Acta Bot. Neerl. 23(3), June 1974, p. 201–207.

FUNCTION OF GOLGI VESICLES IN RELATION TO CELL WALL SYNTHESIS IN GERMINATING PETUNIA POLLEN. III. THE ULTRASTRUCTURE OF THE TUBE WALL

F. M. ENGELS

Botanisch Laboratorium, Universiteit, Nijmegen

SUMMARY

Pollen tubes from germinated *Petunia* pollen were prepared in different ways for electron microscopic investigation of their walls. The ultrastructural features of the tube walls depend strongly on the preparation methods used. The tube wall was found to be composed of two layers or appeared striated after fixation with $KMnO_4$ and OsO_4, respectively. After freeze-etching the tube wall is twice as thick as after chemical fixation and a fine striation is visible. Subsequent extractions dissolve the matrix substances and a network of microfibrils is left, which is not composed of lamellae.

1. INTRODUCTION

In literature a variety of ultrastructures have been described in cell walls of pollen tubes. The different features of tube walls are strongly dependent upon the fixatives used. After prefixation with glutaraldehyde and postfixation with OsO_4 the walls at the very tip of *Lilium* pollen tubes consist of compartments. These compartments are not observed when glutaraldehyde is washed out prior to OsO_4 fixation (ROSEN & GAWLIK 1966). OsO_4 fixation alone shows the tube wall to be composed of two layers (ROSEN et al. 1964, FRANKE et al. 1972); sometimes the outer layer appears to be lamellated (VAN DER WOUDE et al. 1971). This lamellated tube wall has also been found in pollen tube walls from *Impatiens holstii* after aceton fixation and post-staining with $CuBr_2$ (FLYNN 1968). $KMnO_4$ fixation reveals a thick, a thin, or a bilayered tube wall in Lilium pollen tubes, depending on the concentration of $KMnO_4$ (ROSEN & GAWLIK 1966). In micrographs from *Petunia* pollen tubes it has been observed that the tube wall is not homogeneous after $KMnO_4$ fixation (SASSEN 1964, KROH 1967, VAN DER PLUYM & LINSKENS 1966). Altogether, the morphological ultrastructure of pollen tube walls as revealed by chemical fixation remains obscure and difficult to interpret.

After treating pollen tubes of *Petunia* and *Brassica* with strong alkali and acid, a skeleton of microfibrils is found which is assumed to consist of cellulose (SASSEN 1964, KROH 1964). In *Brassica* it has been shown that the skeleton is composed of one lamella of microfibrils (KROH 1964). A systematic breakdown of *Impatiens* pollen tubes by enzyme and chemicals, followed by negative staining, has been carried out by FLYNN (1968). By this treatment the matrix is

dissolved and a skeleton of cellulose microfibrils remains.

In a preceding experiment the pollen tube cell wall of *Petunia* pollen was chemically analysed after a series of extractions (ENGELS 1974). During the subsequent steps of the extraction procedure samples were taken from the same material to make platinum carbon replicas in order to get more information on these pollen tube walls. The present report deals with the results obtained after fixation of intact pollen tubes and with the ultrastructural changes observed in the extracted pollen tube walls.

2. MATERIALS AND METHODS

Germinating *Petunia* pollen was prepared for thin sectioning and freeze-etching as previously described (ENGELS 1973).

Platinum-carbon replicas were made from the surface of pollen tubes after subsequent treatments with ethanol-ether, boiling water, HCl, and KOH under conditions described previously (ENGELS 1974).

Pollen tubes treated with a mixture of acetic-acid and hydrogen peroxide (1:1) were embedded in butylmethacrylate and sectioned. The butylmethacrylate in the sections was dissolved in amylacetate and the remaining parts of the tube walls were shadowed with platinum.

All preparations were studied with a Philips EM 201.

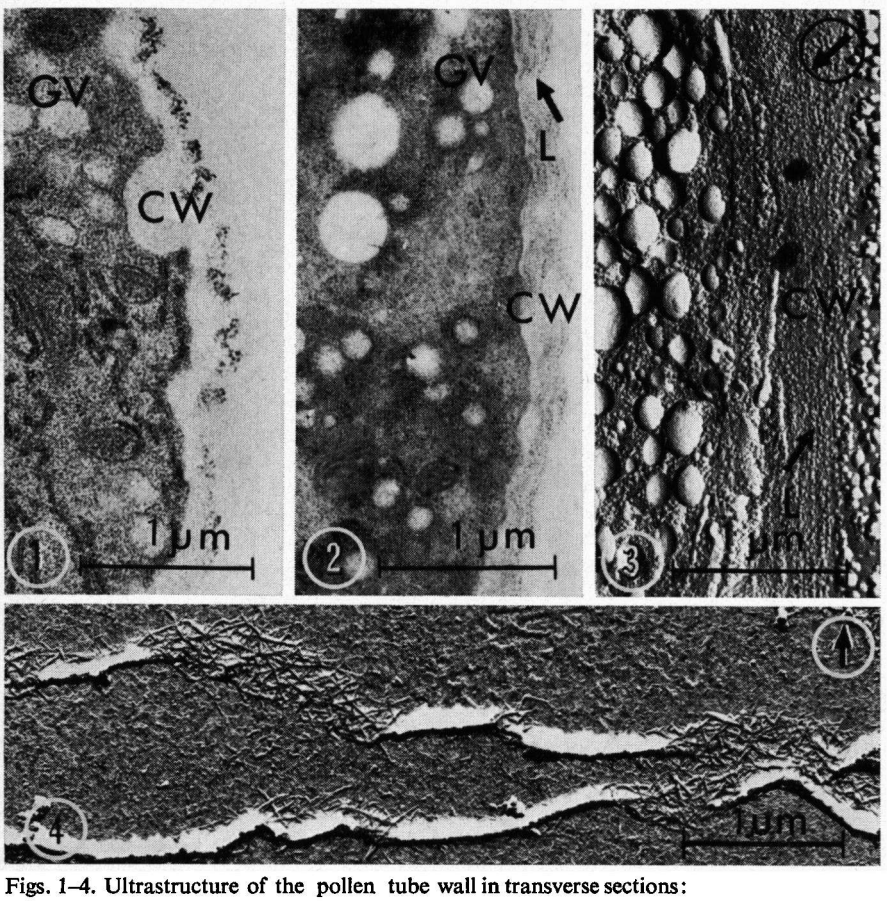
3. RESULTS

3.1. The ultrastructure of intact pollen tube walls after thin sectioning and freeze-etching

Great differences are observed in the tube wall structure, depending on the preparations used for electron microscopy. After fixation with $KMnO_4$ the cell wall shows two layers. The inner layer is electron-transparant, comparable to the contents of the Golgi vesicles. The outer layer is electron dense and has a granular appearence (*fig. 1*). In contrast, pollen tubes fixed with OsO_4 show alternating electron transparant and electron dense lamellae (*fig. 2*). With both fixations undulations of the plasmalemma are clearly visible. These are probably the result of fusion from Golgi vesicles with the plasmalemma. However, it is striking that in freeze-etch preparations the tube wall is nearly twice as thick as a chemically fixed one. Furthermore, a weak indication of lamellation is visible (*fig. 3*).

3.2. Ultrastructure of tube walls treated with acetic-acid and hydrogen peroxide

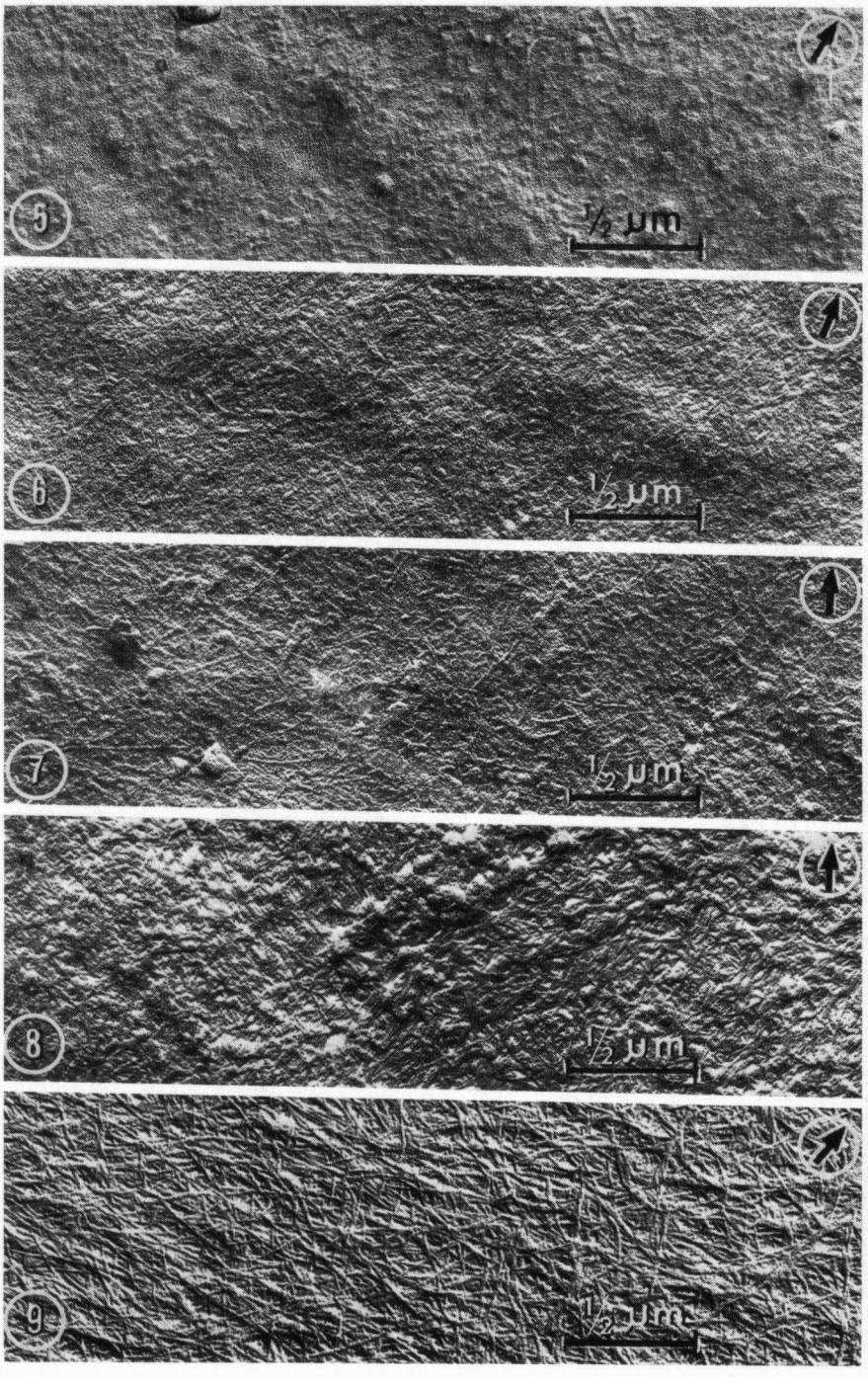
By dissolving the butylmetharylate from thin sections with pollen tubes treated with acetic-acid and hydrogen peroxide, parts of the tube wall are turned over to one side while others stay upright (*fig. 4*). The skeleton of microfibrils shows the same appearence as after KOH treatment. It is composed of one lamella of microfibrils.



Figs. 1–4. Ultrastructure of the pollen tube wall in transverse sections:
Fig. 1. After $KMnO_4$ fixation; CW = cell wall, GV = Golgi vesicle.
Fig. 2. After OsO_4 fixation; in the cell wall (CW) a lamellation (L) is found.
Fig. 3. After freeze-etching; the thick cell wall exposes a fine lamellation (L).
Fig. 4. After treatment with acetic-acid and hydrogen peroxide; a network of microfibrils is exposed.

3.3. Morphological changes after chemical treatments

The surface of the untreated tube wall has a relatively smooth appearence (*fig. 5*). Occasionally indications of short pieces of microfibrils can be seen. Small granules are dispersed over the surface. After treatment with ethanol-ether, microfibrils embedded in amorphous matrix material become visible all over the surface (*fig. 6*). Extraction of the tube wall with boiling water exposes the uppermost microfibrils better and over longer distances (*fig. 7*). Although still more fibrils become visible after extraction with HCl, they can only be followed over shorter distances. Rough granular material is dispersed over the surface (*fig. 8*). After KOH treatment all the matrix substances are removed. The tube wall residue is composed of a skeleton of microfibrils (*fig. 9*). A


5
½ µm
6
½ µm
7
½ µm
8
½ µm
9
½ µm

preferential direction of the fibrils could not be observed. The diameters of the fibrils measure 15–20 nm.

4. DISCUSSION

The bilayered structure observed in pollen tube walls of *Petunia* after $KMnO_4$ fixation is in agreement with earlier findings in pollen tubes of the same plant (SASSEN 1964, KROH 1967, VAN DER PLUYM & LINSKENS 1966) and in *Lilium* (ROSEN & GAWLIK 1966). This structure is also in agreement with that found after OsO_4 fixation in *Lilium* (ROSEN et al. 1964, FRANKE et al. 1972). The lamellated appearence of the tube wall, found after OsO_4 fixation in *Petunia*, corresponds with the observations made by VAN DER WOUDE et al. (1971) on *Lilium* pollen and by FLYNN (1968) on *Impatiens* pollen. From the results it is difficult to decide which of both pictures of the tube wall is the correct one. The different fixation and staining procedures result in different features of the wall.

In tube walls of *Petunia* the lamellation observed after OsO_4 fixation is also found after freeze-etching. However, if one treats the tube walls with acetic-acid and hydrogen peroxide, only one lamella of microfibrils becomes visible. The same result is reported for tube walls of *Brassica* (KROH 1964). The pollen tube wall can be considered as a primary cell wall with randomly distributed microfibrils. By removal of the matrix the fibrils are pressed together and the skeleton therefore appears in sections as one lamella.

Remote from the tip an orientation of fibrils in tube walls has been observed by SASSEN (1964). However, this could not be confirmed in our studies.

A correlation has been made between the lamellae seen after fixation with OsO_4 in the wall of stigmatic papillae of *Brassica* after penetration of pollen tubes into the papilla wall and the cellulose lamellae left in the papilla wall after purification with acetic-acid and hydrogen peroxide (KROH 1964). This correlation may be questioned. In tube walls of *Petunia*, several lamellae are seen after OsO_4 fixation and freeze-etching; in contrast only one lamella is observed in sections after removal of the matrix. From this observation one may conclude that the lamellar structure of the tube wall after chemical fixation does not reflect the cellulose lamellae. A correlation made between EM-structures observed after chemical fixation and their chemical and physical nature "in situ" must, therefore, be handled with caution.

After extraction of the tube wall with boiling acetic-acid and hydrogen peroxide a network consisting of microfibrils remains. Substances which can resist this treatment could not be found. The treatment with acetic- acid and hydrogen

Figs. 5–9. Platinum carbon replicas of the tube walls after different treatments:
Fig. 5. Untreated; a fine granulation is visible all over the surface. Some microfibrils are visible.
Fig. 6. After treatment with ethanol-ether.
Fig. 7. After treament with boiling H_2O.
Fig. 8. After treatment with N-HCl.
Fig. 9. After treatment with 2N KOH. The encircled arrows indicate the shadow direction.

peroxide at lower temperature (SASSEN 1964) may be the cause of insufficient extraction.

A systematic extraction of the pollen tube wall by different solvents has been followed by making platinum carbon replicas. The presence of matrix material and microfibrils could be demonstrated. A fine granulation on the surface of untreated walls which was also observed after $KMnO_4$ fixation is removed by ethanol-ether. The fibrils are now observed distinctly to be embedded in matrix material. After HCl treatment the matrix shows a rough granular structure partly covering the microfibrils. In *Impatiens* pollen tubes a change in dimensions of granular material has been observed by negative staining after several extractions which is considered to be a manifestation of the same wall material (FLYNN 1968). In *Petunia* it is assumed that HCl dissolves the matrix substance and that a part of it precipitates during the drop of temperature preceding the removal of HCl by water washings. This material is then irregularly dispersed over the surface of the tube wall. A network of microfibrils is obtained after the last extraction with KOH. An orientation of microfibrils could not be observed.

After freeze-etching the tube wall is nearly twice as thick as after chemical fixation. In this study only transverse sections of the cell wall were studied excluding the effects of sectioning on wall thickness. Since it is accepted that freeze-etching does not cause artefacts we must conclude that the thin tube walls, seen after chemical fixation, are probably the result of shrinkage during fixation and dehydration. This must be taken into consideration when the thickness of embedded cell walls is measured.

ACKNOWLEDGEMENTS

The author is much indebted to Prof. Dr. H. F. Linskens for his stimulating interest, to Dr. M. M. A. Sassen and Dr. M. Kroh for critical discussions, to Dr. Ir. G. W. M. Barendse for reading and to Mrs. E. R. Tummers-Moesker for typing the manuscript.

REFERENCES

ENGELS, F. M. (1973): Function of Golgi vesicles in relation to cell wall synthesis in germinating Petunia pollen. 1. Isolation of Golgi vesicles. *Acta Bot. Neerl.* **22**: 6–13.

— (1974): Function of Golgi vesicles in relation to cell wall synthesis in germinating Petunia pollen. II. Chemical composition of Golgi vesicles and pollen tube wall. *Acta Bot. Neerl.* **23**: 81–89.

Flynn, J. J. Jr. (1968): *The cell wall fine structure of Impatiens holstii pollen tubes.* Thesis, University of Mass. U.S.A.

FRANKE, W. W., W. HERTH, W. J. VAN DER WOUDE & D. J. MORRÉ (1972): Tubular and filamentous structures in pollen tubes: Possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. *Planta (Berl.)* **105**: 317–341.

KROH, M. (1964): An electron microscopic study of the behaviour of Cruciferae pollen after pollination. In: H. F. LINSKENS (Ed.), *Pollen physiology and fertilization.* p. 221–225. North Holland Publ. Company, Amsterdam.

— (1967): Fine structure of Petunia pollen germinated in vivo. *Rev. Palaeobotan. Palynol.* **3**: 197–203.

PLUYM, J. VAN DER & H. F. LINSKENS (1966): Feinstruktur der Pollenschläuche im Griffel von Petunia. *Der Züchter* **36**: 220–225.

ROSEN, W. G. & S. R. GAWLIK (1966): Fine structure of Lily pollen tubes following various fixation and staining procedures. *Protoplasma* **66**: 181–192.

—, —, W. V. DASHEK & K. A. SIEGESMUND (1964): Fine structure and cytochemistry of Lilium pollen tubes. *Amer. J. Bot.* **51**: 61–67.

SASSEN, M. M. A. (1964): Fine structure of Petunia pollen grain and pollen tube. *Acta Bot. Neerl.* **13**: 175–181.

VAN DER WOUDE, W. J. & D. J. MORRÉ (1968): Endoplasmic reticulum-dictyosome-secretory vesicle association in pollen tubes of Lilium longiflorum Thunb. *Proc. Indiana Acad. Sci.* **77**: 164–170.

—, — & C. E. BRACKER (1971): Isolation and characterization of secretory vesicles in germinated pollen of Lilium longiflorum. *J. Cell Sci.* **8**: 331–351.