Protoplasma 92, 195–210 (1977)

PROTOPLASMA

Effects of MBC on Hyphal Tip Organization, Growth, and Mitosis of *Fusarium acuminatum*, and Their Antagonism by D_2O

R. J. Howard and J. R. Aist

Department of Plant Pathology, Cornell University, Ithaca, New York, U.S.A.

With 12 Figures

Received November 17, 1976

Summary

Effects of treatment with methyl benzimidazole-2-ylcarbamate (MBC) on living hyphal tip cells of *Fusarium acuminatum* were determined with phase contrast light microscopy. These included (i) displacement of mitochondria from hyphal apices, (ii) disappearance of Spitzenkörpers, (iii) reduction of linear growth rate, and (iv) metaphase arrest of all mitoses: responses i–iii were not a result of effects on mitosis. Since all of these responses theoretically could have resulted from an MBC effect on microtubule structure and/or function, heavy water (D_2O) was used to counteract MBC. Treatments of hyphae with MBC + D_2O caused quantitative responses, i–iii above, intermediate between those to the separate reagents, and some nuclei of these hyphae were not arrested at mitosis. Moreover, several nuclei fragmented (multimicronucleation) in a manner apparently similar to mammalian nuclei treated with antitubulin agents. Thus, the effects of MBC on apical organization, Spitzenkörper integrity, hyphal growth and mitosis could have been mediated through interference with microtubules.

1. Introduction

Benomyl is a systematic, fungitoxic, benzimidazole-derivative widely used for the control of plant pathogenic fungi. *Oomycetes* and *Zygomycetes* are generally insensitive (Bollen and Fuchs 1970, Edgington *et al.* 1971). Methyl benzimidazole-2-ylcarbamate (MBC), the hydrolysis product and toxic moiety of benomyl, has been reported to inhibit mitosis in fungi (Hammerschlag and Sisler 1973, Davidse 1973, Richmond and Phillips 1975), higher plants (Richmond and Phillips) and animals (Styles and Garner 1974, Seiler 1975, de Brabander *et al.* 1976 b). The antimitotic activity of MBC in fungi resembles mitotic arrest caused by colchicine in higher plants and mammalian cell cultures (Hammerschlag and Sisler, Richmond and Phillips, Davidse 1973, 1975). Colchicine probably interferes with mitosis in these organisms through its specific binding with the dimeric state of tubulin (Wilson 1975 a) the microtubule subunit protein. Davidse

(1975 and 1977) demonstrated ^{14}C-MBC binding in a protein extract from *Aspergillus nidulans* and provided substantial evidence that this protein was fungal tubulin. We previously reported that MBC causes metaphase arrest of mitosis in *Fusarium acuminatum* (HOWARD and AIST 1976). Because of these similarities with the activity of colchicine, MBC might be a member of a class of compounds called antitubulins. WILSON (1975 b) considered colchicine a prototype of this group of biologically active agents.

The effects of antitubulins on eukaryotic cells are manifested, though not exclusively (WILSON 1975 a), through an interference with microtubule-mediated processes (BRYAN 1974) including mitosis, intracellular transport and subcellular organization (BRYAN 1974, PIPELEERS *et al.* 1976, MOSKALEWSKI *et al.* 1976, DE BRABANDER *et al.* 1976 a). MBC was recently included among the compounds with known antitubulin specificity by DE BRABANDER *et al.* (1976 b) and is thus, presumably, one of many "drugs that interfere with polymerization of tubulin *in vivo*", as defined by MARGULIS (1973). DE BRABANDER and coworkers (1976 b) provisionally designated the capacity for inducing multimicronucleation as a direct indication of a compound's specific interference with microtubule structure or function. STYLES and GARNER (1974) and SEILER (1975) also reported multimicronucleation by MBC. *In vitro* activity of MBC against mammalian tubulin polymerization has been demonstrated (HOEBEKE and VAN NIJEN 1975, DE BRABANDER *et al.* 1976 b), however, the level of inhibition was well below that of standard antitubulins. MBC has, at most, a very low affinity for mammalian tubulin (DAVIDSE and FLACH 1977).

Different antitubulins have synergistic effects on microtubule function when applied simultaneously, whereas agents with opposite effects show antagonism (MARSLAND 1970). Such antagonism has been demonstrated between heavy water (D_2O) and (i) colchicine (GILLESPIE *et al.* 1968, MARSLAND and HECHT 1968, BURGESS and NORTHCOTE 1969, MALAISSE *et al.* 1975, RUBIN and WEISS 1975), (ii) colcemid (INOUÉ *et al.* 1965), (iii) cold (MARSLAND *et al.* 1971), (iv) high hydrostatic pressure (MARSLAND and ZIMMERMAN 1965, LOWE-JINDE and ZIMMERMAN 1969, MARSLAND *et al.* 1971), and (v) vincristine (MALAISSE *et al.* 1975). Considerable evidence demonstrates that D_2O enhances polymerization of tubulin (MARSLAND 1970, INOUÉ and SATO 1967, SATO 1975, SCHNEPF *et al.* 1976). Although the mechanism of this microtubule stabilization is unknown, the effect is well established. Other possible effects of deuterium on biological systems have been reviewed by THOMSON (1963).

In previous cytological investigations on the effects of MBC in fungi (HAMMERSCHLAG and SISLER, DAVIDSE 1973, RICHMOND and PHILLIPS 1975), nuclear stains that require fixed and hydrolyzed fungal cells were employed. Therefore, several early responses to MBC reported here were not detected. We studied living fungal cells, during or shortly after treatment with MBC, with and without concomitant D_2O treatment. The results extend the

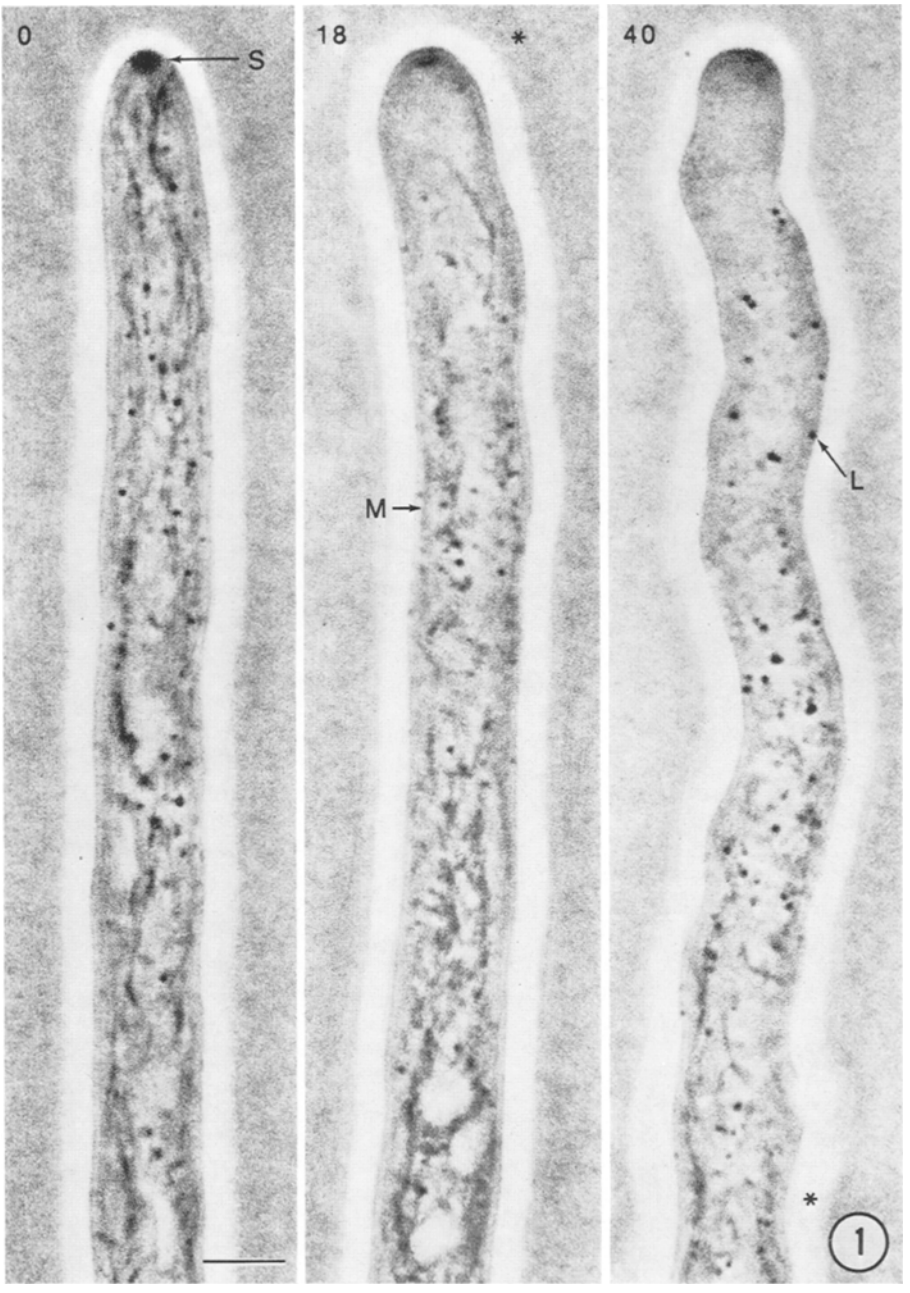

Fig. 1. Phase contrast, time-lapse sequence of the apical region of a living hyphal tip cell of *Fusarium acuminatum* before and after perfusion with 10 µg/ml of MBC. Numbers in the upper left corners show the elapsed time (minutes) from the 0 frame when treatment was begun. By frame 18 Spitzenkörper integrity had been disrupted. The dense area at the apex of the cell in frames 18 and 40 most likely represents partially dispersed component vesicles of the Spitzenkörper. Mitochondria (*M*) were not oriented parallel to the longitudinal axis of the cell, as in frame 0. The asterisks identify the same point in the medium in frames 18 and 40. Lipid bodies (*L*) were apparently unaffected by the treatment. Scale bar = 5 µm

probable cellular sites of action of MBC in fungi and provide experimental evidence for the involvement of microtubules and the "Spitzenkörper" in hyphal extension.

2. Materials and Methods

2.1. Organism and Media

Benomyl-sensitive isolate no. 73 (Smiley and Howard 1976) of *Fusarium acuminatum* Ellis & Everhart (ATCC 32965) was maintained in a lyophilized state, recovered as needed on Difco potato dextrose agar medium (PDA), and stored at 10 °C. Mycelial plugs used in experiments were taken from actively growing margins of colonies subcultured once from PDA-stock cultures.

Martin's medium (MM) no. 137 was prepared as described by Tuite (1969) except that streptomycin and rose bengal were omitted and the pH was 6.0. In experiments involving amended medium, reagents were sterilized by Millipore filtration and incorporated into the autoclaved medium, cooled to about 50 °C.

2.2. Reagents and Procedures

MBC (99.9%) was provided by E. I. du Pont de Nemours & Co., Wilmington, Delaware, U.S.A. It was first dissolved in dimethyl sulfoxide (DMSO) and then diluted with distilled or heavy water to final concentrations of 1.0 μg MBC/ml (5.2×10^{-6} M MBC) and 0.1% DMSO, unless otherwise stated. Heavy water (D_2O), obtained from Sigma Chemical Co., St. Louis, Missouri, U.S.A., was used in a 40% (v/v) solution in distilled water (unless indicated otherwise). This concentration produced optimum antagonistic effects toward MBC in radial growth assays which tested 15, 30, 40, 45, and 60 percent (v/v) D_2O concentrations, each with four replications. Data reported for effects on living cells by D_2O, alone or in concomitant treatment with MBC (D_2O + MBC), were obtained from cells grown on slides coated with 40% D_2O-amended medium.

Methods of aseptic preparation of living hyphal tip cells for light microscope observation were similar to those used by Aist (1969). Standard microscope slides thinly coated with a film of agar medium were placed on bent glass rods over filter paper in petri dishes containing 4 ml distilled water. Suitable colonies were obtained by transferring mycelial plugs, fungus-side down, onto the centers of the slides and incubating the dishes in the dark for 45–50 hours at 24 °C.

Each slide in experimental treatments was prepared by the following procedure. The colony surface was flooded at *time zero* with control (DMSO) or treatment (MBC + DMSO) solution. Precisely 2.5 minutes later, the slide was removed from the petri dish and drained on edge over a paper towel for 15 seconds. All agar and mycelium, except for a 15 × 15 mm square containing colony margin was cut away and removed without disturbing the remaining 5–7 mm lengths of hyphae. A coverslip was applied to the remaining agar square with extreme care. The edges of the coverslip were sealed with paraffin and the preparation was immediately observed. Treatments and controls were staggered in time to allow for observation of one and then the other on two side-by-side Zeiss light microscopes equipped with phase contrast optics, in a room held at about 18 °C.

An alternate method of preparation allowed for continuous observation of the same hyphal tip cells before, during, and after treatment. This involved perfusion of treatment or control solutions under the coverslip through 1 mm wide channels cut into the agar square. In these experiments the two edges of the coverslip perpendicular to the channels were not sealed.

Observations were restricted to cells which had been under the coverslip less than 1 hour. Data were obtained exclusively from hyphal tip cells which were (i) in the upper surface

plane of the agar, thereby assuring good optics, (ii) healthy, as determined by hyphal diameter, cytoplasmic density and the presence of the subcellular organizational zonation described by GROVE *et al.* (1970) as "subapical" and the "zone of vacuolation", and (iii) lacking visible damage as a result of coverslip application. Data were obtained by

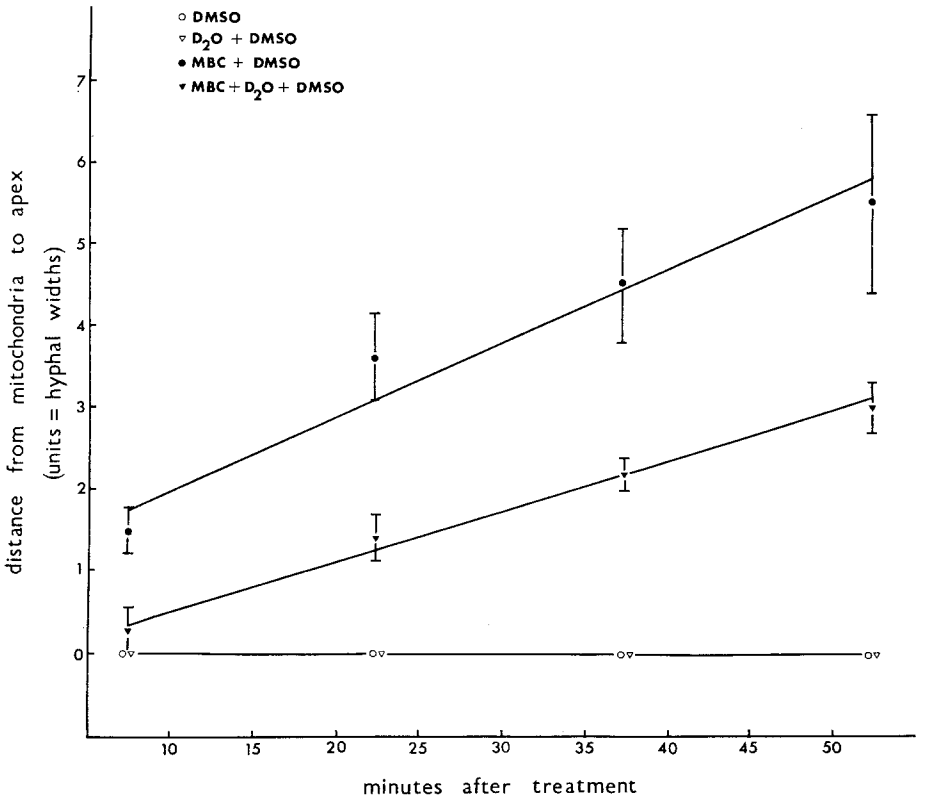

Fig. 2. Increase with time (minutes after initiation of treatment) in the distance between hyphal apices and mitochondria of *Fusarium acuminatum*. For each time interval, the mean distance and its 95% confidence interval for measurements of 100 hyphae are shown. Note: correlation coefficients; MBC + DMSO, $r = 0.98$; MBC + D_2O + DMSO, $r = 0.99$

scanning living tip cells in these preparations at intervals of 5–10, 20–25, 35–40, and 50–55 minutes after treatment. For each treatment an appropriate control preparation was alternately observed during these same intervals. The distance between the hyphal apex and the nearest mitochondrion was estimated in units of hyphal width. This method automatically normalized the data to compensate for differing hyphal widths within treatments. Statistical procedures were obtained from STEEL and TORRIE (1960).

2.3. Growth Rate Determinations

One drop of polystyrene latex, supplied by Dow Chemical Co., Midland, Michigan, U.S.A. (0.26 μm particle diameter), per 10 ml of treatment solution provided visible, stationary points of reference for the measurement of hyphal elongation. Within 10 minutes from *time zero*, several hyphal tip cells were located and their coordinates recorded. Each cell

was photographed at intervals of approximately 10 minutes (the exact time since treatment was recorded for each frame). By measuring the length of hyphal elongation during a known period of time between any two successive photographs of the same hyphal tip cell, the growth rate (μm/min) during that interval could be calculated. Data were analyzed statistically using the Duncan's New Multiple Range Test (Steel and Torrie 1960), $P = 0.05$.

3. Results

3.1. Effects on Organization of Hyphal Apices

The initial, cytological effect of MBC treatments on living hyphal tip cells was a disruption of apical organization (Fig. 1). Several thousand cells were observed and in each case the tip reaction was characterized by a gradual increase in the distance between hyphal apices and mitochondria (Figs. 1 and 2) and a gradual disappearance of the "Spitzenkörper" (Figs. 1 and 3). Most mitochondria were no longer oriented parallel to the cell's longitudinal axis (Fig. 1, compare frames 0, 40), nor did they occupy the normal position just behind the "Spitzenkörper". Since the MBC solution used in treatment of the cell shown in Fig. 1 was introduced by perfusion, it was not possible to precisely control the concentration of MBC to which the cell was exposed. This prohibited meaningful comparisons between Fig. 1 and Figs. 2–4.

When cells grown on D_2O-amended MM were treated with D_2O + MBC, the level of the initial responses to MBC described above were significantly reduced (Figs. 2, 3, and 6). With respect to the "Spitzenkörper", a qualitatively intermediate condition was often observed (Fig. 6) in which the "Spitzenkörper" were approximately two-thirds the diameter of those in controls (D_2O + DMSO). In other respects, these diminished "Spitzenkörper" were identical to those in controls. Cells with a smaller "Spitzenkörper" were easily distinguished from cells without a "Spitzenkörper"; the latter had apices completely devoid of the typical dense area.

3.2. Effects on Growth

MBC significantly reduced the growth rate of hyphal tip cells and colonies (Figs. 4 and 5). In less than 15 minutes after MBC-treatment the mean rate of elongation of 10 hyphae was 65% that of 10 DMSO-treated cells (Fig. 4). At 45 minutes after *time zero*, this fraction of the control growth rate had dropped to nearly 30% (about 0.72 μm/min). Growth rate of D_2O + DMSO-controls was significantly less than that of DMSO-controls (Fig. 4), and a similar effect on colony growth was observed (Fig. 5). The mean growth rate of the cells treated with MBC + D_2O + DMSO was intermediate between, and significantly different from, the values for D_2O + DMSO- and MBC + DMSO-treated cells.

Altered growth form of hyphal tip cells, as a consequence of exposure to MBC, was coincident with loss of "Spitzenkörper" integrity. A photo-

micrograph of a normally growing hyphal tip is shown in frame 0 of Fig. 1. Growth anomalies included undulation (frame 40), typically in three dimensions, an increase in the number of growing points per cell (within 1 hour after exposure to MBC), and a change in the shape of the apical dome (compare frames 0, 18, 40). After 48 hours on MBC-amended medium, tip cells were extensively branched. These off-shoots were curled, twisted and themselves often branched.

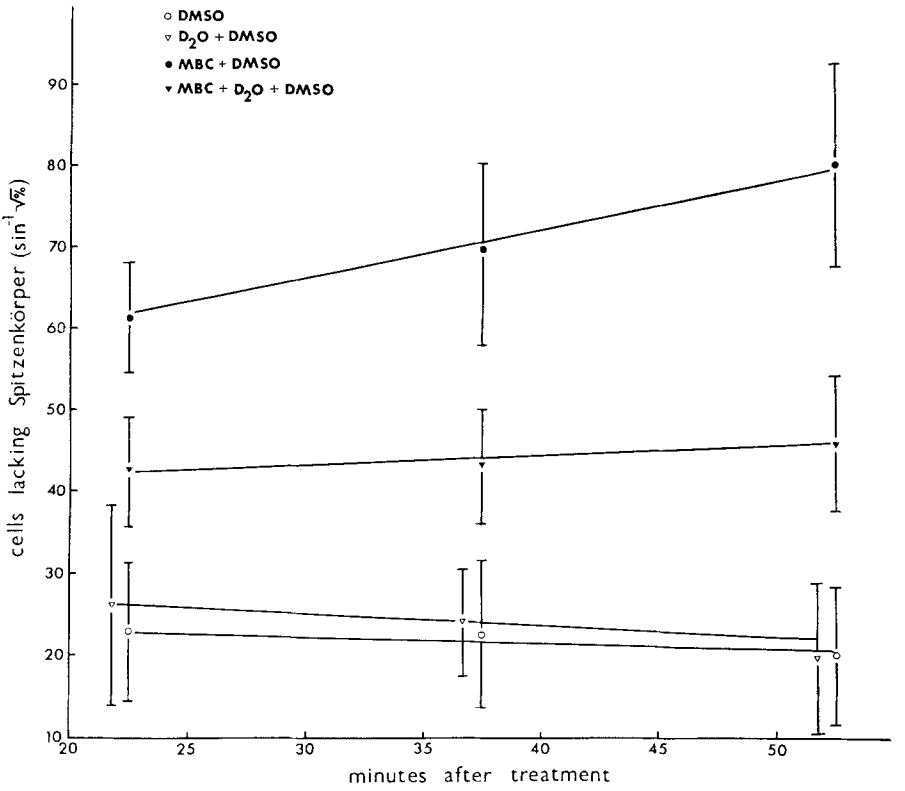

Fig. 3. Spitzenkörper lability in living hyphal tip cells of *Fusarium acuminatum* after various treatments. For each time interval (minutes after initiation of treatment) the mean number and its 95% confidence interval for observations of 100 hyphae are shown. Note: correlation coefficients; MBC + DMSO, $r = 0.99$; MBC + D_2O + DMSO, $r = 0.94$; D_2O + DMSO, $r = -0.99$; DMSO, $r = -0.95$

3.3. Effects on Nuclei

Mitosis in *F. acuminatum* (Figs. 7–9) was very similar to that in hyphal tip cells of *F. oxysporum* Schlect. (Aist 1969). Interphase nuclei were randomly dispersed, often irregular or eliptical in outline, and similar in density to that of the cytoplasm (Fig. 7). When nuclei entered prophase they were about equidistantly spaced, their outlines were nearly circular and they were less

dense than the surrounding cytoplasm (*division density*, Figs. 8 and 9). These criteria were used to recognize cells with predivision nuclei.

Nuclei in MBC-treated cells were consistently blocked at metaphase (Fig. 11). About 30 minutes later, such nuclei reverted to an interphase condition without dividing (Figs. 10 and 12). The metaphase arrest followed an apparently normal interphase and prophase in which the characteristic predivision appearance of nuclei was followed by disappearance of the nucleolus and condensation of the chromosomes (Fig. 11). The interpolar anaphase/telophase spindle (Fig. 9) did not form. During metaphase arrest, nuclear diameter was similar to that before attempted division (compare Figs. 7 and 11). Following reversion to an interphase state the diameter gradually increased to nearly twice that at metaphase arrest (Fig. 10). One or two nucleoli re-formed in reverting nuclei (Figs. 10 and 12), but the division density was retained. These results were consistent in every cell of 200 treated with 10 µg MBC/ml in 1% (v/v) DMSO and in all of 100 cells treated with 1.0 µg MBC/ml in 0.1% DMSO. Mitoses in 100 DMSO- and 50 D_2O + DMSO-treated control cells were identical to those in 50 cells treated with distilled water. Mitotically blocked nuclei migrated (Girbardt 1968, Aist 1969) and septa formed in the presence of MBC.

Several nuclear divisions were observed in different cells after 2.5 minute treatments with 25, 30, 40, and 60% (v/v) D_2O followed by D_2O + MBC, and in treatment with 40%-D_2O + MBC. The effects were variable in that (i) all nuclei in a cell divided, (ii) some nuclei divided while others in the same cell did not, or (iii) none of the nuclei in a cell divided. These results were obtained by observing treated nuclei continuously from interphase through the attempted division until reversion to interphase. In addition, some nuclei treated with D_2O + MBC fragmented into small karyomeres, many containing nucleolus-like bodies (Fig. 10). Intranuclear fusion of nucleoli was observed in two mitotically blocked nuclei reverting to the interphase state.

In D_2O controls, direct attempts to detect effects on the spindle apparatus were unsuccessful. Several investigators have reported that D_2O increases spindle volume and birefringence (see Sato 1975). The spindle apparatus of *F. acuminatum* under polarizing optics was not birefringent before, during, or after any treatment.

4. Discussion

Altered organelle orientation in hyphal tips of *F. acuminatum* after MBC-treatment resembled the effects of oncodazole- (de Brabander *et al.* 1976 a) and colchicine-induced (Moskalewski *et al.* 1976, Lagunoff and Chi 1976) destruction of cytoplasmic microtubules. Davidse (1975 and 1977) correlated MBC- and oncodazole-sensitivities in strains of *Aspergillus nidulans*, and he found that oncodazole and colchicine effectively reduced ^{14}C-MBC-binding to fungal tubulin. Ultrastructural confirmation of such disruption of sub-

cellular organization caused by benomyl was presented by RICHMOND and PRING (1971). They found that organelles in germ tubes of *Botrytis fabae* were drastically reoriented. Unfortunately, their permanganate fixation prevented visualization of microtubules (in either control or treated specimens) and may have been partially responsible for the altered orientation of organelles (GIRBARDT 1969).

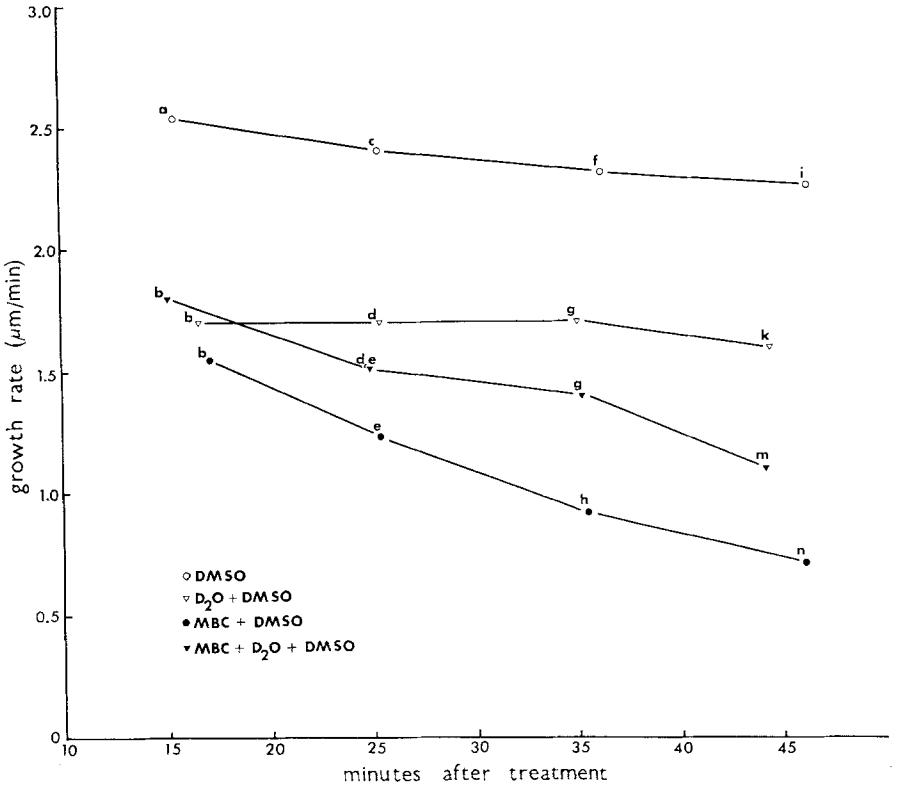

Fig. 4. A comparison of changes in growth rates among four different groups, each of 10 living hyphal tip cells of *Fusarium acuminatum*, as they responded to different treatments. The X-value for each point represents mean time (minutes after initiation of treatment); Y-value, mean rate. Note: Significant differences ($P = 0.05$) between lines at each interval, determined by Duncan's New Multiple Range Test, are indicated by different letter designations

Orientation of organelles parallel to the longitudinal axes of untreated hyphal tip cells was characteristic of *F. acuminatum* (Fig. 1, frame 0), and is generally typical among filamentous higher fungi (GIRBARDT 1969). The destruction of this ordered arrangement almost immediately upon MBC-treatment might have resulted from MBC-interference with microtubule functions; microtubules "... are prime candidates for a role in the system by which a eukaryotic cell

controls its structural organization" (Snyder and McIntosh 1976). Examples of possible microtubule-organelle interactions in fungi have been discussed by Heath (1975). Various mechanisms for such interactions have been proposed (*cf.*, Sheterline *et al.* 1977).

Increased distance of mitochondria from hyphal apices was the first effect of MBC detectable in our system. This distance following MBC treatment was roughly twice that after D_2O + MBC (Fig. 2). Since this distance was

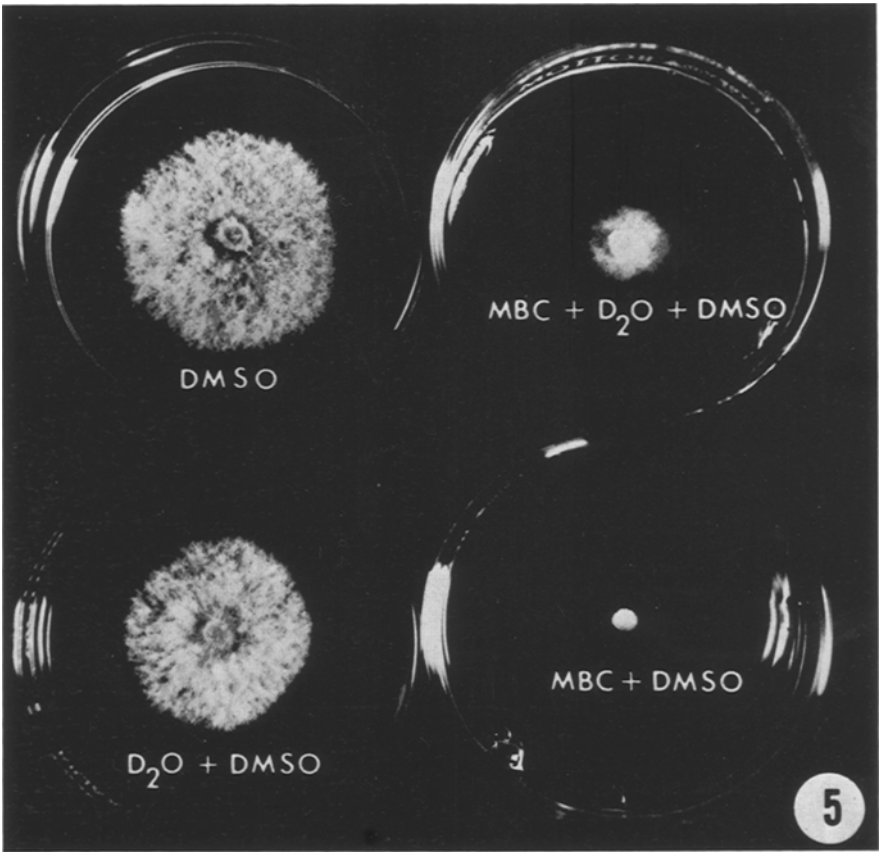

Fig. 5. Radial growth from standard mycelial plugs of *Fusarium acuminatum* after four days on Martin's medium amended as indicated. Concentrations of amendments were as follows: DMSO, 0.1% (v/v); D_2O, 30% (v/v); MBC, 1.0 µg/ml. The experiment was performed twice, with two replicates each time which gave similar results

significantly greater in MBC-treated than in D_2O + MBC-treated hyphae, and since hyphal growth rate was significantly lower after the former treatment than after the latter, then growth of the tip away from mitochondria cannot, *by itself,* account for the increased distance. If it could, one would expect a greater distance in hyphal tips which were growing faster. This was

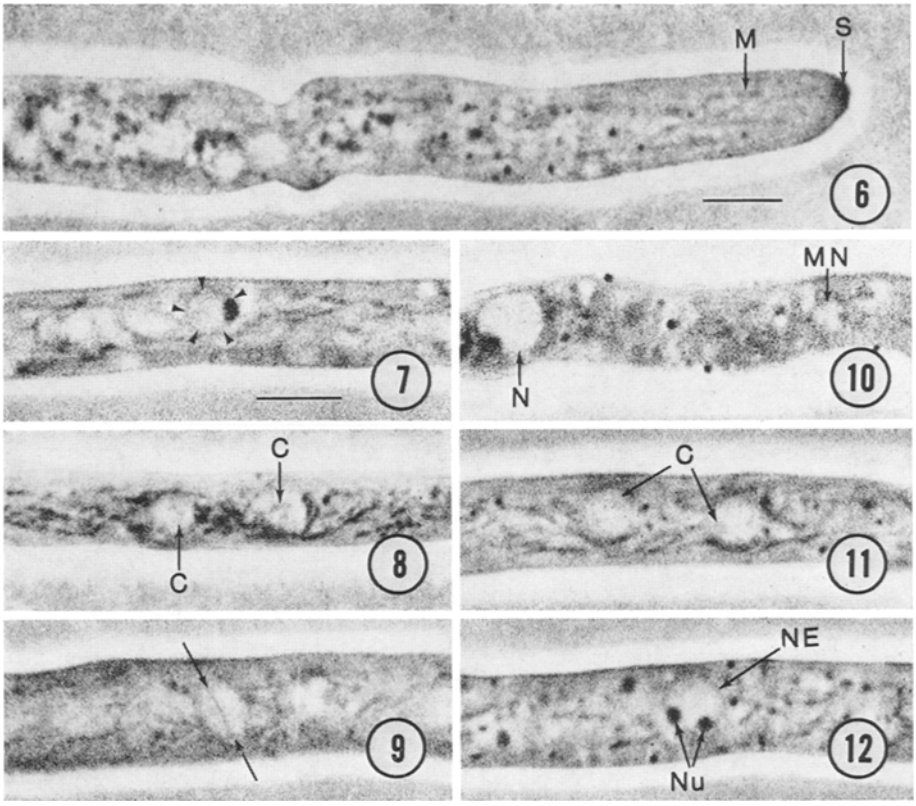

Fig. 6. The apical region of a living hyphal tip cell of *Fusarium acuminatum* 30 minutes after initiation of D_2O + MBC treatment. Mitochondria (*M*) were positioned close to the apex. The Spitzenkörper (*S*) was slightly smaller than in untreated cells. Phase contrast light optics. Scale bar = 5 μm

Figs 7–12. Nuclei in living hyphal tip cells of *F. acuminatum* as seen by phase contrast light optics. Scale bar = 5 μm. Figs. 7–9. Stages of the nuclear cycle in untreated cells. Fig. 7. The interphase nucleus (outlined by arrowheads), with a prominent nucleolus, typically had a density similar to that of the surrounding cytoplasm. Fig. 8. Metaphase nuclei lacked a nucleolus, contained a more or less central clump of condensed chromosomes (*C*), and had nucleoplasm of a lower density (*division density*) than the surrounding cytoplasm. Fig. 9. The spindle (between arrows) was prominent against the nucleoplasm of division density in the anaphase-telophase nucleus. Fig. 10. This micrograph was recorded 60 minutes after initiation of D_2O + MBC treatment. Mitosis in the large nucleus (*N*) was blocked at metaphase about 20 minutes after the treatment began. The nucleoplasm retained division density after reversion to an interphase state. Structures resembling micronuclei (*MN*), each containing a nucleolus-like body, were also observed. Fig. 11. Metaphase arrest of mitosis in an MBC-treated cell. Blocked nuclei lacked a nucleolus, had the usual division density and contained condensed chromosomes (*C*), but the chromosomes were not arranged in central masses as in untreated cells. Fig. 12. This nucleus, shown 40 minutes after MBC-induced metaphase arrest, reverted to interphase with two nucleoli (*Nu*). The prominent nuclear envelope (*NE*) surrounded a nucleoplasm of low density

not the case (compare MBC + DMSO- to MBC + D_2O + DMSO-treated cells in Figs. 2 and 4). Apparently mitochondria are shifted back, away from the apex. This effect, together with reduced growth rate and disappearance of "Spitzenkörper", was not dependent on mitotic arrest since most cells were affected before their nuclei entered prophase. Clearly, the site of action of MBC is not restricted to nuclei.

In the original description of the "Spitzenkörper", Brunswik (1924) considered it to participate in some way in apical extension. The position of the "Spitzenkörper" in the hyphal apex has been correlated with the subsequent direction of growth (Girbardt 1955 and 1957). Incorporation of N-[acetyl-^{3}H] glucosamine in *A. nidulans* occurred almost exclusively at the hyphal tip (Katz and Rosenberger 1971). These observations suggest that the "Spitzenkörper" is involved in apical extension. Our results support this concept in that decreased growth rates of hyphae treated with MBC were temporally correlated with loss of "Spitzenkörper" (Figs. 3 and 4). Since effects of MBC on both the above phenomena were reduced by D_2O (Figs. 3 and 4), normal growth rate and "Spitzenkörper" integrity might require fully functional microtubules.

Girbardt (1955, 1957, and 1969), Grove and Bracker (1970) and McClure *et al.* (1968) reported that apical extension was halted when the "Spitzenkörper" disappeared. In contrast, we found that some growth occurred in the apparent absence of a "Spitzenkörper" in *F. acuminatum* (Figs. 1, 3, and 4). Presumably, the apical vesicles were no longer concentrated enough to be visualized. Wall deposition in the absence of a visibly intact "Spitzenkörper" resulted in disoriented growth (Fig. 1); therefore, one role of the "Spitzenkörper" might be to orient cell wall-component deposition at the hyphal apex, as was suggested by Girbardt's (1955, 1957, and 1969) observations.

Microtubules might be involved in directed linear growth of *F. acuminatum*. Together with the implicit evidence above, this hypothesis is supported by extensive literature on the intracellular transport function of microtubules in a variety of eukaryotic cells (Rebhun 1972, Bryan 1974, Paulson and McClure 1975, Soifer 1975, Pipeleers *et al.* 1976). McClure, Park, and Robinson (1968) suggested that vesicles containing wall materials are formed in a basipetal area of the tip cell and then migrate to the apex and fuse with the plasmalemma. We suggest that cytoplasmic microtubules might play a role in the intracellular transport of such vesicles. Thus, effects of MBC on growth could be interpreted as resulting from disruption of microtubule-mediated transport of wall materials or "Spitzenkörper" integrity, or both. Increased branching as reported here, and the multiple emergence of highly branched germ tubes (*cf.*, Richmond 1975) are logical consequences of such disruptions, since apical vesicles would be prevented from accumulating at a single site.

It is likely that the capacity of MBC to block mitosis at metaphase resulted

from interference with microtubule formation, rather than from depolymerization of microtubules. The apparent MBC-insensitivity of [presumably microtubule-based (GIRBARDT 1968, AIST 1969, RAUDASKOSKI and KOLTIN 1973)] migration of daughter nuclei is consistent with this interpretation; already formed microtubules would still be present to function in nuclear migration. Accordingly, newly expanded tip regions would be devoid of cytoplasmic microtubules (or with reduced numbers) which would limit microtubule-mediated processes (*e.g.*, ordered growth).

Multimicronucleation, in organisms in which the nuclear envelope breaks down prior to metaphase, occurs as a result of multi-polar or apolar nuclear division (STARR 1963). Reconstitution of the nuclear envelope around each clump of chromatin following such division provides for atypical genome compartmentalization. Each karyomere may then contain one (more or less) chromosome (STARR). In filamentous *Ascomycetes*, like *Fusarium* spp., the nuclear envelope remains intact throughout division and directly constitutes the envelopes of daughter nuclei by collapsing around their chromatin (AIST and WILLIAMS 1972). The mechanism by which multimicronucleation occurred in *F. acuminatum* is unknown.

We have extended the list of antitubulins counteracted by D_2O to include MBC. Since it has been repeatedly shown that D_2O affects microtubules (INOUÉ and SATO 1967, SATO and BRYAN 1968, TILNEY and GIBBINS 1969, MARSLAND 1970, SATO and DANIELS 1971, MALAISSE-LAGAE *et al.* 1971, SATO 1975, KAZAMA 1975) and that D_2O counteracts antitubulins (see Introduction), we interpret our results as further evidence that MBC acts as an antitubulin in benomyl-sensitive fungi. The effects of D_2O and MBC on *F. acuminatum* are presently under study at the ultrastructural level to test this interpretation.

Acknowledgements

We thank Dr. RICHARD W. SMILEY for the use of his laboratory and EDWARD J. BRAUN for many hours of stimulating discussion. This work was supported by a grant-in-aid of research from Sigma Xi, The Scientific Research Society of North America, to R.J.H. and by CSRS grants No. 316-15-53 to J.R.A. and Dr. HERBERT W. ISRAEL and No. 216-15-24 to Dr. OLEN C. YODER.

References

AIST, J. R., 1969: The mitotic apparatus in fungi, *Ceratocystis fagacearum* and *Fusarium oxysporum*. J. Cell Biol. **40**, 120—135.

— and P. H. WILLIAMS, 1972: Ultrastructure and time course of mitosis in the fungus *Fusarium oxysporum*. J. Cell Biol. **55**, 368—389.

BOLLEN, G. J., and A. FUCHS, 1970: On the specificity of the *in vitro* and *in vivo* antifungal activity of benomyl. Neth. J. Plant Pathol. **76**, 299—312.

BRUNSWIK, H., 1924: Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*. In: Botanische Abhandlungen, Vol. 5 (GOEBEL, K., ed.). Jena: Gustav Fischer.

Bryan, J., 1974: Microtubules. Bio. Science **24**, 701—711.

Burgess, J., and D. H. Northcote, 1969: Action of colchicine and heavy water on the polymerization of microtubules in wheat root meristem. J. Cell Sci. **5**, 433—451.

Davidse, L. C., 1973: Antimitotic activity of methyl benzimidazol-2-ylcarbamate (MBC) in *Aspergillus nidulans*. Pestic. Biochem. Physiol. **3**, 317—325.

— 1975: Antimitotic activity of methyl benzimidazol-2-ylcarbamate in fungi and its binding to cellular protein. In: Microtubules and microtubule inhibitors (Borgers, M., and M. de Brabander, eds.), pp. 483—495. Amsterdam: North-Holland Publ. Co.

— and W. Flach, 1977: Differential binding of methyl benzimidazole-2-ylcarbamate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of *Aspergillus nidulans*. J. Cell Biol. **72**, 174—193.

de Brabander, M., R. van deVeire, F. Aerts, M. Borgers, and P. A. J. Janssen, 1976 a: The effects of methyl [5-(2-Thienylcarbonyl)-1H-benzimidazole-2-yl]carbamate, (R17934; NSC 238159), a new synthetic antitumoral drug interfering with microtubules, on mammalian cells cultured *in vitro*. Cancer Res. **36**, 905—916.

— — — S. Geuens, and J. Hoebeke, 1976 b: A new culture model facilitating rapid quantitative testing of mitotic spindle inhibition in mammalian cells. J. nat. Cancer Inst. **56**, 357—363.

Edgington, L. V., K. L. Khew, and G. L. Barron, 1971: Fungitoxic spectrum of benzimidazole compounds. Phytopathology **61**, 42—44.

Gillespie, E., R. Levine, and S. E. Malawista, 1968: Histamine release from rat peritoneal mast cells: inhibition by colchicine and potentiation by deuterium oxide. J. pharmacol. exp. Therap. **164**, 158—165.

Girbardt, M., 1955: Lebendbeobachtungen an *Polystictus versicolor* (L.). Flora **142**, 540—563.

— 1957: Der Spitzenkörper von *Polystictus versicolor* (L.). Planta **50**, 47—59.

— 1968: Ultrastructure and dynamics of the moving nucleus. In: Aspects of cell motility, Vol. 22 (Miller, P. L., ed.), pp. 249—259. Cambridge: Cambridge University Press.

— 1969: Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma **67**, 413—441.

Grove, S. N., and C. E. Bracker, 1970: Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. J. Bacteriol. **104**, 989—1009.

— — and D. J. Morré, 1970: An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. Amer. J. Bot. **57**, 245—266.

Hammerschlag, R. S., and H. D. Sisler, 1973: Benomyl and methyl-2-benzimidazole-carbamate (MBC): biochemical, cytological and chemical aspects of toxicity to *Ustilago maydis* and *Saccharomyces cerevesiae*. Pestic. Biochem. Physiol. **3**, 42—54.

Heath, I. B., 1975: The role of cytoplasmic microtubules in fungi. In: Proc. First Intersectional Congr. Int. Assoc. Microbiol. Soc. Vol. 2 (Hasegawa, T., ed.), pp. 92—101. Tokyo: Sci. Counsel Japan.

Hoebeke, J., and G. van Nijen, 1975: Quantitative turbidimetric assay for potency evaluation of colchicine-like drugs. Life Sci. **17**, 591—596.

Howard, R. J., and J. R. Aist, 1976: Cytological aspects of MBC-sensitivity in isolates of *Fusarium roseum* Acuminatum. Proc. amer. Phytopathol. Soc. **3**, abstr. 33.

Inoué, S., and H. Sato, 1967: Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. J. gen. Physiol. **50**, suppl., 259—288.

— — and M. Ascher, 1965: Counteraction of colcemid and heavy water on the organization of the mitotic spindle. Biol. Bull. **129**, 409—410.

Katz, D., and R. F. Rosenberger, 1971: Hyphal wall synthesis in *Aspergillus nidulans:* effect of protein synthesis inhibition and osmotic shock on chitin insertion and morphogenesis. J. Bacteriol. **108**, 184—190.

KAZAMA, F. Y., 1975: Cytoplasmic cleavage during zoosporogenesis in *Thraustochytrium* sp.: ultrastructure and the effects of colchicine and D_2O. J. Cell Sci. **17**, 155—170.

LAGUNOFF, D., and E. Y. CHI, 1976: Effect of colchicine on rat mast cells. J. Cell Biol. **71**, 182—195.

LOWE-JINDE, J., and A. M. ZIMMERMAN, 1969: Heavy water and hydrostatic pressure effects on *Tetrahymena pyriformis*. J. Protozool. **16**, 226—230.

MALAISSE, W. J., F. MALAISSE-LAGAE, E. VAN OBBERGHEN, G. SOMERS, G. DEVIS, M. RAVAZZOLA, and L. ORCI, 1975: Role of microtubules in the phasic pattern of insulin release. In: The biology of cytoplasmic microtubules. New York Acad. Sci., Ann., Vol. 253 (SOIFER, D., ed.), pp. 630—652. New York: New York Academy of Sciences.

MALAISSE-LAGAE, F., M. H. GREIDER, W. J. MALAISSE, and P. E. LACY, 1971: The stimulus-secretion coupling of glucose-induced insulin release. IV. The effect of vincristine and deuterium oxide on the microtubular system of the pancreatic β cell. J. Cell Biol. **49**, 530—535.

MARGULIS, L., 1973: Colchicine-sensitive microtubules. In: International review of cytology, Vol. 34 (BOURNE, G. H., and J. F. DANIELLI, eds.), pp. 333—361. New York: Academic Press.

MARSLAND, D., 1970: Pressure-temperature studies on the mechanism of cell division. In: High pressure effects on cellular processes (ZIMMERMAN, A. M., ed.), pp. 259—312. New York: Academic Press.

— and R. HECHT, 1968: Cell division: combined anti-mitotic effects of colchicine and heavy water on first cleavage in the eggs of *Arbacia punctulata*. Exp. Cell Res. **51**, 602—608.

— L. G. TILNEY, and M. HIRSHFIELD, 1971: Stabilizing effects of D_2O on the microtubular components and needle-like form of heliozoan axopods: a pressure-temperature analysis. J. Cell. Physiol. **77**, 187—194.

— and A. M. ZIMMERMAN, 1965: Structural stabilization of the mitotic apparatus by heavy water, in the cleaving eggs of *Arbacia punctulata*. Exp. Cell Res. **38**, 306—313.

MCCLURE, W. K., D. PARK, and P. M. ROBINSON, 1968: Apical organization in the somatic hyphae of fungi. J. gen. Microbiol. **50**, 177—182.

MOSKALEWSKI, S., J. THYBERG, and U. FRIBERG, 1976: *In vitro* influence of colchicine on the golgi complex in A- and B-cells of Guinea pig pancreatic islets. J. Ultrastruct. Res. **54**, 304—317.

PAULSON, J. C., and W. O. MCCLURE, 1975: Microtubules and axoplasmic transport. Inhibition of transport by podophyllotoxin: an interaction with microtubule protein. J. Cell Biol. **67**, 461—467.

PIPELEERS, D. G., M. A. PIPELEERS-MARICHAL, and D. M. KIPNIS, 1976: Microtubule assembly and the intracellular transport of secretory granules in pancreatic islets. Science **191**, 88—90.

RAUDASKOSKI, M., and Y. KOLTIN, 1973: Ultrastructural aspects of a mutant of *Schizophyllum commune* with continuous nuclear migration. J. Bacteriol. **116**, 981—988.

REBHUN, L. I., 1972: Polarized intracellular particle transport: saltatory movements and cytoplasmic streaming. In: International review of cytology, Vol. 32 (BOURNE, G. H., and J. F. DANIELLI, eds.), pp. 93—137. New York: Academic Press.

RICHMOND, D. V., 1975: Effects of toxicants on the morphology and fine structure of fungi. In: Advances in applied microbiology, Vol. 19 (PERLMAN, D., ed.), pp. 289—319. New York: Academic Press.

— and A. PHILLIPS, 1975: The effect of benomyl and carbendazim on mitosis in hyphae of *Botrytis cinerea* Pers. ex. Fr. and roots of *Allium cepa* L. Pestic. Biochem. Physiol. **5**, 367—379.

— and R. J. PRING, 1971: The effect of benomyl on the fine structure of *Botrytis fabae*. J. gen. Microbiol. **66**, 79—94.

RUBIN, R. W., and G. D. WEISS, 1975: Direct biochemical measurements of microtubule assembly and disassembly in Chinese hamster ovary cells. The effect of intercellular contact, cold, D_2O, and $N^6,O^{2'}$-dibutyryl cyclic adenosine monophosphate. J. Cell Biol. **64**, 42—53.

SATO, H., 1975: The mitotic spindle. In: Aging gametes (BLANDAU, R. J., ed.), pp. 19—49. Basel: S. Karger.

— and J. BRYAN, 1968: Kinetic analysis of association-dissociation reaction in the mitotic spindle. J. Cell Biol. **39**, 118 a.

— and G. DANIELS, 1971: Analysis of microtubule distribution in mitotic spindles. Amer. Zool. **11**, 684 (abstr.).

SCHNEPF, E., G. DEICHGRÄBER, and N. LJUBEŠIĆ, 1976: The effects of colchicine, ethionine, and deuterium oxide on microtubules in young *Sphagnum* leaflets. A quantitative study. Cytobiologie **13**, 341—353.

SEILER, J. P., 1975: Toxicology and genetic effects of benzimidazole compounds. Mutat. Res. **32**, 151—168.

SHETERLINE, P., J. G. SCHOFIELD, and F. MIRA-MOSER, 1977: The effect of secretotogues and 2-methylpentan-2,4-diol on the microtubule-tubulin equilibrium and the release of growth hormone from bovine anterior pituitary slices. Exp. Cell Res. **104**, 127—134.

SMILEY, R. W., and R. J. HOWARD, 1976: Tolerance to benzimidazole-derivative fungicides by *Fusarium roseum* on Kentucky bluegrass turf. Plant Dis. Reptr. **60**, 91—94.

SNYDER, J. A., and J. R. MCINTOSH, 1976: Biochemistry and physiology of microtubules. In: Annual review of biochemistry, Vol. 45 (SNELL, E. E., ed.), pp. 699—720. Palo Alto, California: Annual Reviews Inc.

SOIFER, D. (ed.), 1975: The biolody of cytoplasmic microtubules. New York Acad. Sci., Ann., Vol. 253. New York: New York Academy of Sciences.

STARR, T. J., 1963: Morphological and biochemical integrity of colchicine-induced psittacosis-infected, micronucleated cells. Tex. Rep. Biol. Med. **21**, 412—421.

STEEL, R. G. D., and J. H. TORRIE, 1960: Principles and procedures of statistics. New York: McGraw-Hill Book Company, Inc.

STYLES, J. A., and R. GARNER, 1974: Benzimidazole carbamate methyl ester-evaluation of its effects *in vivo* and *in vitro*. Mutat. Res. **26**, 177—187.

THOMSON, J. F., 1963: Biological effects of deuterium. In: International series of monographs on pure and applied biology, Vol. 19 (ALEXANDER, P., and Z. M. BACQ, eds.). London: Pergamon Press.

TILNEY, L. G., and J. R. GIBBINS, 1969: Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. II. An experimental analysis of their role in development and maintenance of cell shape. J. Cell Biol. **41**, 227—250.

TUITE, J., 1969: Plant pathological methods: fungi and bacteria. Minneapolis, Minnesota: Burgess Publ. Co.

WILSON, L., 1975 a: Microtubules as drug receptors: pharmacological properties of microtubule protien. In: The biology of cytoplasmic microtubules, New York Acad. Sci., Ann., Vol. 253 (SOIFER, D., ed.), pp. 213—231. New York: New York Academy of Sciences.

— 1975 b: Action of drugs on microtubules. Life Sci. **17**, 303—310.

Author's Address: RICHARD J. HOWARD, Department of Plant Pathology, Cornell University, Ithaca, NY 14853, U.S.A.