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The effect of colchicine on microtubules and microfibrils in root hairs

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SUMMARY

Roots of *Equisetum hyemale* and *Raphanus sativus* were grown in concentrations of colchicine that slowed down root hair growth initially, and later stopped growth completely. The treatment effected depolymerization of most of the microtubules. The wall texture in root hairs of *E. hyemale* was helicoidal after treatment with colchicine, as in untreated hairs. In root hairs of *R. sativus* the deposition orientation of the microfibrils changed (prior to microtubule depolymerization) from axial to oblique and transverse, but deposition remained ordered. As ordered wall texture is deposited in orientations that differ from the orientation of the depolymerizing microtubules and also in the absence of microtubules, it is concluded that microtubules do not directly control the orientation of nascent microfibrils.

Key-words: colchicine, microfibrils, microtubules, root hairs, wall texture, *Equisetum hyemale*, *Raphanus sativus*.

INTRODUCTION

The mechanism that controls the ordered deposition of cell wall microfibrils is a matter of controversy (Emons 1982; Traas *et al.* 1985; Hepler 1985; Lloyd & Wells 1985; Derksen *et al.* 1990). In growing plant cells, microtubules and microfibrils are consistently found in parallel to each other; this also occurs in the expanding part of the root hair, i.e. the tip, where both microtubules and microfibrils run in all directions (Emons & Derksen 1986; Emons 1989). This has led to the hypothesis that cortical microtubules direct the nascent microfibrils (for review: Heath & Seagull 1982).

In root hair tubes different types of wall textures have been found: axial, helical, helicoidal (Sassen *et al.* 1981) and crossed polylamellate (Lloyd & Wells 1985). The helicoidal wall is built of a stack of parallel-fibred lamellae. Microfibril orientation in subsequent lamellae is rotated with respect to the previous lamella. In general, cortical microtubules in root hairs are axial or steeply helical (Seagull & Heath 1980; Emons & Wolters-Arts 1983; Lloyd & Wells 1985; Traas *et al.* 1985) though more complicated configurations have been reported for *Allium* (Lloyd & Wells 1985; Traas *et al.* 1985).

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Thus, in root hairs with axial or helical wall texture, microtubules and nascent microfibrils are often in parallel, while in root hairs with helicoidal wall texture such a co-orientation has not been found. In addition, in the green algae with helicoidal wall texture, cortical microtubules are not in parallel with nascent microfibrils (for review: Itoh 1989). The loss of microfibril order after disruption of the microtubules with colchicine is seen as a strong argument for the role of microtubules in microfibril orientation (for review: Robinson & Quader 1982). Such experiments have not been carried out on root hairs and on cells with helicoidal walls.

To study the effect of microtubule depolymerization on wall microfibril deposition in root hairs, young roots of *Equisetum hyemale* and *Raphanus sativus* with growing hairs were treated with the drug colchicine. These species were chosen because they have a well-known root hair wall texture: random until 300 μm from the hair tip and helicoidal in the rest of the hair tube in *E. hyemale* (Emons 1982; Emons & Wolters-Arts 1983) and random until 25 μm from the hair tip and axial in the rest of the hair tube in *R. sativus* (Sassen *et al.* 1985). In young root hairs of *E. hyemale* net axial microtubule alignment was found consistently using different methods: thin-sectioning of chemically fixed material (Emons 1982; Emons & Wolters-Arts 1983), dry cleaving (Traas *et al.* 1985), immunofluorescence (Traas *et al.* 1985) and freeze-substitution (Emons 1989). It is the most thoroughly studied higher plant cell that does not show concomittant microtubule and microfibril alignment. The *R. sativus* root hair also has net axial microtubule alignment. It is the hairs of this plant in which co-alignment between cortical microtubules and nascent microfibrils was first shown (Newcomb & Bonnett 1965; Seagull & Heath 1980).

The present study reports on the effects of a number of concentrations of colchicine on growth, hair morphology, microfibril texture in the tip, microfibril texture in the tube, and on microtubules of two species that possess different root hair wall textures.

MATERIALS AND METHODS

Root hairs of *Equisetum hyemale* (L.) were taken from stem cuttings (*cf.* Emons & Wolters-Arts 1983). Root hairs of *Raphanus sativus* (L.) were taken from seedlings germinated on moist filter paper and grown in water in Petri dishes. Plantlets were placed in special cuvettes in which roots of stem cuttings and intact seedlings could be examined under a light microscope to follow hair initiation and growth, and in which the medium could be changed without disturbing the roots. Only hairs that grew in the medium were used. Roots were exposed to the drug at concentrations of 1, 5, or 10 mM for up to 20 h. This range of concentrations was chosen because within this range microtubules were depolymerized, hair growth did not stop abruptly, and cells did not plasmolyse. Roots were fixed in these cuvettes by adding freshly prepared *p*-formaldehyde up to a concentration of 3%.

Microtubules were studied by means of immunofluorescence (Traas *et al.* 1985). Cell wall texture was examined by means of the dry-cleaving technique for cell walls (Sassen *et al.* 1985). Preparations were examined and photographed using a Phillips EM 300 or 201.

RESULTS

Root hair growth during colchicine treatment was followed by light microscopy. In control hairs of *Equisetum hyemale*, growth was 17–26 μm h^{-1}. Hair growth was arrested after 60–85 min treatment with 10 mM colchicine (three experiments, five hairs each).

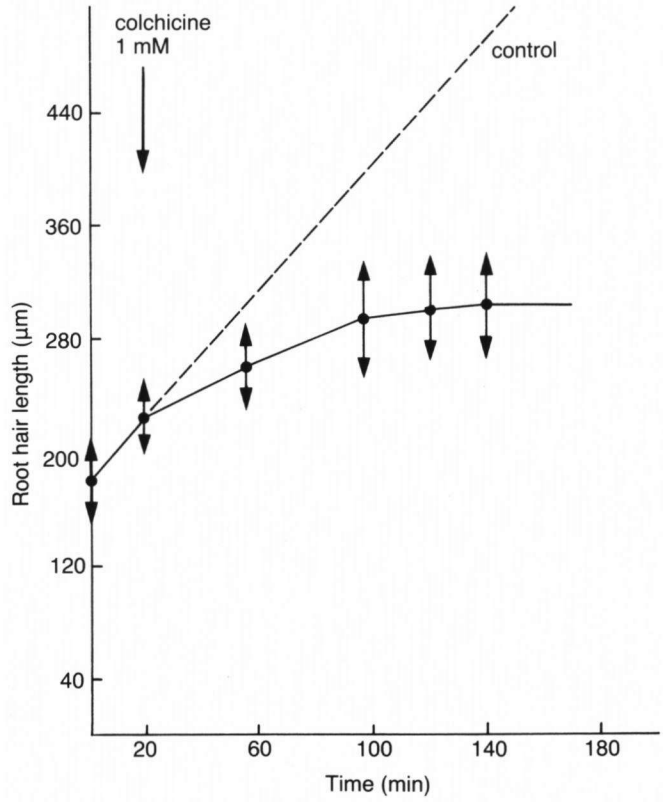

Fig. 1. Growth curve of root hairs of *Raphanus sativus* grown in 1 mM colchicine.

In *Raphanus sativus*, growth in control hairs varied between 48 and 116 $\mu m\ h^{-1}$ (five experiments, five hairs each), and stopped after 60–115 min treatment with 10 mM colchicine. As an example, Fig. 1 shows a growth curve of root hairs of *R. sativus* grown in 1 mM colchicine. Growth at the start of the experiment was 104 μm^{-1}. The growth rate, in the period of active growth of control hairs, was reduced to a minimum at 125 min after application of the drug; however, in some cases growth continued in 1 mM colchicine for up to 3 h.

The morphology of *E. hyemale* root hair tips changed during treatment. The diameter of the part grown during treatment was up to twice the original and the hemisphere flattened. An increase in diameter was not seen in *R. sativus* root hairs.

Microtubules were studied after growth had stopped. Their depolymerization at that time depended on the concentration of colchicine, and is shown for *R. sativus* in Table 1. Figure 2a shows microtubules of *E. hyemale* in an untreated root hair. Microtubules lie net-axial. After 7 h in 1 mM colchicine, most of the microtubules were still present in the original configuration; after 7 h in 10 mM colchicine many microtubules were depolymerized and the orientation of the remaining microtubules deviated more from the axial direction than that in untreated hairs (Fig. 2b). Figure 2c shows the net axial microtubule organization in untreated root hairs of *R. sativus*. In these hairs, the area of the hair tube where the large vacuole is situated seems to be wider because the hair collapses on the slide, while at the tip it remains round. Figure 2d shows a hair of *R. sativus* treated with 10 mM

Table 1. Effect of colchicine on microtubules in root hairs of *Raphanus sativus*. The presence of microtubules was scored by means of immunofluorescence. Quantitative measurements were not possible

Concentration of colchicine (mM)	Duration of treatment (h)		
	1	5	10
1	−	−	−
5	−	+/−	+/−
10	+/−	+	+

(−) Most microtubules present, no effect, (+/−) some microtubules present, partial effect, (+) no or few microtubules present, strong effect.

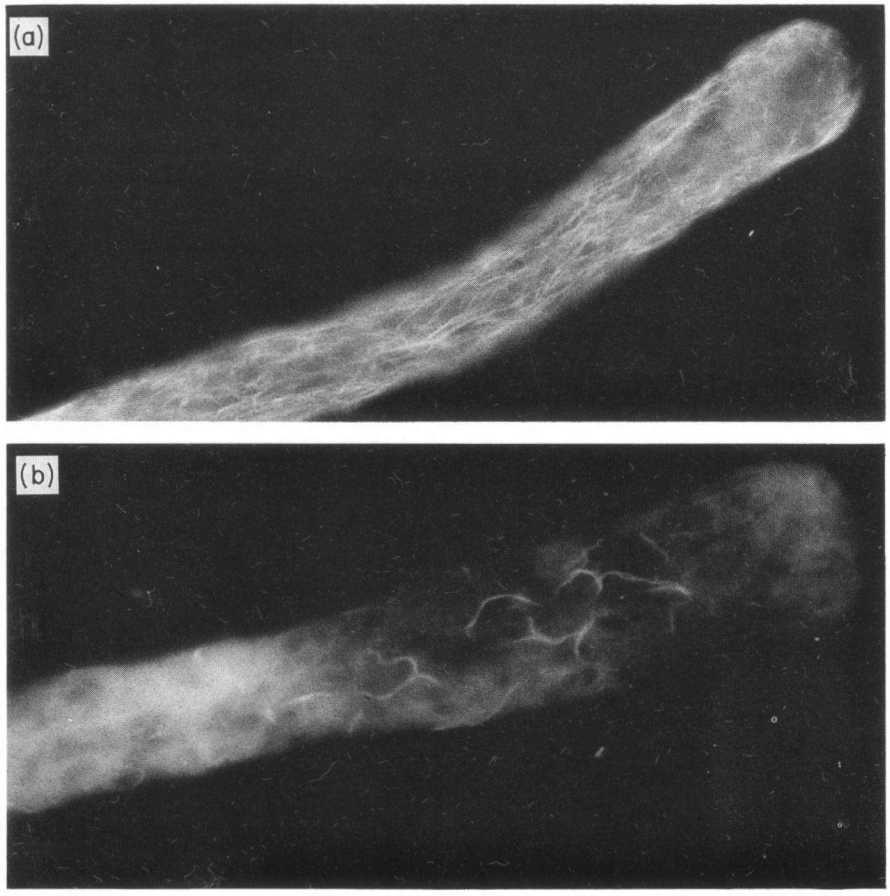

Fig. 2.

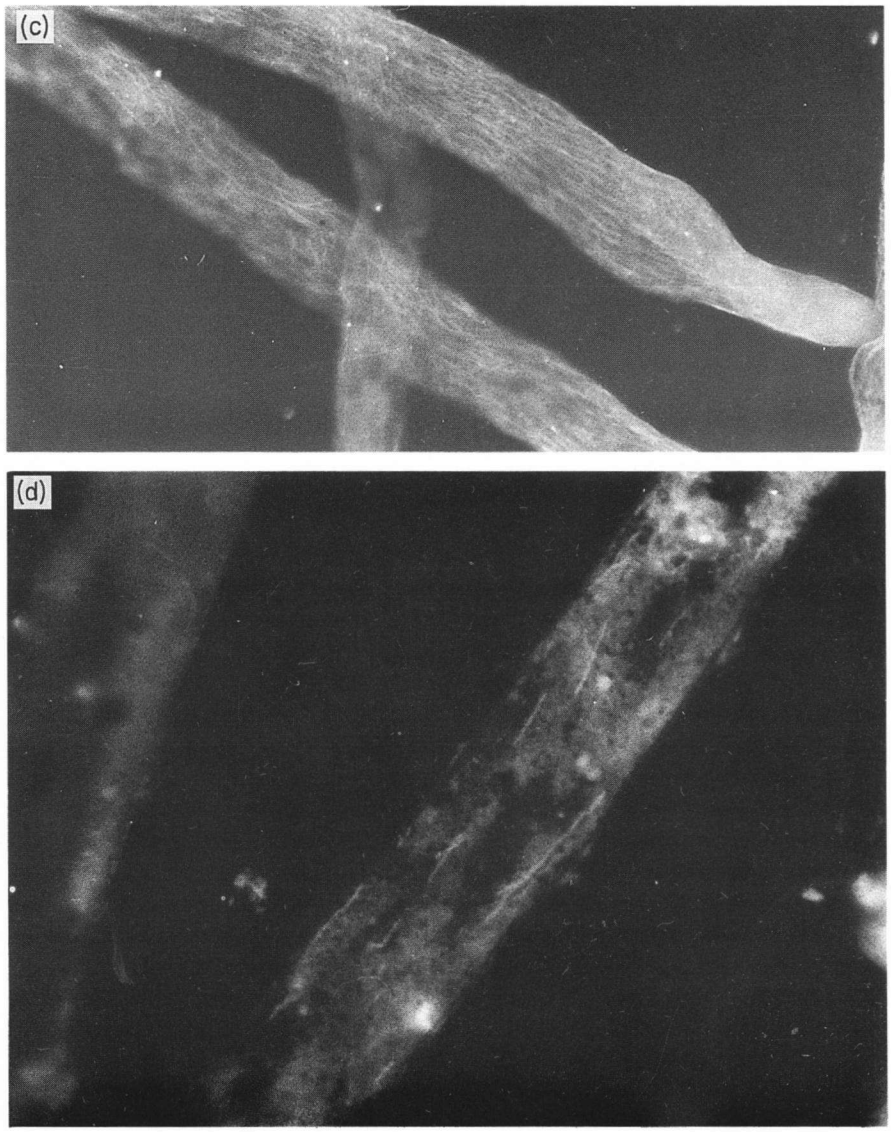

Fig. 2. Microtubules in root hairs visualized by immunofluorescence. (a) *Equisetum hyemale*, control. (b) *Equisetum hyemale* treated with 10 mM colchicine during 7 h. (c) *Raphanus sativus* control. (d) *Raphanus sativus* treated with 10 mM colchicine during 1·5 h. (× 1250.)

colchicine for 1·5 h: only short microtubules in the original longitudinal orientation are observed. Immunofluorescence pictures of colchicine-treated hairs show much background staining (Fig. 2d), probably derived from tubulin monomers as a result of microtubule depolymerization. The occurrence of the short longitudinally oriented microtubules after colchicine treatment was confirmed by electron microscopy (data not shown). After 5 h in 10 mM colchicine, approximately 80% of the hairs of *R. sativus* no longer contained microtubules (Table 1).

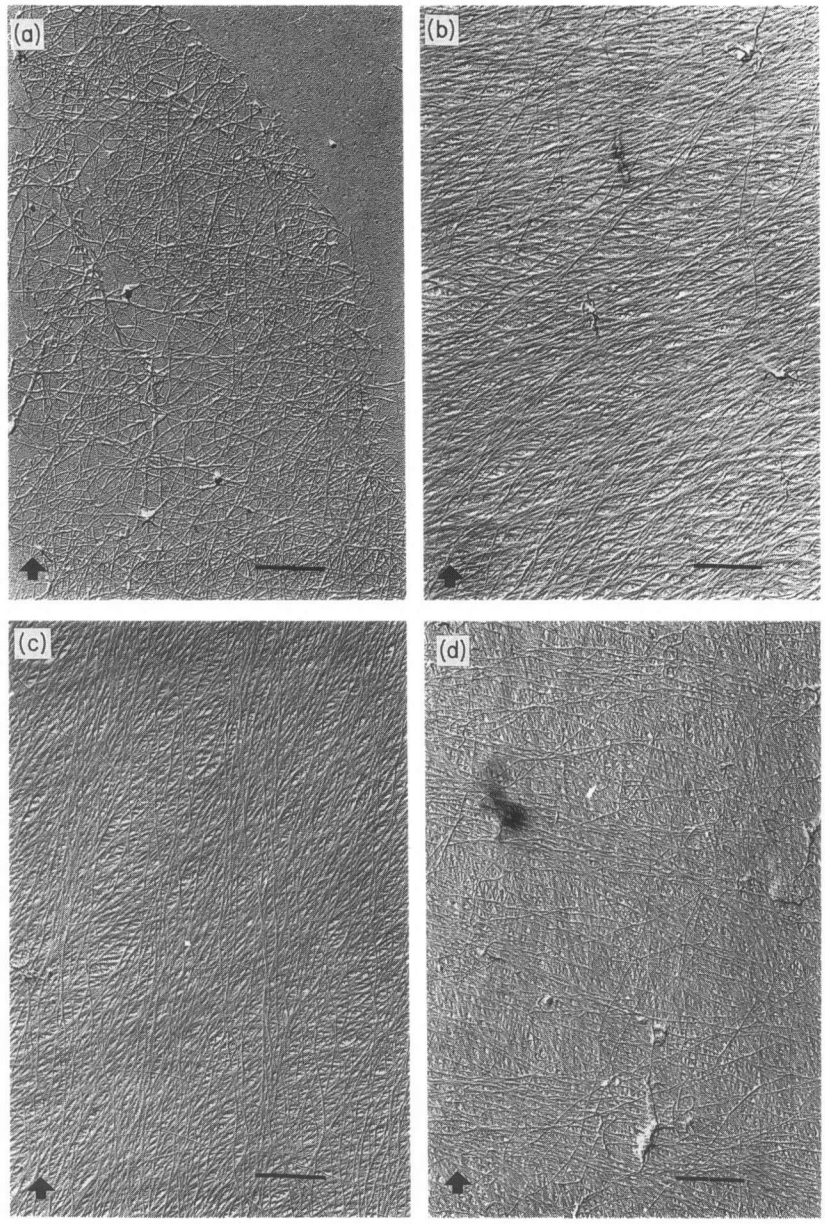

Fig. 3. Surface views of inner cell wall of root hair of *Equisetum hyemale* after treatment with 10 mM colchicine during 6 h. (a) Random texture at the hair tip. (b–d) The last-deposited lamellae with different orientations according to the long axis of the hair. (× 18 144, bar: 500 nm.) Arrow: long axis of the hair.

The cell wall texture of root hairs of *E. hyemale*, observed after 6 h in 10 mM colchicine, was as in the control hairs with a random texture at the hair tip (Fig. 3a), and further into the hair tube the typical lamellae of the helicoidal wall contained longitudinal, oblique and transverse (Figs 3b–d, respectively) nascent microfibrils. The microfibrils in the last-deposited lamella showed different orientations at different locations along the hair, as

found in the control preparations (Emons 1989). The rotation mode of the helicoid was counter-clockwise and the angle between microfibrils in adjacent lamellae (30°) did not differ significantly from the control.

The microfibril orientation in control preparations of *R. sativus* was axial, but in root hairs grown in water the deviation of individual microfibrils from the axial orientation was up to 45°, which is more than in the narrower hairs grown in moist air. After a 1-h treatment with 1 mM colchicine the orientation of the last deposited microfibrils differed from the control (Fig. 4a). In some hairs random nascent wall texture was observed, but in the majority of the hairs the main orientation of the newly deposited microfibrils was oblique to the long axis of the hair (Fig. 4b) with individual microfibrils again deviating by as much as 45° from the main orientation. Areas with axial microfibril orientation were still observed. After 2 h of drug treatment in a concentration of 1 mM, the results did not differ. Active microfibril deposition must have taken place because the helical texture almost reached the hair tip (Fig. 4b); a characteristic that does not occur in growing control hairs in which the 25 μm from the hair tip exhibits random microfibrils. Figures 4c and d are micrographs taken after treatment for 7 h with 10 mM colchicine. Three microfibril orientations can be observed. The deepest, oldest lamella contains longitudinally oriented microfibrils, the microfibril orientation of the subsequent lamella is oblique and the last deposited one is transverse to the long axis of the hair. This new configuration resembles a helicoidal texture and would be half a helicoidal arc in thin section.

DISCUSSION

Treatment with colchicine depolymerized the microtubules, and had a significant effect on root hair growth rate, possibly by disrupting polar organization of the cytoplasm. In *Equisetum hyemale*, which does not contain reinforcing secondary wall microfibrils up to 300 μm from the tip, the treatment effected a wider hair tip. In addition, the hairs derived from the trichoblasts during colchicine treatment had a bulbous appearance.

After treatment with colchicine the newly formed hair tip of *E. hyemale* root hairs had a normal arrangement of randomly oriented microfibrils. The hair tube of these root hairs showed a perfect helicoidal wall texture when grown in colchicine. It could be argued that in the tube of these root hairs a helicoidal wall had been deposited before the drug had entered the cell, and that during drug treatment no new microfibrils had been deposited. This is certainly not the case in *R. sativus*. In the latter, microfibril orientation in the tube shifts after drug treatment and the random texture close to the hair tip becomes covered with helically oriented microfibrils, two phenomena not seen in the control. Because the area with random microfibrils at the tip is so long in *E. hyemale* and the transition from random to helicoidal so gradual, it was not possible to determine by means of dry-cleaving whether this area decreased as a result of the drug treatment.

Weerdenburg & Seagull (1988) reported the generation of microfibril bundles following treatment with 3 mM colchicine in liquid suspension cultures of *Vicia faba*. They suggested that these bundles are the result of characteristics inherent in the cellulose synthesizing machinery and triggered by a disruption of normal microtubule function. We did not find any more bundling of microfibrils in treated cells than in the controls, neither in *E. hyemale* nor in *R. sativus*.

Since the hypothesis that microtubules orientate microfibrils was first postulated (Ledbetter & Porter 1963), more data on the function of microtubules have become available (for reviews: Bershadsky & Vasiliev 1988; Derksen *et al.* 1990). Their possible

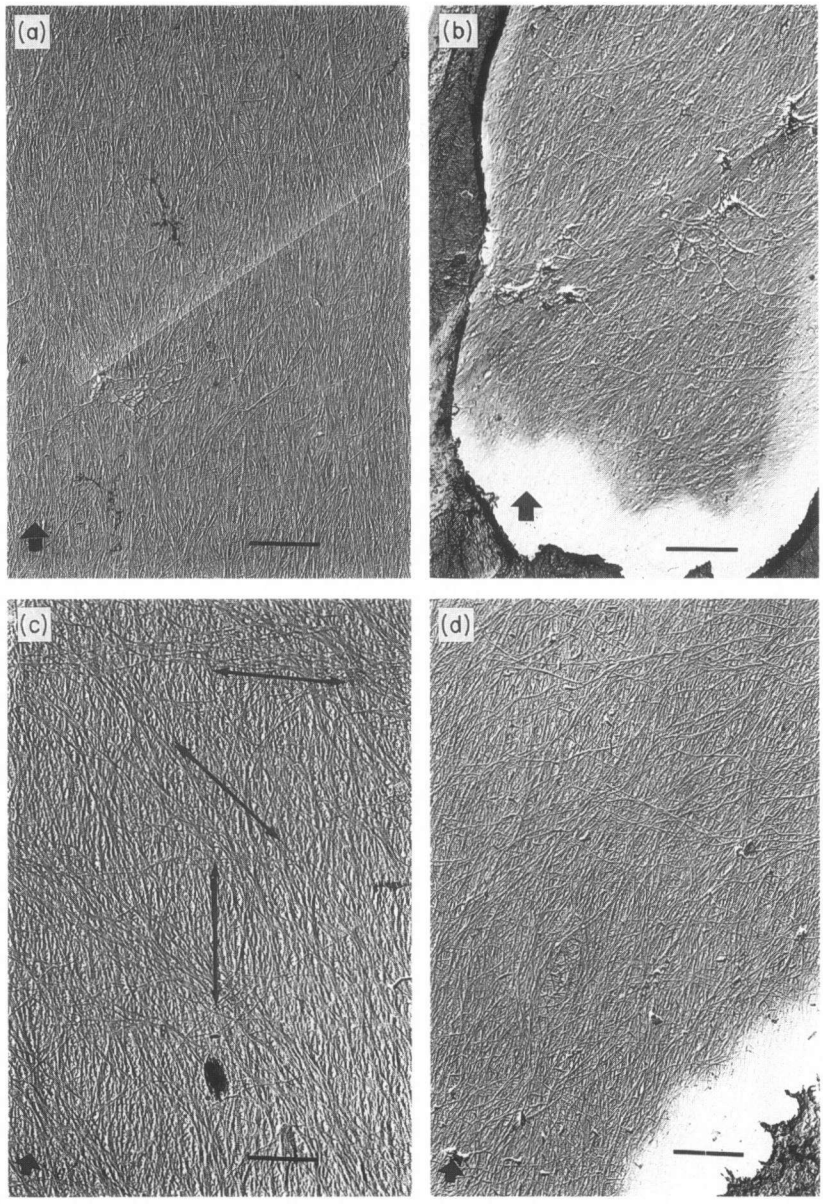

Fig. 4. Surface views of inner cell wall of root hairs of *Raphanus sativus* deposited during colchicine treatment. (a) Longitudinal microfibrils in the control. (b) Oblique microfibrils 5 μm from the hair tip after 1 h in 1 mM colchicine. (c, d) Subsequent longitudinal (original layer before treatment), oblique and transverse orientations in hairs (long arrows in 4c) after 7 h in 10 mM colchicine. (× 18 216, bar: 500 nm.) Short arrows: long axes of hair.

role in exocytosis affects the amount of wall matrix material brought into the wall and probably also the amount of particle rosettes, the putative microfibril synthesizing enzymes, inserted into the plasma membrane (Emons 1985). They serve a morphogenetic role by maintaining cell morphology during growth when the wall is still plastic. These

functions taken into account, one would expect that depolymerization of microtubules affects the direction of microfibrils without postulating that they actually control microfibril orientation by their own direction.

The present experiments offer further evidence that microtubules have a function in cell morphogenesis and maintenance of cell polarity, by which tip growth is possible, but that they do not directly orientate the microfibrils during deposition. It is important to note that the shift in microfibril orientation in *R. sativus* takes place before the depolymerization of the microtubules (Table 1). This could mean that microtubules need to be tightly connected to the plasma membrane to exert their influence on the movement of cellulose synthesizing complexes and that this connection naturally does not occur in a number of cells and is disrupted by colchicine. It also means that microfibrils are committed to ordered deposition without the intervention of cortical microtubules.

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