Observations on the Fine Structure of Developing Microspores of *Tradescantia bracteata*

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With 8 Figures

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Summary

A study of the ultrastructure of the microspores of *Tradescantia bracteata* has been made at all stages of development from the tetrad to the mature pollen grain. The principal observations concern the following structures.

a) The exine which is developed mainly within the tetrad and possesses a regularly orientated fibrillar structure.

b) The intine whose secretion appears to involve the endoplasmic reticulum as well as the Golgi apparatus.

c) The generative cell walls which are transitory and composed of callose.

d) The vacuolar apparatus which appears to have a lytic function.

e) Membrane bounded bodies which are extruded from the microspore nucleus just before pollen grain mitosis.

Evidence is given for interpreting the latter process as a method of DNA extrusion which may provide a vehicle for the conveyance of genetic information to the cytoplasm. Alternatively the possibility that the extrusion bodies may be incipient mitochondria is considered.

1. Introduction

Previous reports of fine structure in the male gametophyte of angiosperms have dealt almost exclusively with short stages of its development. The bulk of the literature is concerned with the meiotic phase of microsporogenesis, or with the ontogeny of the exine, see for example Rowley (1959, 1962, 1963, 1964, 1967), HESLOP-HARRISON (1962, 1963, 1964, 1966, 1968 a, b), WEILING (1965 a, b), SKVARLA and LARSON (1966), GODWIN, ECHLIN, and CHAPMAN (1967), ANGOLD (1967), and ECHLIN and GODWIN (1968 a, b). Other authors have described the ultrastructure of mature, or germinating pollen, for example LARSON and LEWIS (1962), SASSEN (1964 a, b), ROSEN, GAWLIK, and GODWIN (1968 a, b).
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Dashek, and Siegesmund (1964), Larson (1965), Dashek and Rosen (1966), Rosen and Gawlik (1966), and Kroh (1967), while Larson (1963), Maruyama, Gay, and Kaufmann (1965), Gorska-Brylass (1967), Heslop-Harrison (1968), and Angel (1968) have described the formation of the vegetative and generative cells after pollen grain mitosis. This paper is a report of investigations of the general development of the pollen of *Tradescantia* between the time when it is released from the tetrads and the time when it is shed from the anther at anthesis, using both electron microscopy and cytochemical techniques, and it complements an earlier paper dealing specifically with the accompanying development of the tapetum.

2. Material and Methods

A full description of the material, and the methods used in its preparation, have been given in an earlier paper (Mepham and Lane 1969). Here it will suffice to say that the material came from a diploid clone of *Tradescantia bracteata* grown under controlled conditions. Under the chosen conditions distinct cytological stages could be recognized at intervals of approximately 24 hours. Mitosis occurred during the sixth day, and anthesis on the twelfth day after release of the pollen from the tetrads.

Anthers at each daily stage of development were fixed by a glutaraldehyde/osmic treatment, dehydrated through an ethanol series to propylene oxide as antemedium, and embedded in Araldite. As an alternative, dehydration of a small number of preparations was carried out by freeze-substitution. Thin sections for electron microscopy were double stained using uranyl acetate and lead citrate, while thicker sections for optical microscopy were stained with toluidine blue.

Carnoy (6:3:1) fixation was used for the histochemical tests which were performed on pollen extruded from anthers onto glass slides, as well as on de-waxed sections cut at 6 μm thickness. The periodic acid-Schiff (PAS) test was performed as recommended by Barka and Anderson (1963), using acetylation and lipid extraction procedures as controls. Callose was visualized with dilute aqueous lactic acid, and by a modification of Arens' (1949) fluorescence method. Aqueous ruthenium red, and the hydroxylamine-ferric chloride method of Reeve (1959) were used to detect pectins. Cellulosic substances were stained by the iodine/potassium iodide-sulphuric acid procedure recommended by Jensen (1962).

3. Observations

3.1. The Young Pollen Wall

In *Tradescantia* the microspores are liberated from the tetrads by enzymatic degradation of the callose special mother cell wall (Mepham and Lane 1969). Each microspore has a well developed exine, but no intine, while it is still invested by the callose. The young microspore wall gives a negative reaction in all tests for carbohydrates. The microspores increase in volume as soon as they are free in the anther loculi, the wall stretches and the baculae separate. Appertural and non-appertural regions of the ektexine are then more easily distinguished. Fusion of the tops of some baculae while still in the tetrad gives rise to extensive regions of tectate ektexine, and these regions are less susceptible to stretching than the areas which have remained in simple