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Disorganization of dictyosomes by monensin treatment of *in-vitro* germinated pollen of *Malus domestica* Borkh.

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SUMMARY

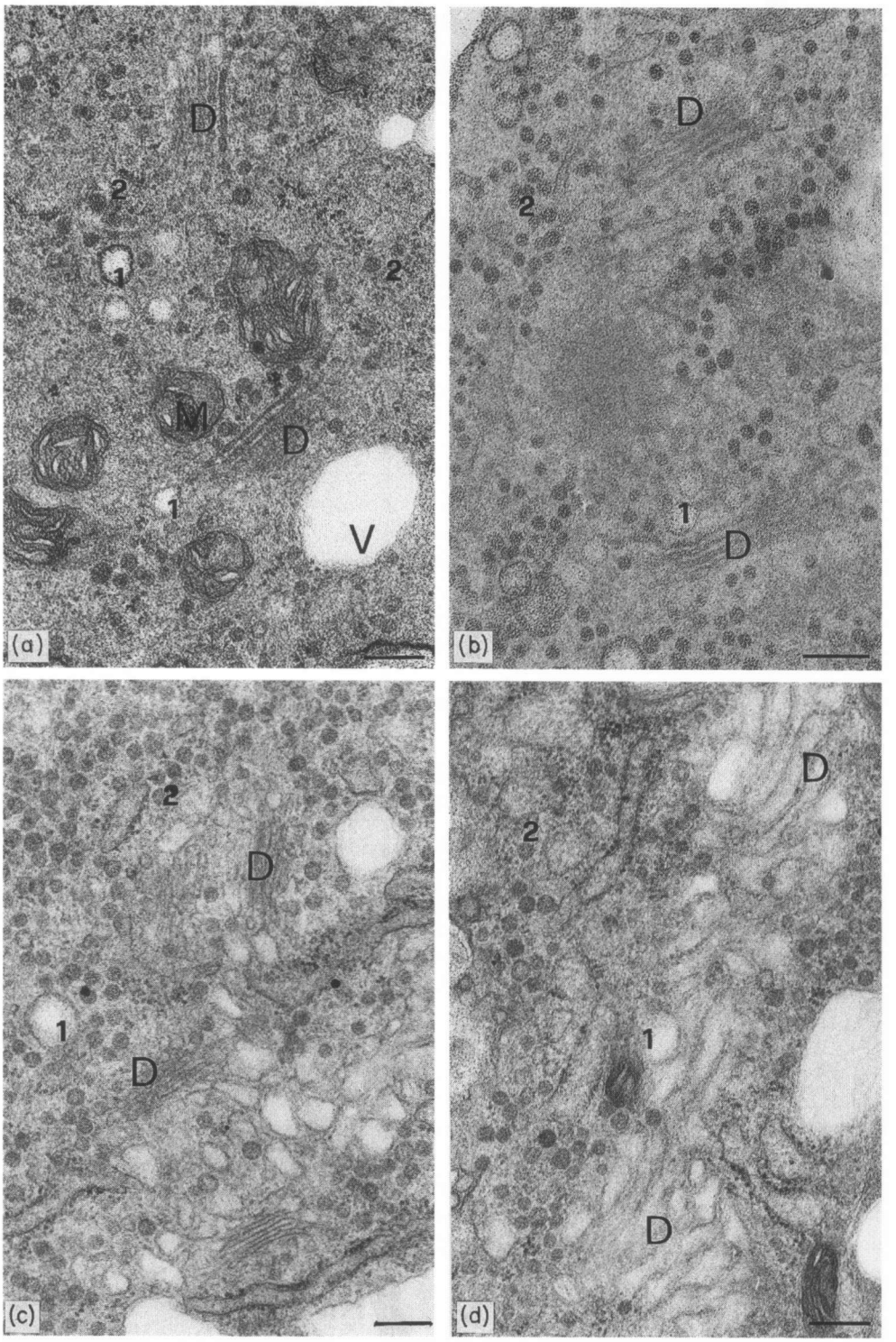
Ultrastructural alterations of dictyosomes after treatment with monovalent ionophore, monensin, were studied in pollen tubes of *Malus domestica* Borkh. The cisternae of dictyosomes in pollen tubes, grown in the presence of 125 nM monensin for 60 min, appear dilated and the interval between two consecutives is enlarged. These changes are stronger towards the pollen tube apex. The number of large vesicles is decreased. In pollen tubes treated with 125 nM monensin for 120 min cisternae are swollen. Unlike the small vesicles, the large ones become hardly recognizable and do not react or react weakly to the polysaccharide test.

Key-words: dictyosomes, *Malus domestica*, monensin, pollen tube, secretory vesicles, tip growth.

INTRODUCTION

Monensin, a monovalent carboxylic ionophore, induces an enlargement of the dictyosome which results from a disturbance in the membrane equilibrium, and thus inhibits the production of vesicles produced by the dictyosome (Ledger *et al.* 1980; Robinson 1981; Mollenhauer *et al.* 1982; Morré *et al.* 1983, 1986; Tartakoff 1983; Boss *et al.* 1984; Shannon & Steer 1984). The swelling response of the dictyosomes to monensin is so distinct that it can be used as an assay for evaluation of cellular injury (such as excision of tissue slices): in fact, isolated dictyosomes, or Golgi apparatuses of injured cells, swell with monensin (Mollenhauer *et al.* 1992).

Pollen tube growth is strongly dependent on secretory vesicles which deliver the materials needed for the construction of new wall and plasma membrane (Heslop-Harrison 1987). A previous paper (Speranza & Calzoni 1992) has described the dose response of *Malus domestica* pollen growth to monensin: the system was very sensitive, since growth showed a rapid exponential decline with increasing ionophore concentration. Both tube emergence and elongation were already significantly inhibited at 50 nM, and were almost completely blocked at 1 μM monensin. The inhibitory effect of monensin on the release of proteins from pollen tubes into the growth medium has also been demonstrated (Speranza & Calzoni 1992). The aim of the present study is to investigate the ultrastructural alteration induced by monensin at different stages of pollen germination and tube growth.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MATERIAL AND METHODS

Plant material

Pollen of *M. domestica* Borkh. cv. Starkrimson was collected from plants grown in experimental plots near Ravenna, Italy. Handling and storage were the same as described by Calzoni *et al.* (1979).

In-vitro *pollen germination*

Following rehydration at 30°C and under 100% relative humidity for 30 min, pollen was incubated in medium containing 0·2 M sucrose, 3·2 mM boric acid and 1·3 mM calcium nitrate in 3 mM McIlvaine citrate-phosphate buffer, pH 6·6 at 30°C for up to 120 min.

Apart from controls, different concentrations of monensin, taken from a stock solution in ethanol (0·1 μg ml^{-1}), were added separately to the medium. The final ethanol concentration in the controls were identical to that of the corresponding monensin-treated samples.

Electron microscopy

Germinated pollen incubated in the presence or absence of 125 nM monensin for 60 or 120 min was centrifuged for about 10 s. The supernatants were decanted and the pelleted germinated pollen was prefixed in a solution containing 3% glutaraldehyde in 0·066 M cacodylate buffer, pH 7·2, at 4°C for 30 min. The samples were rinsed with the same buffer and post-fixed in a solution containing 1% osmium tetroxide in 0·066 M cacodylate buffer, dehydrated in ethanol and then embedded in Spurr resin (Spurr 1969). Sections were obtained using LKB ultramicrotome Ultrotome III, stained with uranyl acetate and lead citrate. The observations were carried out with a JEOL JEM 100 B electron microscope.

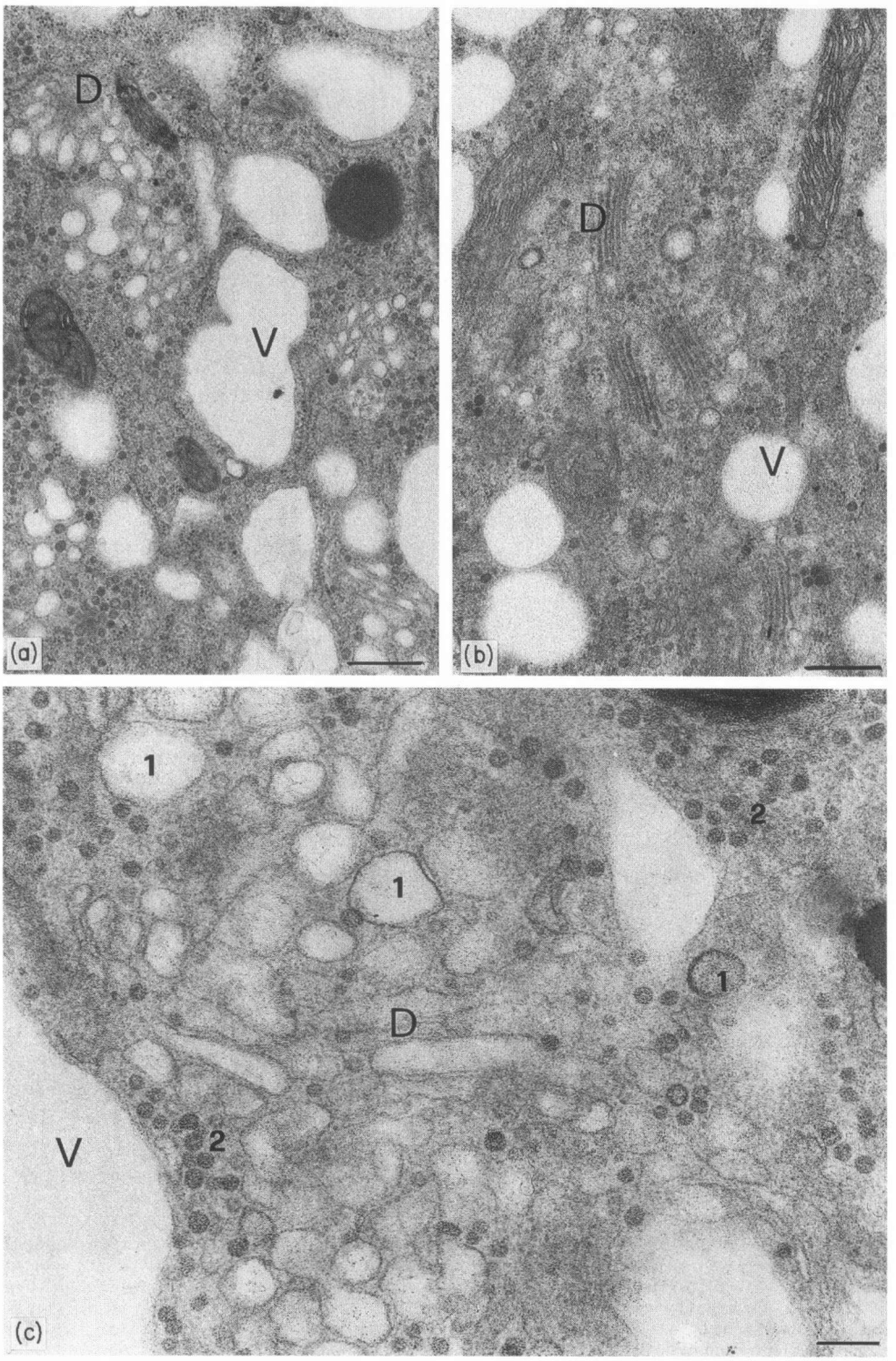
In order to localize polysaccharides, sections collected on gold grids were treated with periodic acid–thiocarbohydrazide—silver proteinate (Thiéry 1967).

RESULTS

In the controls, germinated pollen shows the normal cytoplasmic profiles of RER, vacuoles, roundish mitochondria, amyloplasts which contain big starch grains, and ribosomes aggregated mostly in polysomes. The Golgi apparatus consists of numerous dictyosomes, widespread in every zone of the pollen tube, but more frequent in the sub-apical zone. Each dictyosome consists of about four to five cisternae (Fig. 1a).

The dictyosomes produce two classes of secretory vesicles: small numbers of large vesicles which have a diameter varying between 0·1–0·2 μm and numerous small vesicles with a diameter varying between 0·04–0·06 μm (Fig. 1b). Both kinds of vesicles are randomly distributed in the cytoplasm, and some vesicles can be seen in the proximity

Fig. 1. (a) Portion of pollen tube cytoplasm of *Malus domestica* after 40 min of incubation: dictyosomes (D) produce large (1) and small (2) vesicles; mitochondria (M) and vacuoles (V) are also visible (control). Bar=2 μm (× 42 000). (b) Portion of pollen tube cytoplasm of *M. domestica* after Thiéry's test after 40 min of incubation: the large (1) secretory vesicles react weakly to the test, the smaller ones (2) are strongly positive (control). Bar=2 μm (× 42 000). (c) Dictyosomes in pollen tube very near the grain treated with 125 nM monensin after 60 min of incubation. Bar=2 μm (× 42 000). (d) Dictyosomes in sub-apical portion of pollen tube treated with 125 nM of monensin after 60 min of incubation. Bar=2 μm (× 42 000).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of the plasma membrane or connected with it. The large vesicles, which contain granular materials, are weakly stained after the polysaccharide test, whereas the small vesicles react strongly (Fig. 1b).

The morphology of dictyosomes in germinated pollen, incubated in the presence of 125 nM monesin for 60 min is remarkably altered. The cisternae appear dilated and the distance between two consecutives is enlarged. The alteration is stronger in the sub-apical portion of the pollen tube (Fig. 1d) than in the pollen tube near the grain (Fig. 1c). The number of cisternae does not vary. After the treatment with monensin for 60 min, both kinds of vesicle are visible and reduced in number but the large ones become swollen and difficult to distinguish. They look like little vacuoles which remain around the dictyosome. In the case of treatment with 125 nM monensin for 120 min, cisternae swelling is so striking that some of them are difficult to recognize as they are easily confused with the vacuoles (cf. Figs 2a, b and c). In the cytoplasm there are numerous small vesicles which show a strongly positive reaction to the polysaccharide test (Fig. 2c). They are reduced in number and show the same ultrastructure with respect to the control. Unlike the small vesicles, the large ones show a hardly recognizable morphology. They do not react or react very weakly to the polysaccharide test (Fig. 2c).

In all the samples treated with monensin no other morphological changes can be observed in the pollen tube wall or in the cytoplasm except that the mitochondria appear to have a more electron-dense matrix.

DISCUSSION

It is a general response to monensin in a number of animal and plant cells that the dictyosome cisternae become swollen, which is due to influx of osmotically activated monovalent cations (Ledger *et al.* 1980; Robinson 1981; Mollenhauer *et al.* 1982; Morré *et al.* 1983, 1986; Tartakoff 1983; Boss *et al.* 1984; Shannon & Steer 1984). The present study is the first report on the ultrastructural response to monensin treatment in pollen tubes. It confirms the similar inhibitory effect of monensin on dictyosomes and vesicles. It is interesting to note that the effect of monensin on dictyosomes in germinated pollen appears gradually strengthened from the grain towards the tube tip. The different wall structure of pollen grain, pollen tube and tube tip is possibly the cause of the changing gradient, since such structural differences may result in different uptake rates of monensin. But one should also keep in mind that there are various patterns of cytoplasmic streaming in pollen tubes (Heslop-Harrison 1987). Considering that the swelling of dictyosomes cisternae is also involved in a proton gradient at or near the mature Golgi face (Boss *et al.* 1984), any cytoplasmic gradient, calcium gradient and pH gradient toward the apex of the pollen tube (Heslop-Harrison 1987; Turian 1981) may be related to the gradual alteration of dictyosome structure. The effect of monensin on the production and transport of the large vesicles is apparently both quantitative and qualitative. According to van der Woude *et al.* (1971), in *Lilium longiflorum* pollen tubes the synthetic polysaccharide changes in secretory vesicles occur during migration

Fig. 2. (a) Dictyosomes in the sub-apical portion of pollen tube treated with 125 nM of monensin after 120 min of incubation. Bar=5 μm (× 22 700). (b) Dictyosomes in the sub-apical portion of pollen tube 120 min after sowing (control). Bar=5 μm (× 22 700). (c) Polysaccharide test: sub-apical zone of pollen tube cytoplasm treated with 125 nM of monensin after 120 min of incubation. Small vesicles are strongly positive. The large vesicles react very weakly or do not react at all. Bar=2 μm (× 45 000).

towards the tube tip. In monensin-treated germinated pollen of *M. domestica*, the weakness or the absence of reactivity to the polysaccharide test in large vesicles appears to be related to their size in the sense that the larger the vesicles, the weaker the labelling. This might indicate that they are at an immature stage which may result from the disorganization of the dictyosomes. For the small vesicles no significant alteration can be seen except for the reduction in number. Taking into account that there are two exit sites for secretory products from plant Golgi stacks (Moore *et al.* 1991), it might be that the small vesicles could partially escape from the inhibitory block at the Golgi level by sorting proximally to the site of arrest imposed by monensin. It is known that the apical growth of pollen tubes is associated with the presence of large numbers of vesicles in the tip region. The small vesicles participate in the formation of the pecto-cellulosic part of the pollen tube wall whereas the large ones are involved in the organization of the callosic layer (Cresti *et al.* 1979a,b; Ciampolini *et al.* 1984). Our results present a clear disorganization of dictyosomes and vesicles: this agrees well with the inhibitory effect previously described in apple pollen, showing that monensin strongly decreases both emergence and tube elongation (Speranza & Calzoni 1992). These inhibitory effects on growth were much stronger in the second phase of incubation (120 min). The highly sensitive growth response observed in apple pollen can evidently be related to the perturbation of dictyosome and secretory vesicle features. Moreover the apical growth of the pollen tube also needs the supply of new membrane, plasma membrane associated enzymes and other proteins. Previous results have revealed that there is a massive protein leakage from germinated apple pollen into the medium, under the treatment of monensin (Speranza & Calzoni 1992). Therefore the lack of some basic membrane components in the tip region may also be hypothesized to account for the decrease in the tube growth rate. Notwithstanding these considerable aberrations, pollen tube growth was not completely stopped by monensin treatment (Speranza & Calzoni 1992). This is probably because wall-precursor material originated through dictyosome activity is not the only source for wall formation (Heslop-Harrison 1987) and some vesicles still play their role. Further study on the changing composition of vesicles and pollen tube walls after treatment with monensin is needed in order to understand the mechanism of the inhibitory effects of monensin on apple pollen tube growth.

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