, however, been assigned sites within the pollen grain using immunofluorescence. These include: (1) Antigen E of ragweed which is in both intine and exine wall sites, a finding demonstrated in both freeze-sectioned and freeze-substituted grains (Knox *et al.*, 1970; Knox & Heslop-Harrison, 1971; Howlett *et al.*, 1973); (2) Group 1 allergen (glycoprotein 1) and (3) Antigen A (glycoprotein 2) of ryegrass pollen (Johnson & Marsh, 1965; Smart & Knox, 1980; Howlett & Clarke, 1981a,b) which are present in the walls and cytoplasm, a finding based on methanol-fixed or freeze-dried anthers (Knox *et al.*, 1980; Vithanage *et al.*, 1980; Howlett *et al.*, 1981).

These pollen wall macromolecules diffuse rapidly from the grain on moistening and this has presented problems during immunofluorescence studies. Aqueous fixatives such as paraformaldehyde or glutaraldehyde have proved to be unsatisfactory for two reasons: (1) they leach material from wall sites, and (2) glutaraldehyde reduces the antibody-binding capacity of the pollen antigens (Howlett *et al.*, 1981). In contrast, freeze-drying appears to minimize diffusion artifacts and in the present study this technique has been adapted for the localization of pollen glycoproteins, including Group 1 allergen by immunoelectron microscopy. This has been necessary as the wall

sites of these glycoproteins cannot be defined precisely by fluorescence microscopy because they are at the limits of resolution: the wall of ryegrass pollen is much thinner than that of ragweed pollen (2  $\mu\text{m}$  compared to 5  $\mu\text{m}$  widths).

A further difficulty encountered in immunocytochemical studies is the determination of specificity of antibodies used to localize particular antigens (Knox & Clarke, 1978; Knox, 1982). This problem becomes very important when the high resolution of transmission electron microscopy is employed. Radioimmunoassay techniques have revealed antigenic cross-reactivity between the ryegrass pollen glycoproteins due to the occurrence of common sugar sequences in the carbohydrate components of these glycoproteins (Howlett & Clarke, 1981b). In this paper, specificity of the antisera is examined, by radioimmunoassay, at concentrations equivalent to those used in immunocytochemical studies.

## Materials and methods

### *Specificity of antisera*

Antisera to purified Group 1 allergen and Antigen A were prepared and the immunoglobulin G (IgG) fraction of each and of pre-immune rabbit serum was isolated as described previously (Howlett *et al.*, 1981). The radioimmunoassay of Marier *et al.* (1979) was used to investigate the cross-reactivity of these antisera. The use of this assay for pollen antigens has been described by Howlett *et al.* (1981) and Howlett & Clarke (1981a,b). Solutions of Antigen A or Group 1 allergen (40  $\mu\text{l}$  of 50  $\mu\text{g}/\text{ml}$ ) were added to wells of a polyvinyl chloride microtitre tray (Cooke Laboratories, Virginia, U.S.A.). After 4 h at room temperature, the solutions were removed and the wells washed three times with 200  $\mu\text{l}$  aliquots of 1% bovine serum albumin (BSA; Sigma Chemical Co., Missouri, U.S.A.) in phosphate-buffered saline (PBS). Three-fold serial dilutions of specific antisera or of pre-immune serum in 1% BSA in PBS (75  $\mu\text{l}$ ) were then added to the antigen-coated wells and incubated overnight at 4° C. After thorough washing of the wells (three times with BSA in PBS, eight times with water), Staphylococcal Protein A (Pharmacia, Uppsala, Sweden) – which was iodinated with  $^{125}\text{I}$  by the technique of Fraker & Speck (1978) – was then added (75  $\mu\text{l}$  containing 30 ng, 24 000 c.p.m.) to detect the antigen-IgG complexes. After 5 h, the wells were thoroughly washed, drained on a tissue paper and then cut and counted in a gamma counter (Packard 5110 auto-Gamma scintillation spectrometer).

### *Tissue preparation and immunoferritin methods*

The post-embedding staining technique of immunocytochemistry was used. Anthers from field-grown plants of *Lolium perenne* were freeze-dried and embedded in JB4 resin as described by Howlett *et al.* (1981). Transverse sections of anthers embedded in JB4 (20–30  $\mu\text{m}$  thick) were cut with a glass knife in a Cambridge Rocker microtome. Solutions of IgG [100  $\mu\text{l}$  of 2 mg/ml in 0.01 M sodium phosphate–0.15 M NaCl, pH 7.4 (PBS)] were incubated with the sections for 30 min at room temperature. The sections were then washed with PBS (4  $\times$  500  $\mu\text{l}$ ) over a 15 min period. Ferritin-conjugated IgG of goat serum directed against rabbit IgG (Capell Laboratories, Pennsylvania, U.S.A.) in 0.05 M sodium phosphate, pH 7.5, was centrifuged (10 000g, 5 min, Beckman Microfuge) to remove any large aggregates of ferritin. The undiluted supernatant was reacted (100  $\mu\text{l}$ ) with the sections for 30 min at room temperature. Sections incubated with pre-immune rabbit IgG instead of immune IgG, followed by the ferritin conjugated goat anti-rabbit IgG or with unconjugated ferritin, were employed as controls. All sections were then washed thoroughly in PBS (4  $\times$  500  $\mu\text{l}$  for 15 min).

*Osmication and embedding in Spurr's resin*

The JB4 sections were post-fixed in 1% osmium tetroxide in 0.05 M sodium phosphate buffer, pH 7.2, for 1 h at room temperature, followed by a wash in the phosphate buffer twice over a 10 min period. The sections were removed from the phosphate buffer and washed in 0.05 M sodium maleate buffer, pH 5.2 ( $2 \times 1$  ml during a 20 min period). A solution of 0.5% uranyl acetate in the maleate buffer was incubated with the sections for 2 h at room temperature in the dark. The sections were then washed twice in the maleate buffer and dehydrated through a series of increasing ethanol concentrations (30, 50, 70, 90 and 100%) and the sections were incubated for 15 min in a fresh solution of 100% ethanol. The sections were transferred into ethanol-acetone (1:1v/v) for 30 min and then into 100% acetone for another 30 min period. They were then embedded in a graded series of solutions of Spurr's resin in acetone from 10–100% in 10% steps. The infiltration was performed over a total period of three days.

When JB4 sections are incubated in organic solvents, such as ethanol or acetone, they undergo some deformation and become partly curled and distorted. Consequently, exposure to these solvents was kept to a minimum. Attempts to avoid this problem by embedding the JB4 sections directly in Spurr's resin without the use of organic solvents were unsuccessful since the resin failed to infiltrate the JB4 section. These JB4 sections embedded in Spurr's resin were placed in aluminium trays and cured at 60° C for 7 h, thus allowing complete polymerization of the resin. Ultra-thin sections were cut at right angles to the original JB4 section using an ultra microtome (Porter Blum II) fitted with a diamond knife and the sections were mounted on grids. Sections were then examined in a Siemens 102 transmission electron microscope. To enhance the contrast of cellular membranes, some sections were post-stained with lead citrate (Venable & Coggeshall, 1965).

**Results**

## DETECTION OF ANTIGENIC CROSS-REACTIVITY BY RADIOIMMUNOASSAY

At the particular concentrations of antibody employed in the immunocytochemical study, there was extensive cross-reactivity between the antigens and heterologous IgG fractions (Table 1). Under the same conditions, there was no significant cross-reactivity with bovine serum albumin, and pre-immune IgG bound at background levels to the antigens. The anti-Antigen A IgG bound in high amounts to whole pollen diffusate, as well as to Group 1 allergen and Antigen A. Anti-Group 1 allergen IgG bound to its homologous antigen and in lesser amounts to whole pollen extract and Antigen A.

## IMMUNOELECTRONMICROSCOPICAL LOCALIZATION OF GLYCOPROTEINS

*Ultrastructural preservation of pollen grains*

Scanning electron micrographs of solvent-washed pollen of *Lolium perenne* established the surface features of the grains, including the single germinal aperture (Fig. 2). Freeze-drying of the pollen grains within the anther resulted in variable ultrastructural preservation of individual grains. The shape of grains, when viewed by light microscopy, ranged from near spherical to shrunken (Howlett *et al.*, 1981). In the present study, some grains showed excellent preservation of both wall and cytoplasmic structure (for example, Figs. 3, 4, 6, 7) whilst other grains had poorly preserved cytoplasm (for example, Fig. 9).

**Table 1.** Antigenic cross-reactivity of Group I allergen and Antigen A from ryegrass pollen.

| <i>Antibody</i>       | <i>Antigen</i>                   | [ <sup>125</sup> I]Protein A bound<br>(c.p.m. × 10 <sup>-3</sup> ) |
|-----------------------|----------------------------------|--------------------------------------------------------------------|
| Anti-Group I allergen | Group 1 allergen                 | 25.5                                                               |
|                       | Antigen A                        | 12.0                                                               |
|                       | <i>L. perenne</i> pollen extract | 15.0                                                               |
|                       | BSA                              | 0.4                                                                |
| Anti-Antigen A        | Group 1 allergen                 | 15.6                                                               |
|                       | Antigen A                        | 25.9                                                               |
|                       | <i>L. perenne</i> pollen extract | 42.0                                                               |
|                       | BSA                              | 0.4                                                                |

Wells of a microtitre tray were coated with Group 1 allergen, Antigen A, BSA (40 µl of 50 µg/ml in PBS) or *L. perenne* pollen extract (40 µl of 200 µg/ml in PBS). IgG fractions of antisera to Group 1 allergen, Antigen A or pre-immune serum were added to the antigen-coated wells at the concentrations used for the localization studies (50 µl of 2 mg/ml in 1% BSA in PBS). After 16 h at 4° C, the IgG solutions were removed and [<sup>125</sup>I]Protein A added (50 µl, 60 ng, 60 000 c.p.m.). Each determination was performed in triplicate. Pre-immune serum bound to each antigen at background levels (400 c.p.m.).

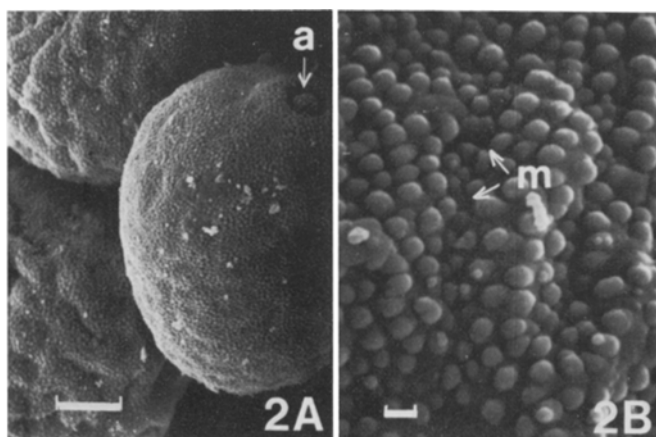


**Fig. 2.** Surface morphology of pollen grains of ryegrass, *Lolium perenne*, shown by scanning electron microscopy. (A) General view of grains. (B) Detail of exine surface. a, aperture; m, micropores. Scale bars: (A) 10 µm, (B) 0.5 µm.

#### *Antibody binding and penetration of JB4 sections*

The ultra-thin sections were cut at right angles to the original JB4 sections which had been incubated in antisera. The original cut surface was identified by the presence of 'cut ends' of the pollen wall, that is, sudden terminations of the tissue likely to be produced by shearing at the surface (see Figs. 5, 8, 11, 12). Such cut surfaces invariably

were labelled with ferritin in sections treated with either test or control IgG. In Fig. 11, labelling is dense on the external surface of the grain and much less within the arcades of the exine, and one explanation is that the outer face of the exine is at the cut surface of the JB4 section.

In pollen grains with discrete, localized ferritin labelling, cut ends were present, suggesting that the grain formed part of the exposed surface of the original section as in Figs. 11 and 12. Unlabelled grains are interpreted as false negatives, that is, whole grains within the plastic section which had not been exposed to the antibody, since no cut ends were present. It appears that the immunocytochemical reagents did not penetrate whole grains.

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**Figs. 3–23.** Immunoelectron microscopical localization of pollen glycoproteins. Abbreviations: a, aperture; b, bacula; i, intine; m, micropore; n, nexine; ob, osmiophilic bodies; op, operculum; or, orbicules; os, osmiophilic organelle; p, pollenkit; s, starch grain; t, tectum; v, vesicles; z, Z-layer.

**Figs. 3–6.** Binding of unconjugated ferritin to sections of *L. perenne* anthers. All micrographs are of the same grain which had a cut surface exposed to the ferritin. Scale bars: 1  $\mu\text{m}$ .

**Fig. 3.** Section of pollen grain showing wall layers and cytoplasm.

**Fig. 4.** Detail of pollen wall in region of aperture.

**Fig. 5.** Detail of pollen grain wall surface of original JB-4 section, showing cut end of exine, and ferritin particles associated with cut surface (arrow).

**Fig. 6.** Detail of cytoplasm showing ferritin particles in ground substance (arrows) and osmiophilic organelles.

**Figs. 7–10.** Binding of pre-immune IgG to sections of *L. perenne* anthers. JB-4 sections were exposed first to pre-immune IgG and then to ferritin-labelled anti-antibody. Scale bars: 0.5  $\mu\text{m}$ .

**Fig. 7.** Ferritin particles (arrows) are associated with the wall layer and cytoplasm in section of grain which had a cut surface exposed to reagents. Ferritin particles are randomly dispersed in the exine arcades, and associated with spherical osmiophilic bodies and the ground substance of the cytoplasm.

**Fig. 8.** Ferritin particles associated with the cut surface, both exine and cytoplasm, in a different grain from that shown in Fig. 7.

**Fig. 9.** Absence of ferritin labelling in section of pollen grain which apparently had no cut surface exposed to reagents.

**Fig. 10.** Detail of exine from same section as Fig. 9. Note material in arcades has no ferritin labelling.

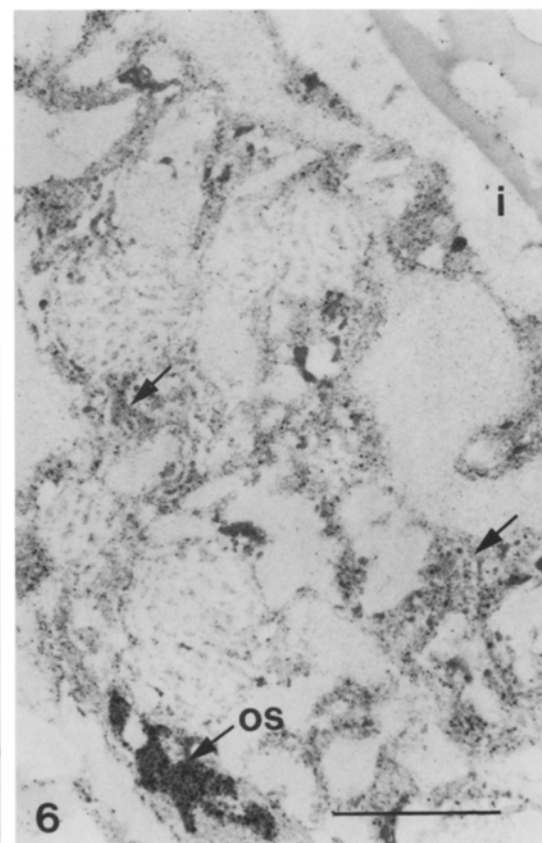
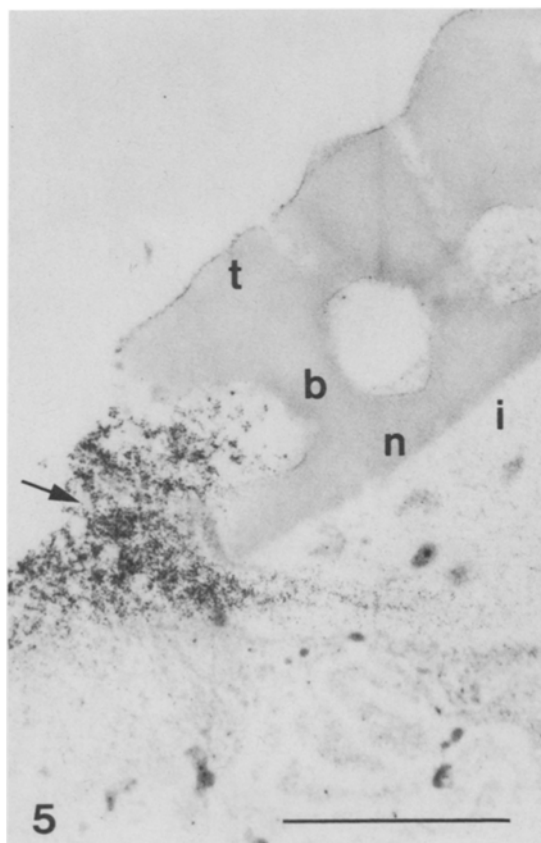
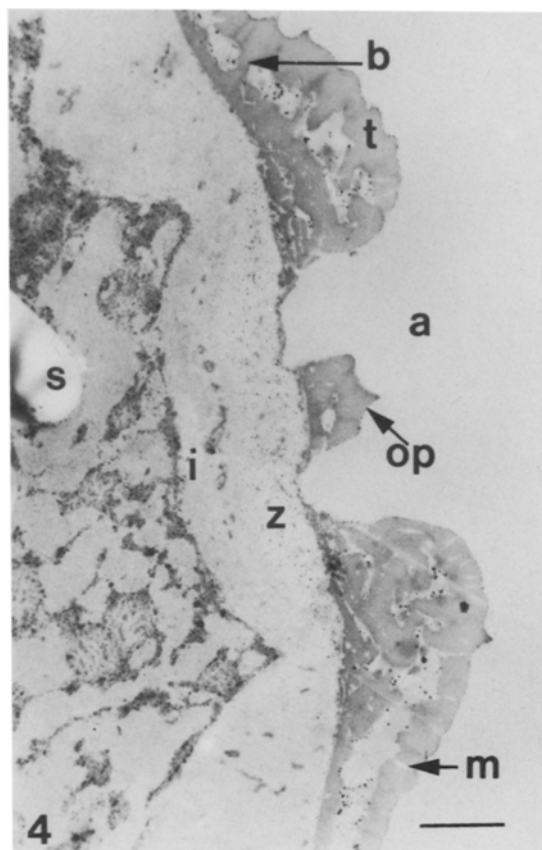
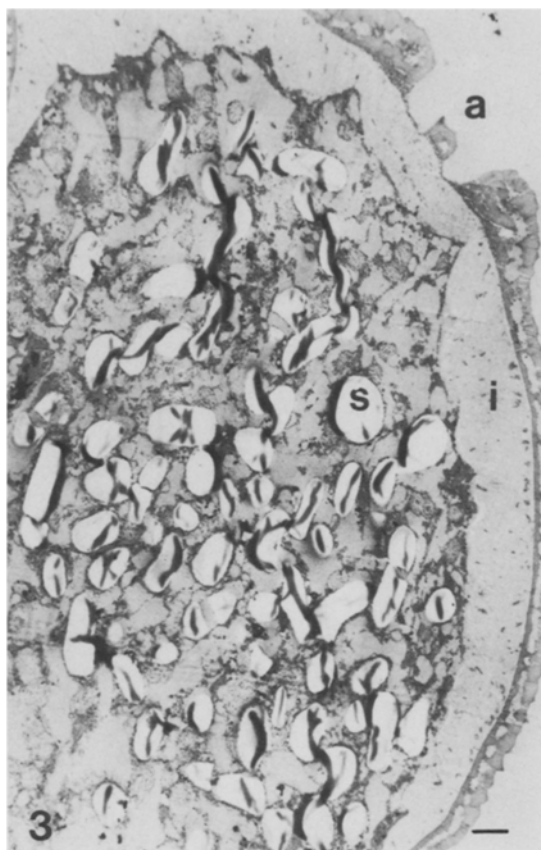
**Figs. 11–19** Immunoelectron microscopical localization of pollen glycoproteins with anti-Group I allergen IgG. JB-4 sections were incubated first in anti-Group I allergen IgG, then in ferritin-labelled anti-antibody.

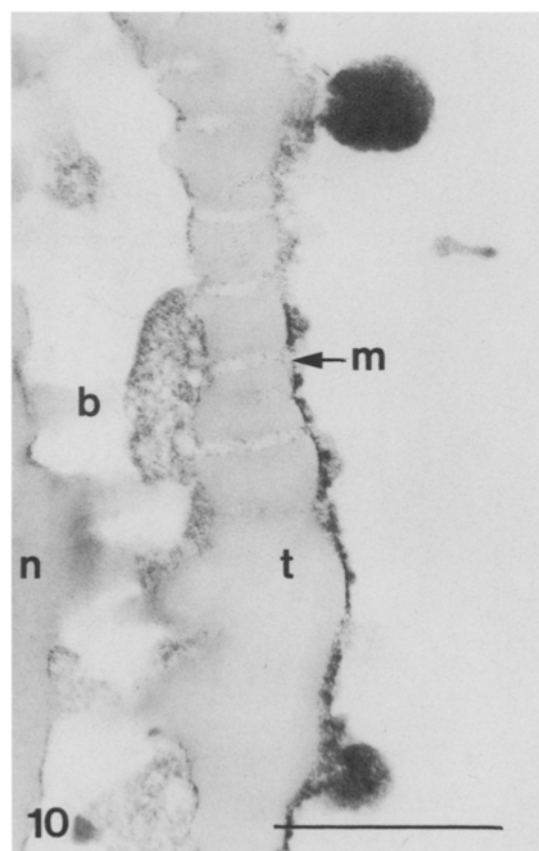
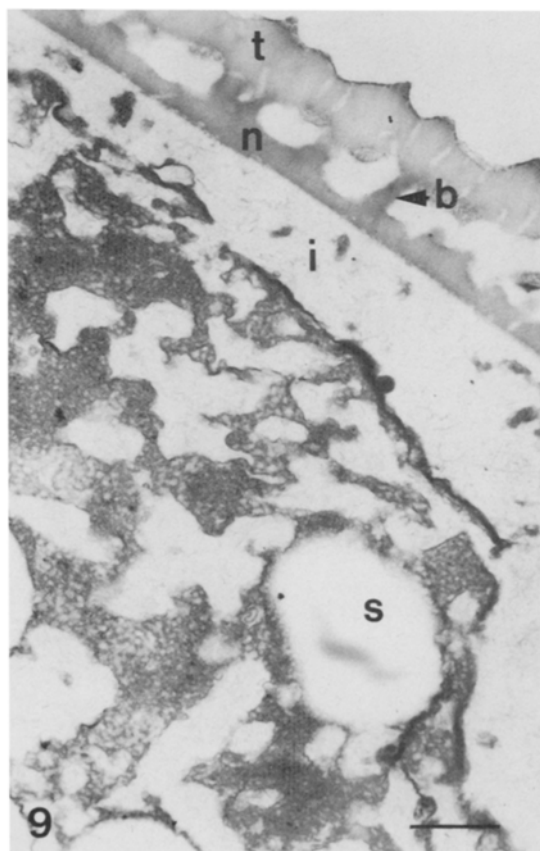
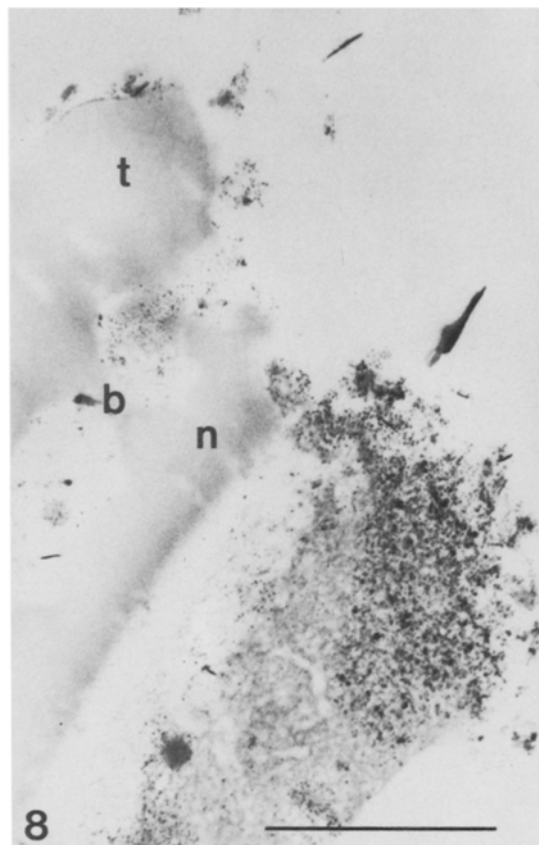
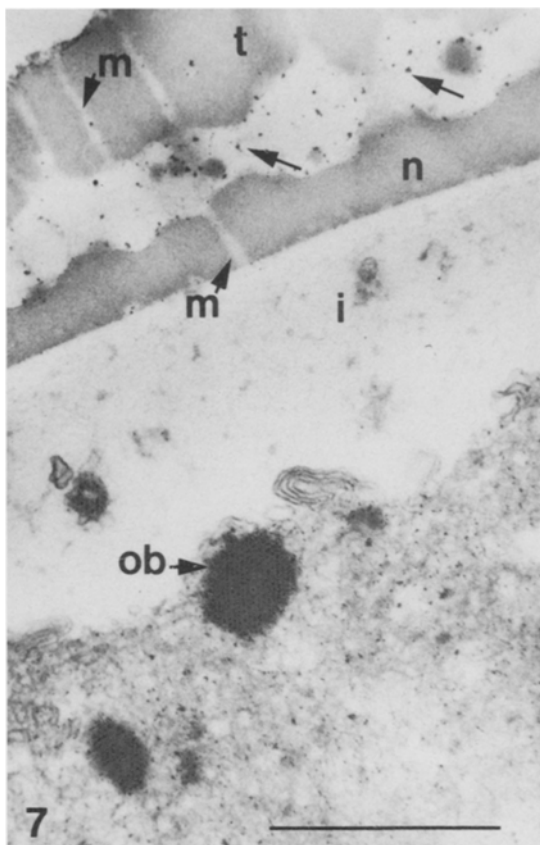
**Fig. 11.** Tectum of exine at cut surface of JB-4 section, showing ferritin particles associated with the grain surface; oblique section. Scale bar: 0.5  $\mu\text{m}$ .

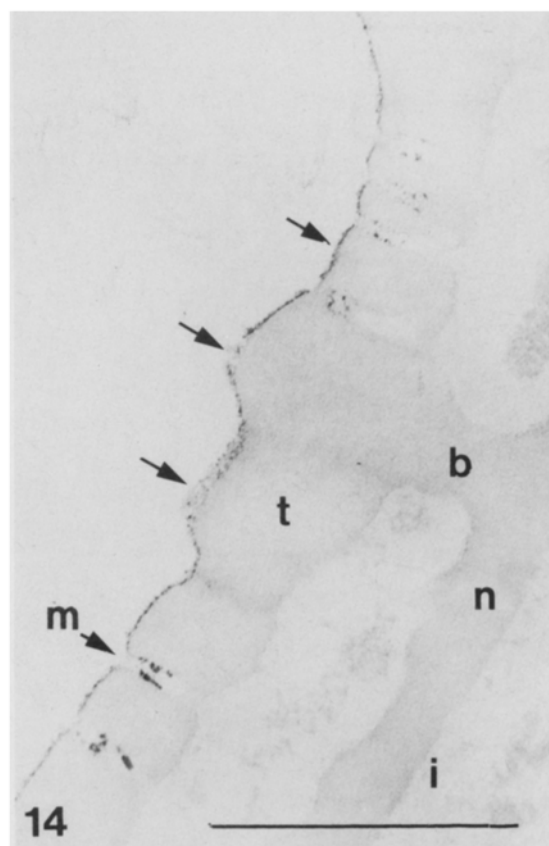
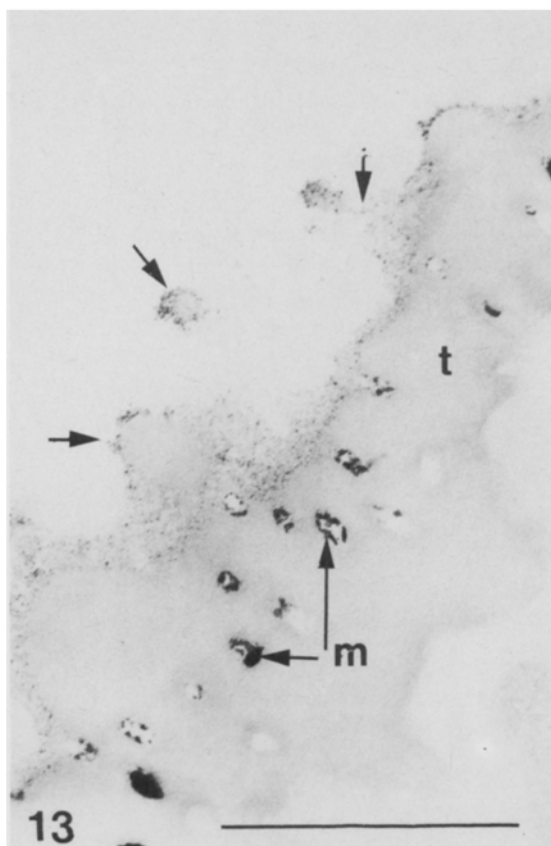
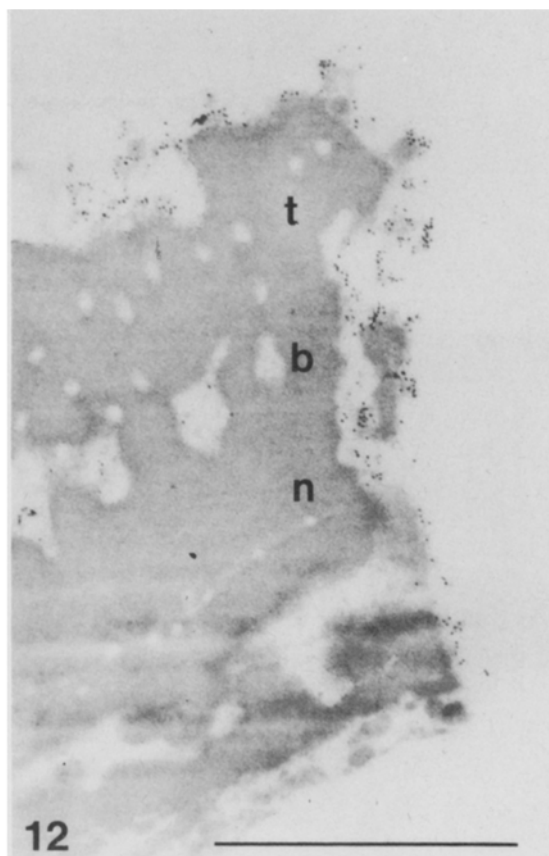
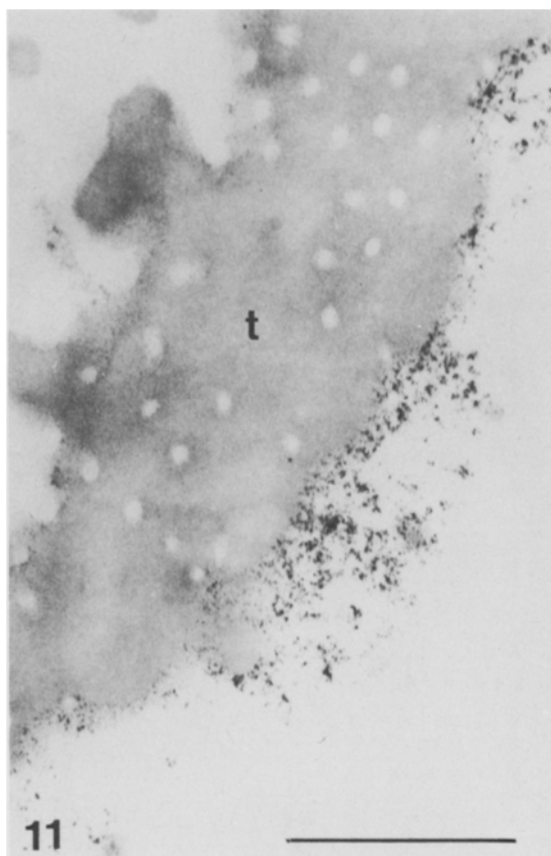
**Fig. 12.** Ferritin particles associated with the cut surface of both exine and cytoplasm in a different grain from that in Fig. 11. Scale bar: 0.5  $\mu\text{m}$ .

**Fig. 13.** Ferritin particles present at exine surface (arrows) and in outer micropores (m); oblique section. Scale bar: 0.5  $\mu\text{m}$ .

**Fig. 14.** Ferritin particles present at exine surface (arrows) and within the outer micropores (m) but absent from the exine arcades and intine; radial section. Scale bar: 0.5  $\mu\text{m}$ .







*Specificity of immunocytochemical staining*

*Incubation in ferritin.* Sections incubated in ferritin showed pollen grains with either a random scattered deposition of ferritin particles or with no detectable labelling at all. In the exine, the ferritin particles when present were dispersed on the external surface, and diffusely scattered throughout the arcades. Scattered particles occurred in the intine in some grains associated with tubular inclusions (Figs. 3, 4) and especially with the Z-layer (Fig. 4). In the cytoplasm, ferritin particles were scattered over the ground substance but not associated with the starch grains or lipid droplets (Fig. 3, 4). An organelle similar in its elongate shape to a plastid had depositions of ferritin particles over electron-dense regions (Fig. 6).

*Incubation in normal (pre-immune) IgG.* The pattern of ferritin labelling in these control sections was essentially similar to that described above for ferritin controls. The density of particle deposition was generally very much reduced and was totally absent in many grains. This was especially evident in the cytoplasm where only scattered particles were detected (Fig. 7). The exine arcades and surface contained material of low electron density (Figs. 7, 9, 10) which usually had no ferritin labelling associated with it. The intine contained various tubular structures, and at the interface with the cytoplasm, membranous evaginations were present (Fig. 7).

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**Fig. 15.** Ferritin particles associated with the wall layers and peripheral cytoplasm in a grain with a cut surface in JB-4 section. Ferritin is associated with spherical material in the arcades (arrows), and with vesicles and osmiophilic bodies at the interface of intine and cytoplasm. Scale bar: 0.5  $\mu\text{m}$ .

**Fig. 16.** Ferritin particles (arrows) associated with the pollen wall and external pollenkit lying in the anther cavity between the pollen grain and the orbicules. Note that the ferritin is associated only with components of high electron density in the pollenkit. Scale bar: 0.5  $\mu\text{m}$ .

**Figs. 17–18.** Detail of labelled material in the exine arcades. Scale bar: 0.1  $\mu\text{m}$ .

**Fig. 19.** Wall of grain with cut surface in JB-4 section. (A) Ferritin particles are associated with material in the exine arcades and in vesicles in the intine; oblique section. (B) After post-staining with lead citrate vesicles within the intine layer are shown to be bounded by a membrane. Scale bar: 0.25  $\mu\text{m}$ .

**Figs. 20 and 21.** Immunoelectron microscopical localization of pollen glycoproteins with anti-antigen A IgG. Scale bars: 0.25  $\mu\text{m}$ .

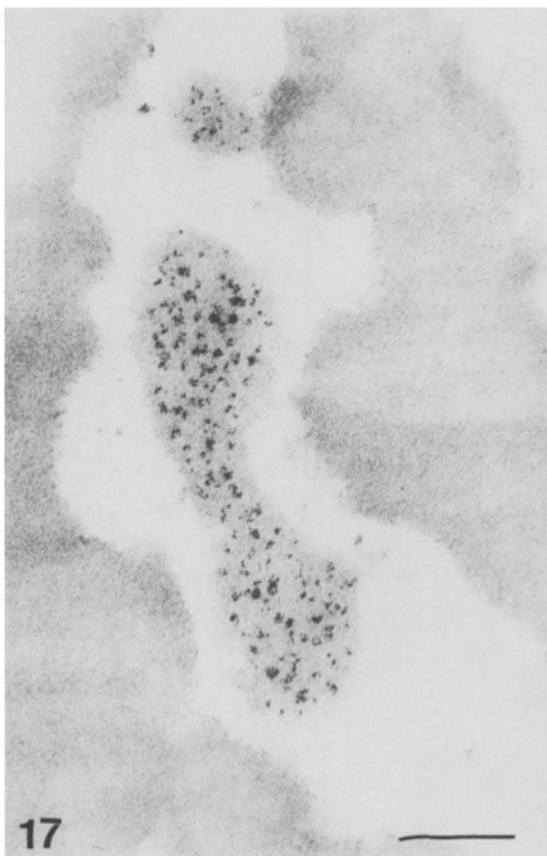
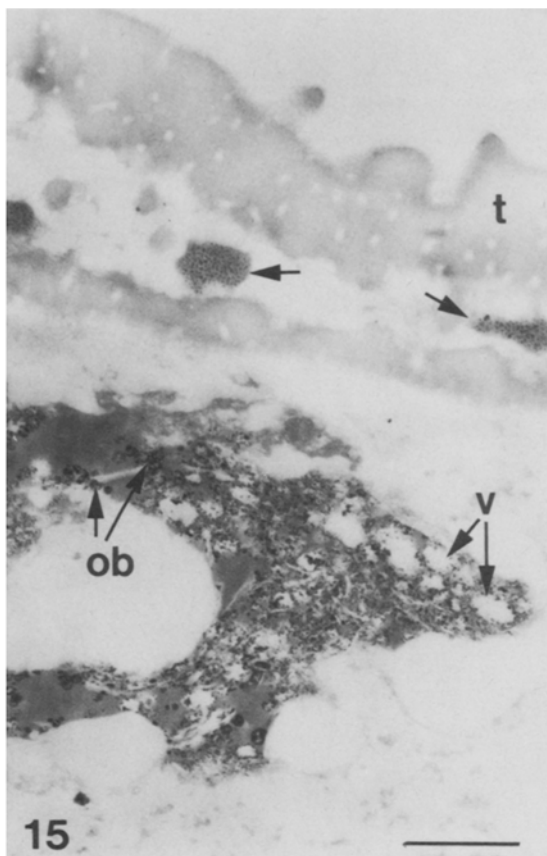
**Fig. 20.** Ferritin labelling associated with surface tectum and pollenkit of exine surface; tangential section.

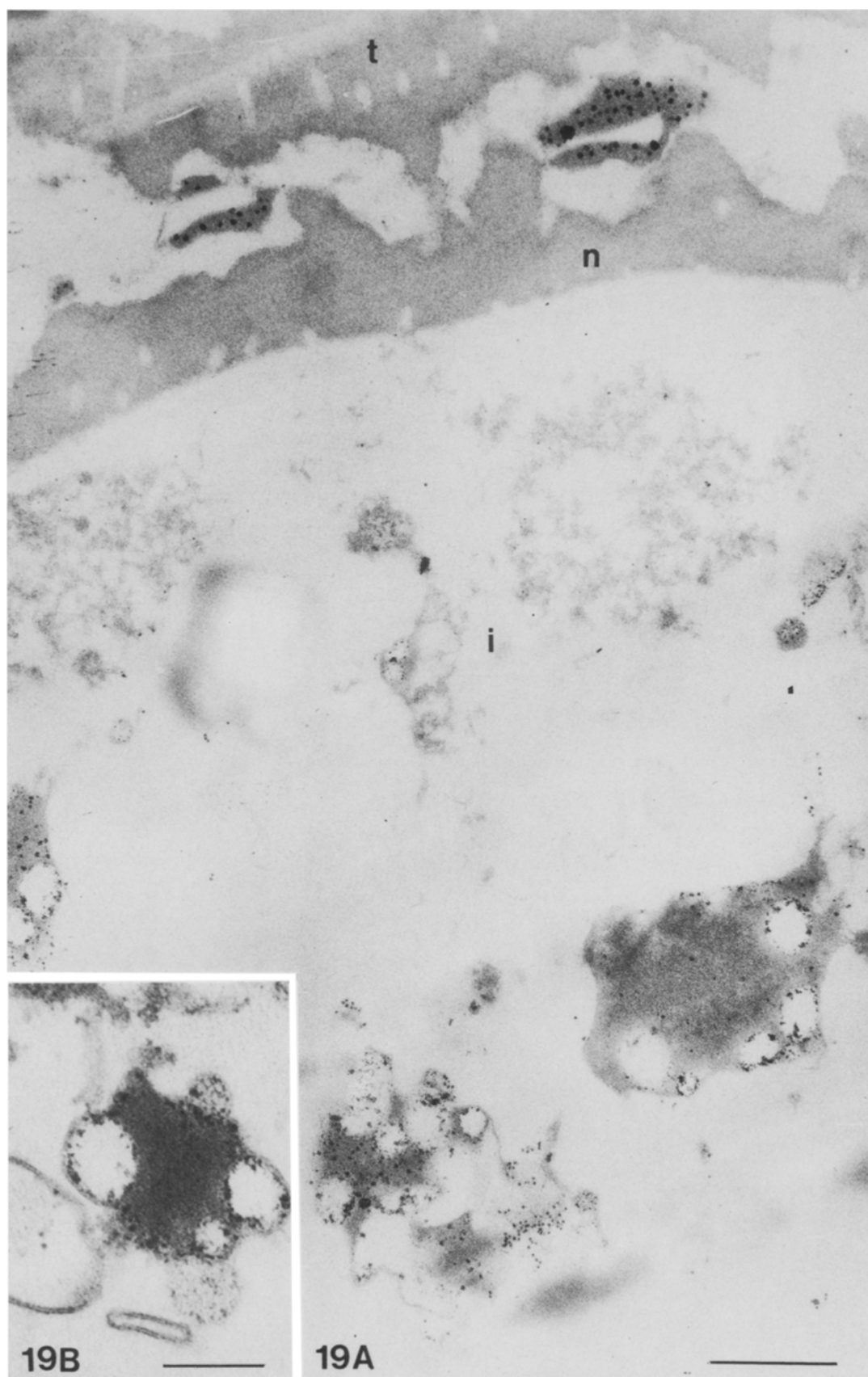
**Fig. 21.** Ferritin labelling on exine surface of pollen wall; radial section.

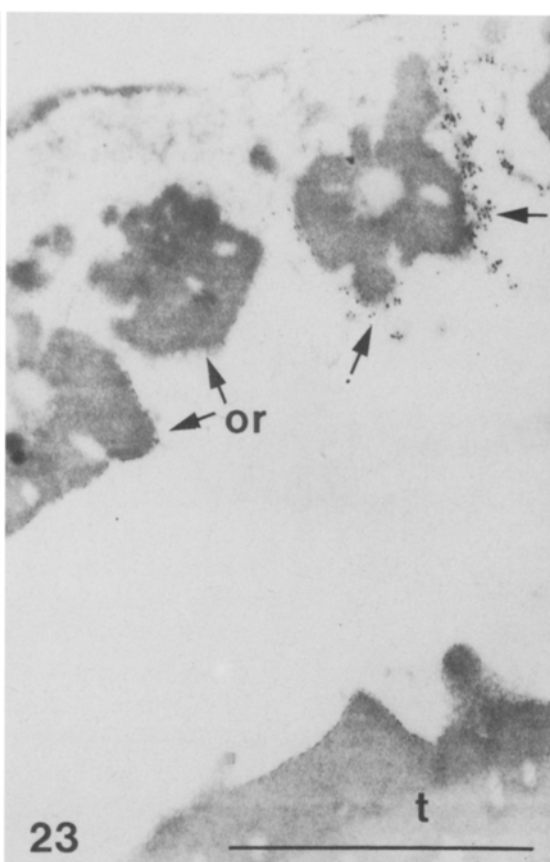
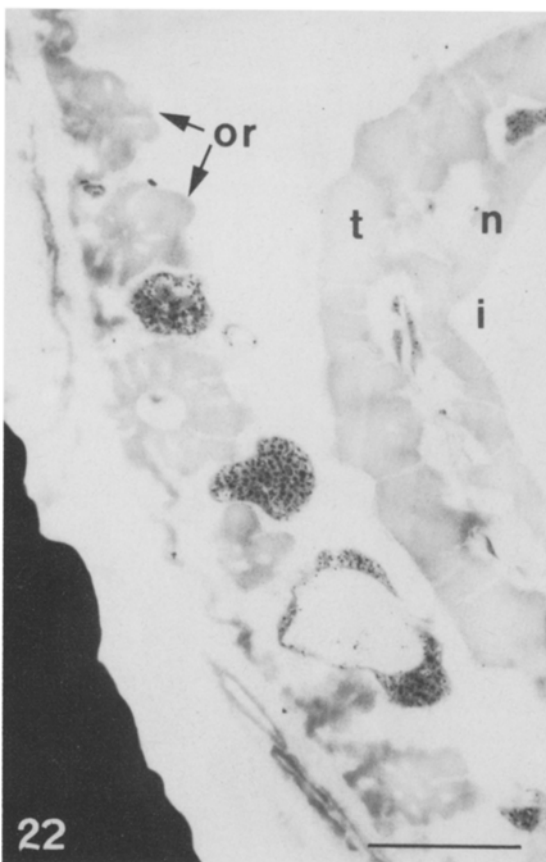
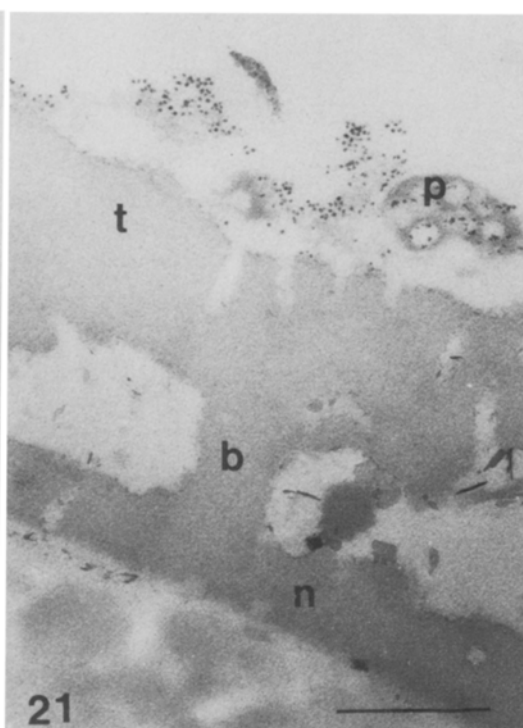
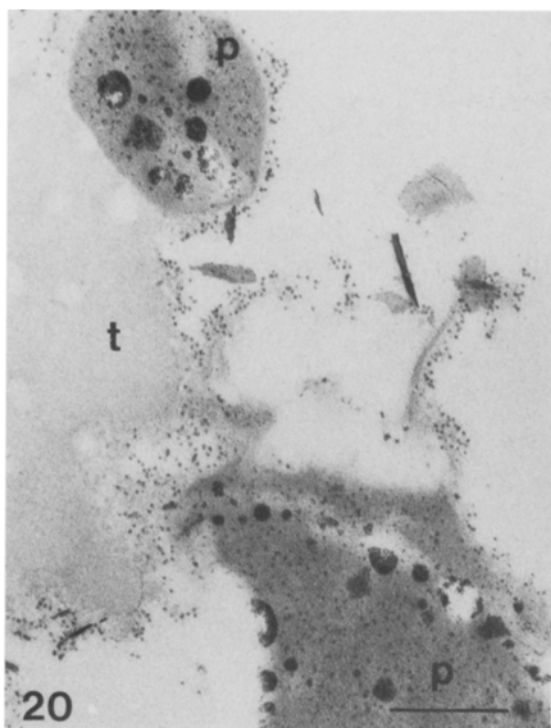
**Figs. 22 and 23.** Ferritin particles associated with the orbicules lining the anther cavity. Scale bars: 0.5  $\mu\text{m}$ .

**Fig. 22.** Ferritin is associated with pollenkit on and between the orbicules. Ferritin is absent within the orbicules in this micrograph, but in some sections, it occurs around the wall of the inner spherical chamber of the orbicules.

**Fig. 23.** Ferritin is scattered over the orbicule surfaces (arrows).







*Incubation in anti-Group 1 allergen IgG.* Ferritin labelling is associated with four sites in the anther sections: (1) the exine and intine wall layers of the pollen; (2) cytoplasmic sites within the grain; (3) the orbicules and anther loculus; and (4) the anther cuticle.

Within the arcades of the exine, labelling is especially intense in material of low electron density (Figs. 15, 16). In some grains, the label resembles a fine precipitate (Fig. 17), while in others the labelling is aggregated into small spheres or vesicles within an otherwise unlabelled material (Fig. 18). Ferritin is also present in the inner or outer micropores which connect the arcades to the intine or exterior of the grain respectively. In Fig. 13, ferritin particles are seen clustered in the outer micropores of the exine, and massed on the external surface of the exine. This is an oblique section, and the particles are seen in radial section in Fig. 14.

The label is present in the inner polysaccharide intine layer. Here it is associated with vesicular structures which possess a membrane (Fig. 19) and are either embedded in the wall or at the interface with the cytoplasm.

The external surface of the pollen grain wall and the periphery of the anther loculus contain ferritin particles which are present in a variety of characteristic patterns. Individual particles may be massed over the exine surface (as in Figs. 11, 12, 14). Alternatively, particles may be present in spheres within material of low electron density assumed to be pollenkit. In this case, the ferritin particles may be present in discrete areas or over the entire surface of the material (Figs. 16, 22). These same patterns of labelling are observed with the orbicules (Figs. 22, 23).

Ferritin labelling has been observed on the surface of the cuticle, and in the cytoplasm of epidermal cells of the anther. The cuticle surface labelling is virtually absent in control sections but epidermal cell labelling is present in both test and control sections.

*Incubation in anti-Antigen A IgG.* The pattern of ferritin-labelling was identical to that found in anthers incubated in anti-Group 1 allergen IgG. The label was present in arcades of the exine and on the grain surface where it was associated with pollenkit (Figs. 20, 21).

## Discussion

In immunocytochemical studies, a problem often encountered is the assessment of the specificity of the antibodies used to localize the particular antigens. This specificity is usually established by immunodiffusion methods, which are less sensitive than the enzyme-linked, immunosorbent assay (ELISA) or radioimmunoassay. These latter techniques detect binding of antigens to single antibody molecules in a similar way to the immunofluorescence and immunoferritin reactions. We have used immunodiffusion to examine specificity of antisera to rye grass pollen glycoproteins, and no antigenic cross-reactivity was detected (Howlett *et al.*, 1981). The antiserum specificity has now been examined by radioimmunoassay following development of the method by Howlett & Clarke (1981a). At the high concentrations of antisera used in localization

studies, both Group 1 allergen and Antigen A bind to heterologous antisera. However, antisera to Group 1 allergen are more specific than antisera to Antigen A. Some of the cross-reacting antigenic determinants of the glycoproteins are probably saccharides (Howlett & Clarke, 1981b). We did not attempt to absorb the antisera, since only minute amounts of the purified glycoproteins were available (Howlett & Clarke, 1981a). These quantitative methods of antiserum analysis have only been applied recently to immunocytochemical studies of plant antigens. Craig *et al.* (1980), using ELISA, demonstrated low levels of cross-reactivity between two storage glycoproteins of pea seed (legumin, 1% carbohydrate, vicilin, 0.3% carbohydrate; Basha & Beevers, 1976). It seems likely that antigenic cross-reactivity will pose problems in localization studies of plant glycoproteins which contain significant levels of carbohydrate. A potential solution to this problem is the use of monoclonal antibodies.

Because of their ready solubility in aqueous media, the pollen glycoproteins have been particularly difficult to localize in their cellular sites by immunocytochemistry. We have been able to take advantage of the dehydrated state of mature pollen (15–35% water; Stanley & Linskens, 1974) within the anther to freeze-dry the grains with reasonable preservation of ultrastructure and process the material under anhydrous conditions to the antibody incubation steps. In pollen treated under these conditions, both glycoproteins are present predominantly in the pollen wall in similar sites, which was predictable in view of the cross-specificity of the antisera. The localization sites include vesicles of the intine, arcades of the exine, the exine surface and the orbicules of the anther cavity. These intine vesicles are bounded by a unit membrane and are likely to be evaginations of the plasma membrane incorporated into the wall during wall deposition. Similar vesicles have been described in germinating pollen of *Secale* by Heslop-Harrison (1979) and in *Zea mays* (Kress & Stone, 1982). In the exine arcades, the glycoproteins appear to be associated with pollenkit of low electron density which is characteristic of wind-borne pollen types (Hesse, 1979). They are also present in the pollenkit on the grain surface and on the orbicules.

In a previous study, ryegrass pollen grains fixed in cold anhydrous methanol contained Antigen A in similar sites (Knox *et al.*, 1980). A feature of the present study is the greatly improved ultrastructural preservation and resolution of both wall and cytoplasm following freeze drying, when compared with methanol fixation.

The appearance and distribution of ferritin in this study is unusual in that the particles were mostly clustered in a non-random fashion within the pollenkit and in vesicular components of the intine. In some cases, however, a random distribution was observed on the surfaces of the exine and of the orbicules, similar to that in immunocytochemical studies in animal cells (Nicolson *et al.*, 1971).

The presence of the grass pollen glycoproteins in exine cavities of mature pollen is a surprising result in view of the absence of proteinaceous material in the exine cavities of other grass pollens when viewed by transmission electron microscopy (Rowley, 1964; Skvarla & Larson, 1966; Christensen *et al.*, 1972; Christensen & Horner, 1974; Barlow, 1978; Heslop-Harrison, 1979; Young *et al.*, 1979). In these studies, the pollen proteins

may have been lost during aldehyde or osmium tetroxide fixation, since the grass pollen proteins are remarkably mobile. However, recently Kress & Stone (1982) have shown that *Zea mays* pollen, after glutaraldehyde fixation, had proteins in the exine cavities of the wall.

The site of synthesis of the glycoproteins could not be determined from the present study. They may have originated in the cytoplasm, like many other wall components of plant cells and been transported across the membrane: the mechanism of such extracellular secretion is still only poorly understood (for review, see Chrispeels, 1976). Alternatively, they may have been synthesized in the tapetum, the parent tissue, which surrounds the grains and provides nutrients to them. The presence of tapetally derived proteins has been reported in several pollen types including *Malva viscosa* (Heslop-Harrison *et al.*, 1973) and *Raphanus* (Dickinson & Lewis, 1973).

Regardless of their origin, the glycoproteins, once they are in the wall, are probably able to move freely from intine to exine sites via the exine micropores. These sites which are readily accessible to the grain surface are consistent with the rapid induction of allergic responses in the nasal mucosa of man by the allergen. The role of allergens within the plant is not known, but they are strategically sited for immediate contact with the stigma surface, where they may be involved in incompatibility responses, or in the early stages of pollen germination and growth in the stigma.

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