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The Growth of the Grass Pollen Tube: 1. Characteristics of the Polysaccharide Particles ("P-Particles") Associated with Apical Growth

J. HESLOP-HARRISON* and Y. HESLOP-HARRISON

Welsh Plant Breeding Station, Plas Gogerddan, nr. Aberystwyth

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Summary

Numerous polysaccharide-rich particles ("P-particles") occur in the tip region of growing grass pollen tubes, where they apparently contribute to the extending wall. In other families the corresponding bodies have been shown to originate from dictyosome activity during pollen tube growth. However, in the grasses the main synthesis precedes anthesis; the P-particles represent up to 30% of the reserves of the vegetative cell of the dormant grain, numbering over one million in the pollen grain of rye. Their membranes are incomplete. The polysaccharide content, which is initially coarsely granular but becomes microfibrillar with hydration, is readily extracted with ammonium oxalate, and is probably pectic in nature. Simple methods for isolating the particles in relatively pure populations are described. Hydrolysis yields principally galactose, arabinose, glucose, and rhamnose. Apart from proteins derived from the original bounding membranes, a protein fraction is tenaciously bound to the polysaccharide. Isolated P-particles move anodically in an electrical field, and the possibility that their movement from the grain to the tube tip during growth depends on a potential gradient, already demonstrated for lily pollen tubes, is considered.

Keywords: Pollen tube growth; Microfibrillar pectins; Dictyosome vesicles; *Gramineae; Secale cereale; Pennisetum typhoideum.*

1. Introduction

Since the work of SASSEN (1964) and ROSEN, GAWLIK, DASHEK, and SIEGESMUND (1964), it has been known that the apical growth of pollen tubes is associated with the presence of populations of vesicles in the tip region. The evidence of electron microscopy, although necessarily circumstantial, strongly suggests that these vesicles are derived from dictyosomes, and that they fuse with the wall in the extending region and contribute precursor materials. The mode of growth is therefore essentially the same as in other tip-growing plant cells; close parallels may be found, for example, in the coenocytic alga, *Vaucheria* (OTT and BROWN 1974), in fungi such as *Pythium* (GROVE, BRACKER, and MORRÉ 1970) and, among vascular plants, in root hairs (BONNETT and NEWCOMB 1966).

While it is not in doubt that the vesicles concerned in the tip growth of pollen tubes contain polysaccharides as in other instances where material is transferred into the wall by dictyosome activity (NORTHCOTE and PICKETT-HEAPS 1966, PICKETT-HEAPS 1967), there is some diversity of views as to the nature of the precursors actually conveyed. In a comprehensive cytochemical and fine-structural investigation of the pollen tubes of *Lilium longiflorum*, ROSEN and GAWLIK (1966) and DASHEK and ROSEN (1966) showed that the vesicles contained a polysaccharide which reacted positively in the hydroxylamine $FeCl_3$ test of ALBERSHEIM, MÜHLETHALER, and FREY-WYSSLING (1962), regarded as specific for pectins, and, moreover, that the contents were wholly removed by pectinase digestion and by extraction with ammonium oxalate. They drew the natural conclusion that the principal polysaccharide present was pectic in nature. VAN DER WOUDE, MORRÉ, and BRACKER (1971) developed a method for the extraction of the vesicles from growing pollen tubes of

* Correspondence and Reprints: Welsh Plant Breeding Station, Plas Gogerddan, nr. Aberystwyth SY23 3EB, U.K.

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the same *Lilium* species, and showed that they contained polysaccharides high in galacturonic acid; their findings were therefore in general agreement with those of ROSEN and his colleagues. In contrast, NAKAMURA, MIKI-HIROSIGE, and IWANAMI (1979) referred to the same bodies in *Lilium* pollen tubes as "callose grains", and the corresponding bodies in pollen tubes of *Petunia hybrida* were considered to be callosic also by CRESTI and VAN WENT (1976). Yet another view came from ENGELS (1973, 1974a, 1974b, 1974c), who concluded on the evidence of X-ray diffraction patterns that the bodies in *Petunia* pollen tubes contained cellulose. Uronides were, however, also found.

While most of the accounts of the fine-structure of the vesicles associated with pollen-tube wall growth have referred to a coarsely granulate or flecked content, VAN DER WOUDE, MORRÉ, and BRACKER (1971) encountered an exceptional pollen tube with a microfibrillar content, and speculated that this might indicate a capacity for cellulose synthesis. DE NETTANCOURT, DEVREUX, BOZZINI, CRESTI, PACINI, and SARFATTI (1973) and CRESTI and VAN WENT (1976) illustrated particles with a very marked microfibrillar content associated with the tube tip in *Lilium*, and the bodies illustrated by CRESTI and VAN WENT (1976) from *Petunia* were of a similar character. Such structures were described from *Oenothera organensis* by DICKINSON and LAWSON (1975, 1976), who offered a scheme for the formation of the pollen tube wall suggesting that dictyosome vesicles contributed to larger "fibrillar bodies", which in turn transferred their contents into the wall. The microfibrillar material of these bodies as shown in the electron micrographs of DICKINSON and LAWSON closely resembles that in the exceptional pollen tube of *Lilium* illustrated by VAN DER WOUDE *et al.* (1971) and in pollen tubes of *Lycopersicum peruvianum* by DE NETTANCOURT *et al.* (1973).

The investigations of the apical growth system hitherto have concerned species in which pollen germination and tube growth are comparatively leisurely processes. The initial activation following upon hydration in these species involves the progressive restoration of dictyosome activity, and thereafter most—in some instances perhaps all—of the vesicles transferred to the tube tip are produced during the actual course of growth. VAN DER WOUDE and MORRÉ (1967) estimated that vesicles are produced at a rate of 2,150 per minute during tube extension in *Lilium longiflorum*, and, more recently, using an ingenious method based upon the inhibition of tube growth by cytochalasin D, PICTON and STEER (1981) have reached a figure of 5,388 vesicles per minute for the growing tube of *Tradescantia virginiana*. The recorded pollen tube growth rates were 12 μm min^{-1} for *Lilium* and 6 μm min^{-1} for *Tradescantia*.

The *Gramineae* are strikingly different in respect to rate of pollen hydration, germination and tube growth (HESLOP-HARRISON, 1979a). In rye (*Secale cereale*), germination may occur within 2 min of the onset of hydration, and tube growth rates of 72–120 μm min^{-1} are attained thereafter. Growth rates up to 240 μm min^{-1} are probably achieved in *Zea mays*—40 times greater than in *Tradescantia*. Dictyosomes are infrequent in extending grass pollen tubes, and, so far as can be judged from electron mciroscopy, they are relatively inactive (HESLOP-HARRISON 1979a, CASS and PETEYA 1979). Yet, from simple proportional extrapolation from the data of PICTON and STEER (1981), it may be surmised that maintaining the minimum tube growth observed in rye would require the enormous synthesis rate of 60,000 vesicles per minute.

The disparity is accounted for by major differences in the economy of the grass pollen grain, associated with the specialisation of the reproductive system for extremely rapid development following upon anthesis (HESLOP-HARRISON 1979b). The principal dictyosome activity actually occurs during the later phases of maturation of the pollen in the anther, before the beginning of desiccation and the onset of temporary dormancy, and not during the course of tube growth. In consequence, the grain at the time of dispersal contains a large population of vesicle-derived storage bodies similar in electron-microscopic appearance to those associated with pollen tube tip growth in *Lilium* and other species. The bodies are packed tightly in the partly dehydrated grain, and as a result are pressed into polyhedral shapes. Moreover, with the onset of rehydration it may be seen that they are often not invested in continuous bounding membranes, although initially the content retains a compacted form. Since these bodies are not in fact vesiculate in the mature grain, we have referred to them as polysaccharide particles (P-particles; HESLOP-HARRISON 1979a), and this name is retained here.

Because the ungerminated grass pollen grain contains such large populations of P-particles all at the same stage of development, it offers unusual advantages for investigating their properties. In the present paper, the first of two concerned with the growth of the grass pollen tube, we describe simple methods for the extraction of relatively pure samples of P-particles, and contribute some further data towards their characterisation.

2. Materials and Methods

2.1. Plant Material

The principal observations were made on rye (*Secale cereale* L. cv Rheidol) and millet (*Pennisetum typhoideum* Rich.). Maturing inflorescences were brought into the laboratory, where anthesis was induced as required by exposure to infra-red lamps. For rye pollen, which does not readily germinate in liquid culture, a semi-solid medium (1% agar, 20% sucrose, 10^{-3} M $Ca(NO_3)_2$ and 10^{-3} M H_3BO_3) was used. After the required periods, the germinated grains and emerging pollen tubes were scraped from the surface of the medium and washed free of agar with buffered sucrose or with fixative before collection by centrifugation at 1,000 × g for 5 minutes. Millet pollen was germinated in a liquid medium (10% sucrose, 0.5×10^{-3} M $Ca(NO_3)_2$ and 10^{-3} M H_3BO_3) under gentle agitation, and collected directly by centrifugation for extraction or fixation at the intervals required.

For the bulk extraction of P-particles from ungerminated grains, pollen collected from freshly dehiscing anthers was rapidly dried at room temperature in a desiccator (a treatment which quickly reduces germinability by its effect on the membranes of the vegetative cell; SHIVANNA and HESLOP-HARRISON 1981), and stored until required.

2.2. Extraction of P-Particles

All media used in the extraction and subsequent procedures were made up with de-ionised, glass distilled water, and all reagents were analytical grade. Pollen samples (250 mg, 500 mg or 1 g) were suspended in water (10 ml per 1 g), and agitated vigorously for 2-5 minutes on a vibrator to extract sugars, wall proteins and soluble wall pectins (HESLOP-HARRISON 1979a). For one preparation, sugars were extracted by agitation in 70% ethanol before water extraction; this treatment made no discernible difference to the final yield. The pollen was collected by centrifugation, and homogenised in a glass homogeniser until more than 70% of the grains were disrupted. Several methods for extracting the P-particles were tested; of these the following provided the quickest and cleanest preparations, although not necessarily the highest yields.

Walls, nuclei, starch grains and larger protoplast fragments were removed by passage through 47 mm Whatman glass fibre filter GF/D. Any remaining broken starch grains were then sedimented by centrifugation at 2,500 g for 5 minutes, and the supernatant was passed through glass fibre filter GF/C. The P-particles were finally collected either (a) by centrifugation for 30 minutes at 7,000 g, or (b) on laminated micropore filters with 0.2 μm pore size (Schleicher & Schull). At the latter stages of the extraction procedure, the yield was examined by optical and electron microscopy to check what cell fractions were present. Testing with I/KI indicated that the final preparation still contained occasional minute starch fragments, but the great bulk was made up of uncontaminated, intact P-particles.

The movement of the isolated P-particles in an electric field was investigated using a 4 mm × 2 mm × 0.5 mm micro-electrophoresis cell with platinum electrodes. The washed particles were suspended in 0.1 M MES buffer at pH 5.6, and the movement in the cell observed with phase contrast optics with p.d. up to 75 v.

2.3. Polysaccharide and Protein Extraction

For pectin extraction, samples of the P-particles were refluxed in 0.1 M ammonium oxalate-citrate buffer (pH 4.0), 10 ml per g of the original dry pollen, for 1-2 hours (JARVIS, HALL, THRELFALL, and FRIEND 1981). The insoluble residue was removed by centrifugation at 7,000 × g for 20 minutes, and the solubilised fraction was dialysed against deionised glass-distilled water for 12-15 hours in Visking tubing (cut off, 5,000 daltons). The dialysed solution was then concentrated as required by molecular filtration using membranes with a cut off of 10,000 daltons.

For the solubilisation of membrane proteins, samples of the P-particles were suspended in 0.1 M phosphate buffer, pH 7.0, containing 1% Triton X (1 ml per 1 g original dried pollen), and extracted for 12 hours or more at 4-5 C. Polysaccharide was then sedimented by centrifugation at 7,000 × g for 20 minutes, and the supernatant was diluted 10× with water and re-concentrated to the required volume by molecular filtration for electrophoresis.

2.4. Carbohydrate Determination

Uronides were determined by the procedure of BLUMENKRANTZ and HANSEN (1973) with citrus pectin (Sigma) as a standard. Protein was estimated by the method appropriate for dilute solutions described by SCHAFFNER and WEISSMANN (1973), with BSA as a standard.

2.5. Sugar Composition

Samples of the P-particle preparation equivalent to 250 mg original pollen sample were hydrolysed in 1 ml 2 M trifluoroacetic acid at 112 °C for 2 hours in a sealed tube. The yield, which included the sugars and derivatives of the P-particle protein, was then dried by evaporation under reduced pressure at 60 °C. The soluble components were redissolved in water, and subjected to thin-layer chromatography on Anachem GHL silica gel plates soaked in 1 m NaH_2CO_3 and activated 1 hour at 110 °C, or on Whatman MK6F silica gel plates activated for 1 hour at 110 °C without pretreatment. Two solvent systems were used, acetone:water:chloroform:methyl alcohol, 16:1:2:2 by volume (LATO, BRUNELLI, GIUFFINI, and MEZZINI 1969), and 1-butanol:pyridine:water, 6:4:3 by volume. Sugars were detected by dipping in naphthoresorcinol-H_2SO_4 or p-anisidine-HCl reagents and heating at 110 °C. Tentative identifications were made by comparing R_F and colour reactions with those of authentic samples.

2.6. Electrophoresis

Cellulose acetate-membrane electrophoresis was carried out on Shandon Cellogram membranes in 0.1 M barbital buffer, pH 8.8, at 30 V cm^{-1}. Proteins were detected with Coomassie blue (0.25% in 30% methanol with 2% acetic acid), and polyanionic polysaccharides with 1% alcian blue 8GX in 3% acetic acid.

Microgradient polyacrylamide gel electrophoresis was performed essentially as described by RÜCHEL (1976). Gels, 10-12 mm in length, were cast in segments of glass capillaries, 0.8 mm i.d., and matched before use. Runs were for c. 10 minutes at 15-20 V mm^{-1}. Xylene cyanole and bromophenol blue were used as markers, and proteins were localised with Coomassie blue. The gels were calibrated with ovalbumin, ribonuclease and BSA oligomers as molecular weight markers.

2.7. Electron Microscopy

Where required for observation of general structural detail, ungerminated and germinated pollen was fixed in 1.5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.0 containing 8% sucrose, and, after washing in buffer, post-fixed in 1% OsO_4 in the same buffer for 1-3 hours at 4-5 °C. Thereafter the material was dehydrated through an alcohol series and passed through propylene oxide before embedding

in araldite. The sections were stained in uranyl acetate (saturated in 70% ethanol) for 4-8 minutes followed by lead citrate for 5-10 minutes. In addition, sections were stained with PTA (1% in 3% HCl), alone, or in combination with the standard electron stains. In our hands this stain has been found to impart electron density mainly to pectin-rich wall components and not appreciably to any other cell structure (Y. HESLOP-HARRISON and J. HESLOP-HARRISON 1979).

Material to be used in the enzyme digestion and chemical extraction experiments was fixed in glutaraldehyde as above, and washed, dehydrated and embedded directly without OsO_4 postfixation.

Isolated P-particles were prepared for electron microscopy without sectioning. Washed samples were stained for c. 30 minutes in PTA made up as above, collected by centrifugation at $7{,}000 \times g$ for 30 minutes, washed free of the stain and concentrated as necessary, again by centrifugation, before being dispersed on Formvar-coated slot-grids for examination. The linear dimensions and volumes of the isolated particles were determined from electron micrographs of whole isolated P-particles with a digitiser and Apple II microcomputer.

To investigate the effects of various chemical and enzymic extraction procedures on the electron microscopic image of the P-particles, grids bearing unstained sections of the unosmicated material were inserted into drops of the appropriate media for incubation at 37 °C. The treatments were as follows: Dimethyl sulphoxide (DMSO) for 15 hours; freshly made up cuprammonium hydroxide for 15 hours; oxalate-citrate buffer as above for 5 hours; cellulase (*Aspergillus niger* source, 100 mg/ml at pH 5.6) for 15 hours, and pectinase (*Aspergillus niger* source, 100 mg/ml at pH 4.4) for 15 hours.

The isolated P-particles were also exposed to enzymic digestion before staining. Samples were suspended in cellulase and pectinase as above, and in the proteolytic enzyme complex, Pronase (BDH), made up at 2 mg/ml at pH 7.2, for 30 minutes. The particles were then collected by centrifugation, washed, re-concentrated, and dispersed on slides for optical microscopic observation, or stained in PTA and collected on Formvar-coated slot grids for the electron microscope.

3. Results

3.1. Morphology of the P-Particles

Micrographs of ungerminated pollen grains of rye suggest that the P-particles occupy 20-30% of the volume of the grain. They are closely packed, and, following standard preparation procedures for the electron microscope, they appear as roughly spherical or polyhedral bodies with a coarsely granular or flecked content (HESLOP-HARRISON 1979 a). PTA post-staining enhances the contrast of the granulation, as may be judged from Figs. 1 and 2, of germinating pollen grains of millet 2 minutes from the beginning of hydration. Fig. 3 shows the intact P-particles in a partly purified preparation from rye, with a starch grain providing an index of size. A sample of a finally purified preparation virtually free from starch and membrane fragments is illustrated in Fig. 4. In such samples the particles retain their roughly spherical form, and they disperse readily in the staining solution without coagulating. Mean diameter is $0.34 + 0.066\ \mu m$, with a range of 0.14-$0.6\ \mu m$. Assuming close packing and a mean volume of $2 \times 10^{-2}\ \mu m^3$, a mature hydrated but ungerminated pollen grain of rye with a vegetative cell volume of $9 \times 10^4\ \mu m^3$ must therefore contain well over one million P-particles, supposing that they account for 30% of the content.

In the micro-electrophoresis cell, P-particles suspended in MES buffer at pH 5.6 moved anodically.

3.2. Chemical and Enzymic Extraction

Oxalate-citrate buffer reduced and finally wholly eliminated the coarsely granulate PTA-staining content of P-particles sectioned *in situ* (Figs. 5 and 6). Cuprammonium hydroxide, while modifying the image of both intine and exine and causing some damage to membranes and organelles, did not remove the granulate content, and DMSO was also without effect. To the extent that reliance can be placed upon the specificity of the extraction procedures, these results suggest that the polysaccharide component of the P-particles is unlikely to be cellulose or callose, but strongly indicate that it is pectic in nature.

Cellulase digestion as applied in this investigation was without effect on the P-particles in sectioned pollen, and the granulate, PTA-staining content was not wholly removed by *Aspergillus* polygalacturonase. Exhaustive digestion with this enzyme did, however, destroy the structure of isolated P-particles. Digestion with the non-specific protease, pronase, led to a clumping of the isolated P-particles, which nevertheless retained a generally spherical structure, those at the edge of the mass sometimes showing clear evidence of the polysaccharide content (Fig. 7).

3.3. Polysaccharide Composition

Subjected to electrophoresis on cellulose acetate membranes, the dialysed, concentrated oxalate-citrate fraction of the isolated P-particles gave three very vague protein-staining bands, two anodic and one cathodic, and a rather more heavily staining cathodic band very near the origin. This largely immobile component also stained heavily with alcian blue.

The uronide content of the oxalate-citrate extract amounted to 0.78 mg per 1 g dry pollen. The principal sugars identified by TLC in the complete TFA hydrolysate of the isolated P-particles were arabinose and galactose, with a lesser amount of glucose and traces of rhamnose. A further unidentified neutral

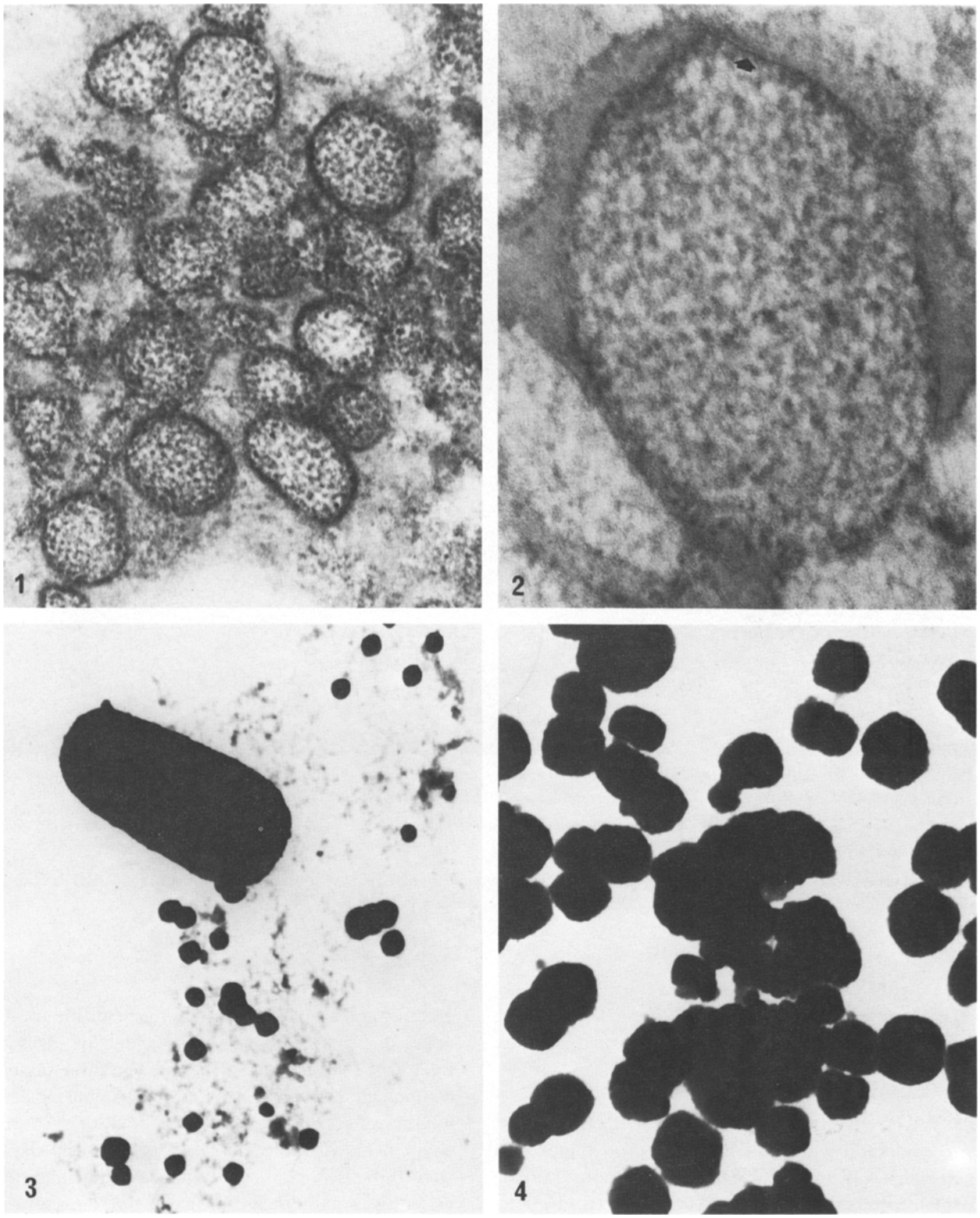

Fig. 1. Transmission electron micrograph of P-particles in germinating pollen of *Pennisetum typhoideum*, fixed 2 minutes from the beginning of hydration. GDA-OsO_4 fixation, standard staining followed by 10 minutes PTA. × c. 42,000

Fig. 2. As Fig. 1, × c. 160,000. A residual stretch of the single bounding membrane may be seen at the arrow

Fig. 3. Partly purified preparation of intact P-particles from ungerminated pollen of *Secale cereale*, PTA staining. A single intact starch grain is present, and wisps of membranous material. × c. 8000

Fig. 4. Final P-particle preparation from pollen of *Secale cereale*. × c. 28,000

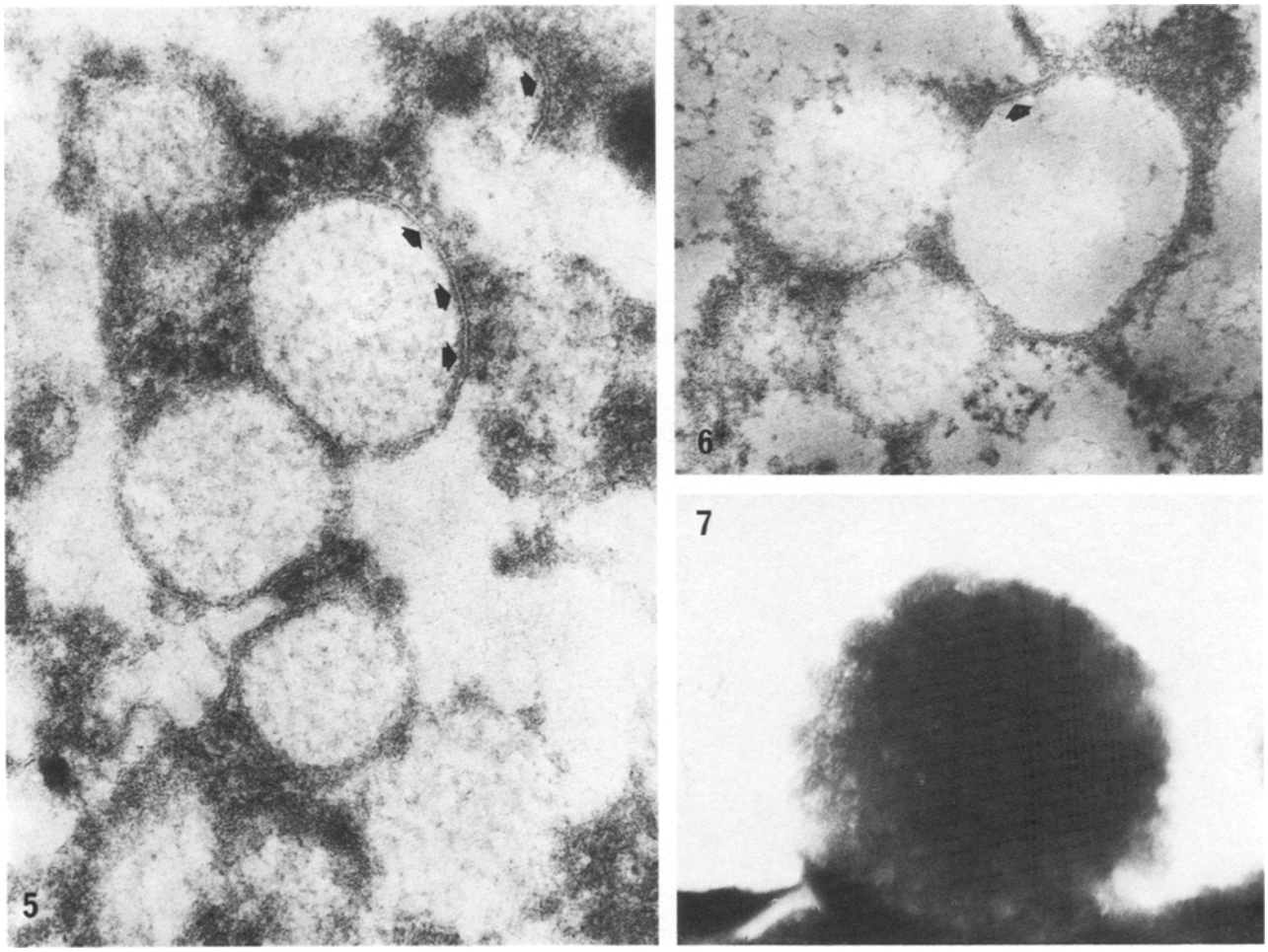

Fig. 5. P-particles from germinating pollen of *Pennisetum typhoideum*, preparation as in Fig. 1, but partly extracted with oxalate-citrate buffer. Discontinuous stretches of the original bounding membranes can be seen at the arrows. × c. 73,000

Fig. 6. As Fig. 5, but with virtually complete extraction after exposure for 5 hours to oxalate-citrate buffer at 37 °C. Stretches of membrane still persist. × c. 73,000

Fig. 7. Whole P-particle from rye pollen, following digestion with pronase. This particle, at the edge of an amorphous clump, has lost its smooth outline, and shows evidence of the polysaccharide contents. PTA staining. × c. 125,000

sugar appeared consistently, although in trace amounts, on the chromatograms. Xylose and mannose were not identified.

3.4. P-Particle Protein

A considerable part of the P-particle protein remained associated with an insoluble fraction which survived the oxalate-citrate extraction. As measured by the procedure of SCHAFFNER and WEISSMAN (1973), the protein content of the oxalate-citrate extract itself was 0.09 mg per 1 g dry rye pollen, BSA equivalent. Since with the amounts available the method was near the limits of its sensitivity, this figure has to be accepted as no more than a general estimate.

Electrophoresis on gradient microgels of the proteins released from isolated P-particles by detergent treatment gave 15 bands (Fig. 8). The three principal components (arrows) are in the approximate molecular weight range of 120-250,000 daltons. Two further components of molecular weight 12-16,000 daltons were also observed; these lie outside of the range of the gel in Fig. 8. No direct evidence of the source of these proteins can be given, but it may be surmised that they were derived mainly from the residual membranes of the P-particles (Fig. 2), although it is not excluded that the extraction procedure does permit some organelle and endoplasmic reticulum residues to reach the final preparation.

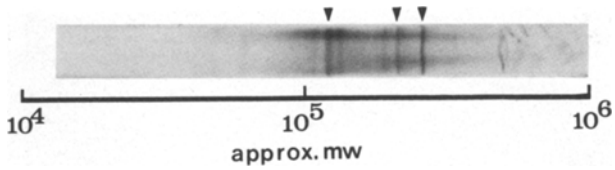

Fig. 8. Band pattern of Triton X solubilised proteins from rye pollen P-particles separated on microgradient polyacrylamide gel; Coomassie blue staining

3.5. P-Particles in Germinating Pollen

Electron microscopy shows that the membranes investing the P-particles in viable pollen grains are frequently discontinuous (Fig. 5), and with full hydration and the onset of germination the particles begin to coalesce and to lose almost all associated membrane. At the same time, the polysaccharide content expands, and the coarsely granulated appearance in the partly dehydrated grain gives place to a microfibrillar aspect (HELSOP-HARRISON 1979 a). Examples of the residual particles in a pollen grain of rye during the active phase of pollen tube growth are seen in Fig. 10; this also shows larger polysaccharide aggregates derived, presumably, by fusion. The content is markedly microfibrillar, and may be compared with that of the P-particles illustrated in the inset Fig. 9, from an ungerminated grain.

The PTA-staining microfibrillar component both of the individual P-particles and the fusion aggregates was eliminated when pollen at the stage of that of Fig. 10 was extracted with oxalate-citrate buffer (Figs. 11 and 12). The microfibrillar content was not removed by cuprammonium hydroxide, however.

4. Discussion

The chemical and cytochemical evidence given here indicates with reasonable certainty that the principal polysaccharide of the P-particles of the ungerminated grass pollen grain is pectic in nature. This conclusion is in accordance with the early cytochemical findings of DASHEK and ROSEN (1966) and the later analytical data of VAN DER WOUDE *et al.* (1971) for the corresponding bodies in *Lilium*. Furthermore, the similarity of the cytochemical reactions of bodies derived from the P-particles in the germinating grass pollen grain and tube suggests that their microfibrillar content is also a pectic polysaccharide. We find no support for the view that cellulose is present in the P-particles of grass pollens; and this is in contrast with the conclusion reached by ENGLER (1974 c) for vesicles associated with apical growth in the pollen tube of *Petunia*. Nor is the P-particle content likely to be callosic, another possibility suggested in recent publications (CRESTI *et al.* 1976). Although it has been generally supposed that pectins are amorphous and without fibrillar structure, microfibrillar forms were described some thirty years ago by ROELOFSEN and KREGER (1951) from collenchyma, and more recently COLVIN and LEPPARD (1973) have provided strong evidence that the microfibrillar coatings occurring between, and sometimes within, the walls of plant cells in suspension culture are mostly composed of "polygalacturonic acid or its salts". This material proved not to be susceptible to pectinase digestion, a fact which COLVIN and LEPPARD (1973) suggest might result from the association of the pectin with protein, present to the extent of 6% in the fibrils associated with cultured cells of *Ipomoea*. In the present work, the PTA staining component of the P-particles was not eliminated by pectinase digestion, possibly for the same reason. Protein was invariably found to be present in oxalate-citrate extracts, in an amount corresponding approximately to 12% of the uronide-reacting polysaccharide. This protein may represent residual, structural, membrane material strongly adsorbed to the P-particle polysaccharide; but it is perhaps more likely that it is an integral part of the molecule.

As we have seen, several earlier authors have called attention to the diverse aspects of the P-particle content observed in electron micrographs. DICKINSON and LAWSON (1975) have suggested that in *Oenothera* the coarsely granulate appearance of the compacted particle gives place to the microfibrillar aspect on fusion immediately before the transfer of the contents into the wall. In the grasses, this transition may be seen in the later stages of germination and tube growth, and indeed it occurs in particles released into aqueous media from disrupted grains (Plates 13, 14, and 17 in HESLOP-HARRISON 1979 a). While the change may reflect some metabolic activity in the particles, it seems probable that the diverse aspects simply reflect the physical state of the pectins, and especially their degree of hydration. The microfibrils may thus be the product of the "unspooling" of the aggregates present in the deeply staining nodules of the ungerminated grain.

Associated with the presence of large reserves of P-particles in the ungerminated grass pollen grain is the fact that the emerging tube does not develop the conspicuous zonation in the immediate sub-apical zone so clearly seen in *Lilium longiflorum* (VAN DER WOUDE *et al.* 1971) and *Lycopersicum peruvianum* (CRESTI *et al.* 1977). In these latter species, the particles are concentrated in the extreme tip region, distal to a zone of

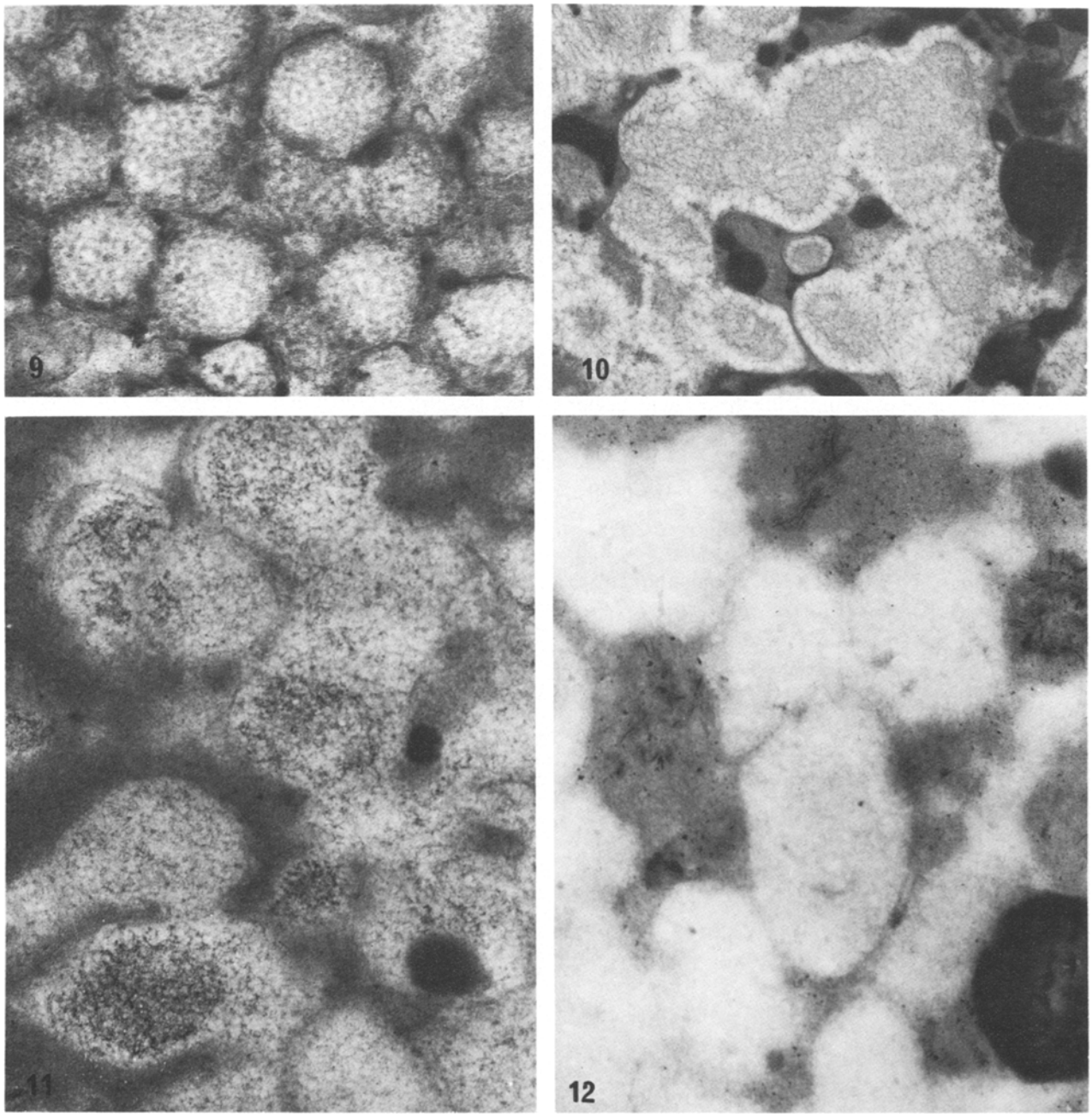

Fig. 9. Sectioned P-particles in an ungerminated pollen grain of *Secale cereale*, GDA-OsO_4 fixation, uranyl acetate-lead citrate post-staining but without PTA staining, showing the close packing, the absence of well-resolved membranes, and the coarsely granulate contents. × c. 40,000

Fig. 10. As Fig. 9, germinating pollen of *Secale cereale*. With identical fixation and staining, the expanded particles now show microfibrillar contents, and there is also evidence of fusion to form larger microfibrillar bodies. × c. 32,000

Fig. 11. Sectioned P-particles in a germinated pollen grain of *Pennisetum typhoideum*, GDA fixation without osmication, 10 minutes PTA post-staining to increase the contrast of the microfibrillar contents but without standard staining. × c. 60,000

Fig. 12. Section from the same preparation as that of Fig. 11, identical staining treatment, but extracted with oxalate-citrate buffer for 5 hours at 37 °C before transfer to PTA. The particles are now without microfibrillar contents. × c. 60,000

active dictyosomes; in the grass pollen tube the P-particles are scattered throughout, and there is no zone of active dictyosomes (HESLOP-HARRISON 1979a). Evidently the reserve stock in the grain is distributed through the tube largely by active cyclosis. There is some sorting out of cytoplasmic components in the apical region, since large organelles do not penetrate into the ultimate 20–25 μm. In the tip region, as throughout the grain, the P-particles show vigorous random movement; yet clearly there is a net vectorial flow, since the grain and the proximal stretch of the tube are ultimately drained of their populations, and this cannot be accounted for simply by cyclosis. The apparent flow may result from the fact that the growing tip provides the only unloading point for the cycling cytoplasm, in which case the transport operates simply on a "conveyor belt" principle. However, the vectorial movement could be based upon a more subtle mechanism if the P-particles, shown here to have a net negative charge, were moving in an electrical field. The observations of WEISENSEEL, NUCCITELLI, and JAFFÉ (1975) are of considerable significance in this connection. These authors showed that a steady current, of the order of a few picoamperes, is driven from base to apex during the course of extension in growing pollen tubes of *Lilium longiflorum*. If this current is not a consequence of the vectorial movement of other cytoplasmic components – and this appears to be ruled out by the observation that it is not eliminated by cytochalasin B at a concentration adequate to block apical growth – then it may in fact be the *cause* of the apparently purposeful migration of the P-particles to the tip region. This and other aspects of pollen tube growth in the grasses will be considered at greater length in our second paper.

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