Action of Taxol on Mitosis: Modification of Microtubule Arrangements and Function of the Mitotic Spindle in *Haemanthus* Endosperm

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ABSTRACT We have studied the effect of taxol on mitosis in *Haemanthus* endosperm. Immuno-Gold Stain (IGS), a new immunocytochemical method (17), was used to visualize microtubules (MTs) in the light microscope. Observations on MT arrangements were correlated with studies in vivo. Chromosome movements are affected in all stages of mitosis which progresses over at least 10^4 range of taxol concentrations. The three most characteristic effects on MTs are: (*a*) enhancement of the lateral associations between MTs, seen especially during the reorganization of the polar region of the spindle, (*b*) promotion of MT assembly, leading to the formation of additional MTs in the spindle and MT arrays in the cytoplasm, and (c) an increase in MT stability, demonstrated in their increased cold resistance. In this report, the emphasis is on the primary, immediate effects, occurring in the first 30 min of taxol action.

Effects are detected after a few mins, are reversible, and are concentration/time dependent. The spindle and phragmoplast are remarkably modified due to the enhancement of lateral associations of MTs and the formation of abundant nonkinetochore and polar, asterlike MTs. The equatorial region of the interzone in anaphase may be entirely depleted of MTs, and the spindle may break perpendicular to the spindle axis. Mitosis is completed in these conditions, providing evidence for the motile autonomy of each half-spindle. Trailing chromosome arms in anaphase are often stretched and broken. Chromosome fragments are transported away from the polar regions, i.e., in the direction opposite to that expected (5, 6). This supplies the first direct evidence of pushing by elongating MTs in an anastral higher plant spindle. These observations draw attention to the relation between the lateral association of MT ends to assembly/disassembly and to the role of such an interaction in spindle function and organization.

Taxol, a low molecular weight microcyclic alkaloid, was isolated from the bark of the western yew (*Taxus brevifolia*) by Wani et al. in 1971 (68). It is a potent drug which lowers the critical concentration of tubulin subunits which are required for the polymerization of MTs and shortens the lag time for microtubule (MT) assembly in vitro (59). Taxol binds specifically and reversibly to polymerized tubulin, i.e., MTs, and affects the allosteric transition of the receptor from the dimeric to polymeric state (52). Taxol-treated MTs are stabilized through either a lowered rate of dissociation (52, 61, 65) or a decreased rate of steady-state tubulin flux (66). All work on taxol, except that of Wolniak et al. (69), has been done on animal cells, and the data on mitosis in progress are still fragmentary (14, 15, 16). In animal culture cells, taxol treatment results in the formation of abundant and unusually arranged MTs (2, 15, 16, 45, 60, 63). In such exponentially growing cultures, the cell cycle (in G_2) and/or mitosis is inhibited by taxol (1, 16, 58, 60). The short duration of mitosis and the small spindle make it difficult to study the initial action of taxol on MT spindle organization at the light microscopic level.

We have used *Haemanthus* endosperm, which has a spindle at least 2–4 times larger than most animal cells in tissue culture. The course of normal (control) mitosis in *Haemanthus* has been studied extensively both in vivo and with the electron microscope (EM) (reviewed in reference 6, see also reference 39). This large body of data made it easier to detect the more complex and newly discovered features of taxol's action, e.g., the increased lateral association of MT ends and the rearrange-

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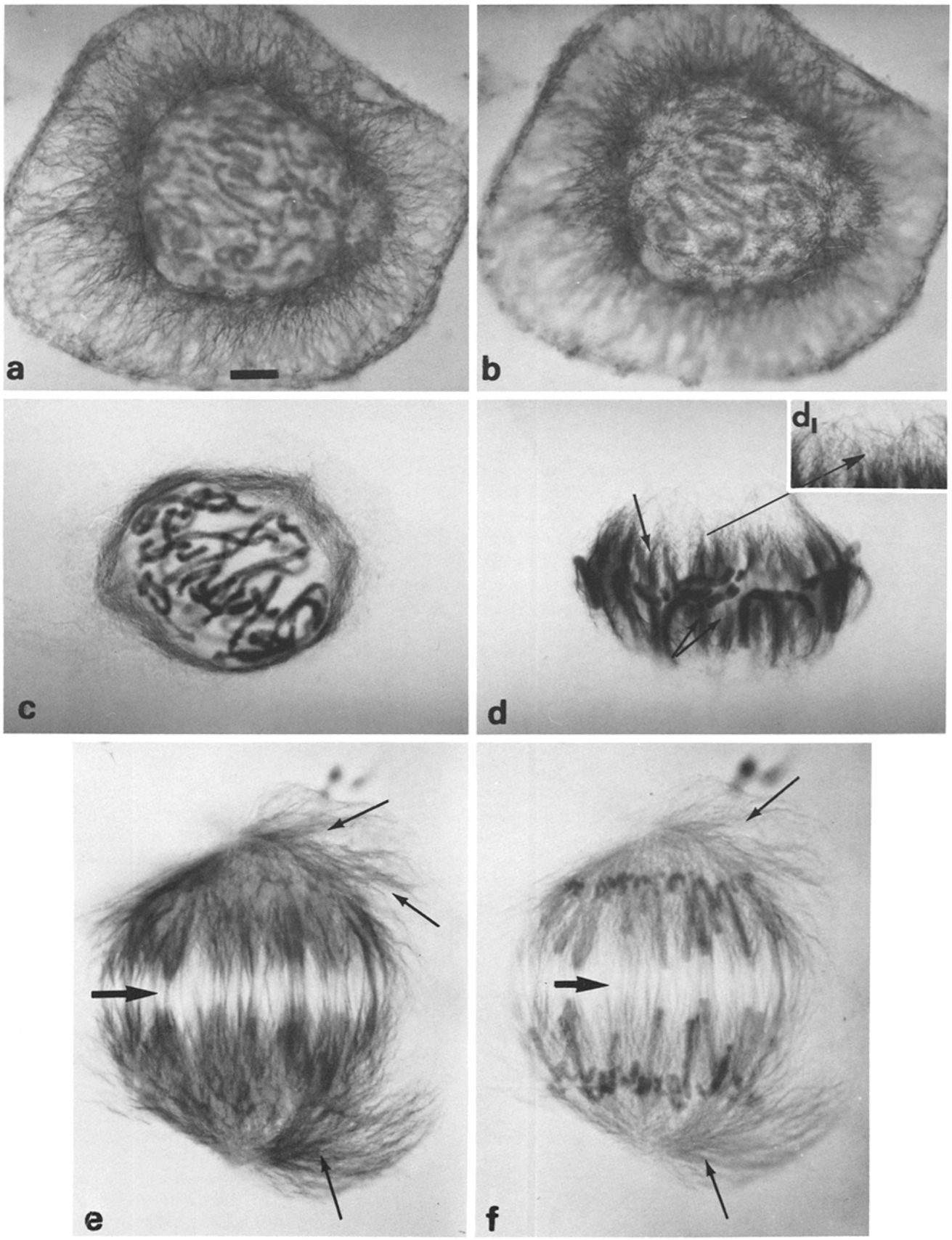


FIGURE 1

ment of MTs within kinetochore (chromosomal) fibers. Other results concern the pushing and breaking of chromosomes by assembling MTs, anchorage of chromosomal fibers, and the role of MT assembly in anaphase (42, 62). The data presented here offer an explanation for the formation of "star configurations" reported both in normal mitosis (4, 35, 38, 51) and as an effect of numerous drugs (19–21, 27, 51).

MATERIALS AND METHODS

Endosperm of *Haemanthus katherinae* Bak was used as a material. The technique for the studies in vivo has been described previously (10, 11, 49). (In the experiments reported here, the basic control medium was 4% glucose buffered with 0.05 M citric acid buffer to pH 5.1.) Cells in nonsterile preparations divide for at least 18 h at room temperature (21°–23°C), at which all experiments (except on low temperature) were performed.

The size of the spindle and chromosomes varies considerably both in the same and different ovules. Most of the spindles and phragmoplasts reproduced are of an average or large size. Spontaneous mitotic aberrations (bridges, fragments) in such untreated control cells are below 3%.

Taxol, obtained from the Department of Health, Education and Welfare, Public Health Service, National Cancer Institute, was dissolved at a concentration of 10^{-2} M in 100% dimethyl sulfoxide (DMSO) and stored at −20°C. This served as a stock solution. Working solutions, used for no longer than 3 d, were stored at 4°C. The concentration of DMSO in the medium ranged from 0.1% to 0.01%. The course of mitosis in media with 0.5% DMSO alone is undistinguishable from controls.

The following taxol concentrations were studied most extensively: 5×10^{-5}, 5×10^{-6}, 10^{-6}, 10^{-7}, and 10^{-8} M. In all concentrations, preparations were fixed 10 and 30 min after incubation in taxol solutions. For some concentrations, additional fixation was done after 3–5, 45, and 180 min. The results described here concern mostly effects which occur during the first 30 min of taxol action at a concentration 10^{-7} and higher. Studies on the longer effects (45 min to 3 h) which demonstrate that division progresses under these conditions and parallel EM studies will be published elsewhere (C. Jenson and J. Molè-Bajer. Manuscript in preparation.)

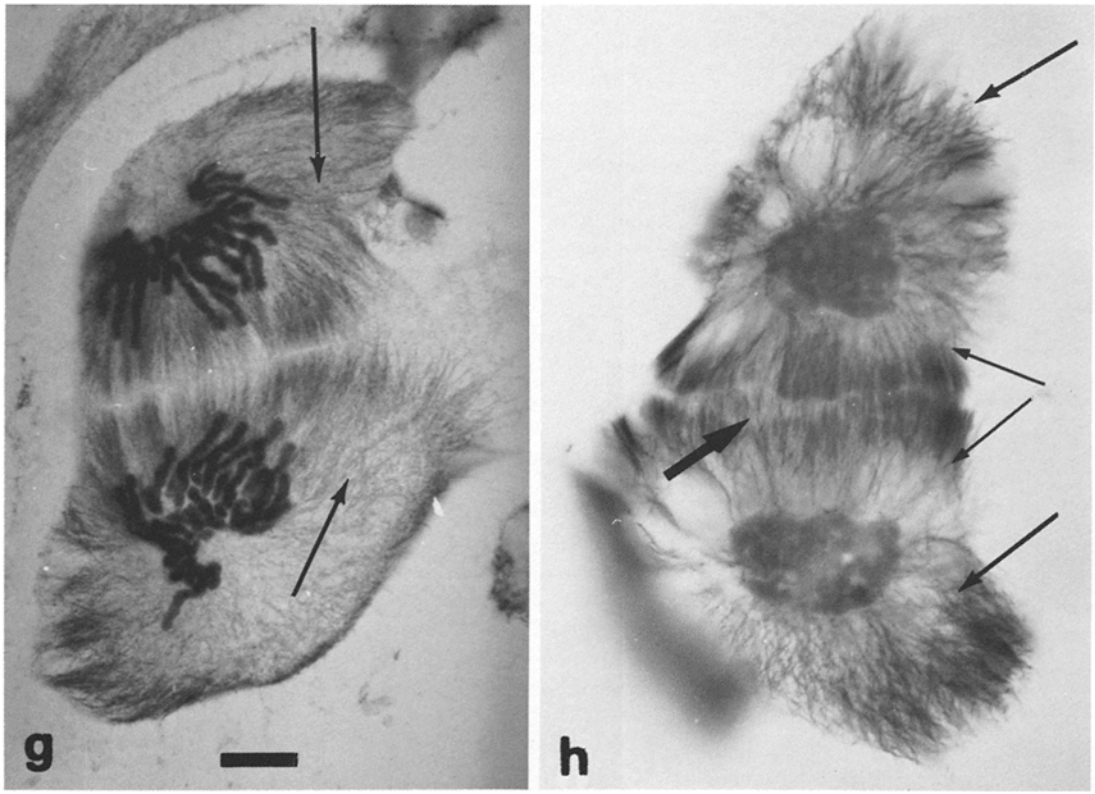


FIGURE 1 Normal mitosis in untreated cells. (*a* and *b*) Early prophase. The same cell at different optical sections. Early stages of the formation of a "clear zone." There is a denser accumulation of MTs around the nucleus than in the cytoplasm. They still form an irregular meshwork. (*c*) Prophase. "Clear zone" — A dense accumulation of MTs around the nucleus is more advanced. It is a rare example of a clear zone with three poles. Two poles on the right side of the cell are in a process of fusing. There are no MTs in the cytoplasm. (cf. Fig. 4 *d*). (*d*) Metaphase. Nonuniform distribution of MTs in this partly flattened cell permits the detection of individual kinetochore fibers (arrows) which intermingle with each other as well as with nonkinetochore MTs, especially at the polar regions. MTs also form an irregular meshwork on the surface of the spindle as shown on the inset (d_1). (Part *d* and the inset show the same cell with different filters: *d* with Y and d_1 with G-B). (*e* and *f*) Anaphase. Micrographs of the same cell with understained chromosomes taken with different filters: *e* with W-G and *f* with O. Well-developed half-spindles with an abundance of new polar MTs (arrows) which form a dome- or umbrella-like structure around the polar regions. "Polar MTs" are located predominantly at the surface of the spindle. Depletion of MTs in the interzone is characteristic of this stage (thick arrow). (*g*) Telophase in flattened cell. Newly formed polar MTs continue to grow in all directions but predominantly toward the equator (arrows). This leads to the formation of "asterlike" structures. Notice splaying of distal ends of polar MTs. (*h*) Late telophase. Chromosomes have already contracted, and form two sister nuclei. "Asterlike" structures (arrows) and the phragmoplast (thin arrows) are well developed. The cell plate, forming a ring, is not stained and appears as a white line (thick arrow). MTs of the "aster" are not laterally associated at their distal ends (splay: cf. Fig. 5 *b* and *e*) G-B filter. Resolving power is increased with narrow band filter in *e*–*f* and *g*. Bars, 10 μm. × 1,000.

Each experiment at a given concentration was repeated at least three times which corresponded to 150–500 cells in different mitotic stages. This supplied several thousand cells in which variations of the same general effects were analyzed.

Taxol solutions either were peryfused or preparations were incubated by floating in small Petri dishes filled with the desired solutions. The latter technique was used for studies on short-term taxol action (3–10 min) and is more effective than peryfusion. In experiments on recovery, cells were also peryfused one to four times with the basic medium without taxol.

To study the progress of mitosis in individual cells, areas with abundant mitoses were marked and the effects of taxol were monitored every 5–10 min. This permitted us to follow in vivo several cells simultaneously and allowed semiquantitative estimates of taxol action before fixation. Parallel detailed studies on single cells (16 mm and video time-lapse) will be presented separately (C. Cypher and J. Molè-Bajer. Manuscript in preparation: see also reference 1).

Experiments at low temperature were performed in a covered water-bath (Forma Scientific, Inc., Marietta, OH; Model 20 95), and cells were fixed with isothermal fixative.

IGS was performed as described (18), or with the following modifications: Immuno Gold GAR G 20 diluted 2 ×, reused up to 5 ×, and staining was done on ice. The following primary rabbit antitubulin antibodies, generous gifts from Drs. J. De Mey (Janssen Pharmaceutica, Beerse, Belgium), K. Fujiwara (Harvard University), and J. B. Olmsted (University of Rochester), respectively, were used: (*a*) antibody against dog brain tubulin (18), (*b*) antibody induced against vinblastine paracrystals isolated from sea urchin eggs (26), and (*c*) antibody against cilliary tubulin from *Tetrahymena* (67).

The IGS technique for *Haemanthus* does not allow us, due to loss of cells, to follow the same cell in vivo and after IGS processing. The number of mitotic cells remaining in our preparations varied between 50 and 900, with an average of about 200.

Micrographs were taken with a Zeiss Photoscope II equipped with a Plan-Apo objective, 63× N.A. 1.4, a Plan-Apo condenser, N.A. 1.4, and a 0.63× nosepiece. Kodak Photomicrography negative film 2415 was processed in Kodak HC 110 developer (formula B) for 4.5 min at 20°C. The visibility of chromosomes was suppressed by a narrow band interference filter (547 nm) in conjunction with a photographic filter, 82B (further abbreviated G-B), and MTs by a 576-nm filter (further abbreviated Y). Both interference filters had transmissions of ~80% (Baird Atomic, Cambridge, MA). The light source was HBO 100 high pressure mercury arc. Some micrographs were taken with a Zeiss wide band interference filter (550 nm) in conjunction with light conversion filter (abbreviated W-G) and Zeiss OG2 (abbreviated O).

RESULTS

MTs Arrangement in Control Cells

The arrangement of spindle MTs, which is visualized with IGS, and unknown features of *Haemanthus* spindle have been described previously (10, 18). Here, only the features of normal mitosis (Fig. 1) which are modified by taxol and are morphological criteria for an estimation of taxol's effects will be summarized.

The IGS method draws attention to the variability of response in endosperm cells. Large numbers of cells remain present on each preparation, and a permanent IGS label is an advantage in evaluating the results. It permits us both to establish the extremes of taxol action and to relate spindle MT organization (arrangement) to the degree of cell flattening in controls. The flattening of cells somewhat slows the course of mitosis but, unless it is very extensive, does not result in any abnormalities. Due to the large number of MTs (over 4,000) in each half-spindle (39, 40), MT arrangement is often obscured in unflattened cells. The following observations are based on flattened cells which often permit following the change of MT organization within a single chromosomal fiber.

Interphase

An elaborate system of MTs is present in all cells, either as a uniform or somewhat denser network of MTs radiating from the nucleus periphery or as a ring of shorter MTs adjacent to the plasmalemma. The latter is observed in cells which presumably have recently completed mitosis.

Prophase

The cytoplasmic MT network disappears concurrent with the formation of a denser microtubular zone (Fig. 1 *a* and *b*) around the nucleus (clear zone; see references 3 and 36). These MTs often form a spindle-shaped structure before the breaking of the nuclear envelope. A multipolar clear zone, composed of more than three poles (Fig. 1 *c*), is rare and, as a rule, transforms into a bipolar spindle (44). The breaking of the nuclear envelope lasts 1–2 min and is followed by a characteristic contraction stage (3). This stage marks the onset of prometaphase, and the whole chromosome group is often pushed to the center of the nucleus as clear zone MTs penetrate into the nuclear area.

Prometaphase/Metaphase

The cytoplasmic MT system disappears and, only rarely, remnant arrays of MTs are present. The shape of the spindle is related to the degree of flattening of the cell: the less flattened the cells, the more pronounced the spindle shape retained. In flattened cells which contain barrel-like spindles, the density of MTs at the poles, or diffused polar regions (Fig. 1 *d*), is low. MTs form also a more or less irregular meshwork, especially at the surface of the spindle (Fig. 1 d_1, *inset*). MTs within single kinetochore fibers are straight or arranged in gentle arcs, and the image is similar to that seen in a polarizing microscope (36). MTs of neighboring fibers intermingle with each other, especially at the polar regions.

The density of MTs and the degree of their lateral association decrease toward the poles, and the images are consistent with MT counts (40). Nonkinetochore MTs (continuous fibers) are often well distinguished at the equatorial plane. They fan out toward the pole and intermingle with neighboring kinetochore fibers. During the progress of prometaphase, chromosome arms tend to align themselves parallel to the spindle axis, following the general orientation of MTs.

Chromosomal fibers located centrally determine the long axis of the spindle. Those on the sides curve gently toward the center of the polar region. This arching, less pronounced in flattened cells, may lead to the formation of indistinct "subpoles." The "subpoles" are composed of a few chromosomal fibers and gradually fuse during anaphase. Multipolar divisions are rare (<0.3%).

Anaphase

The MT arrangement in early anaphase resembles the one in metaphase. However, the divergence and intermingling of individual chromosomal fibers increases during the progress of anaphase; gradually, their individuality is retained only in the proximity of kinetochores. Due to these pronounced changes in orientation, the IGS image of the half-spindle increasingly differs from that obtained in polarizing microscope (36).

From mid-anaphase on, the interzone is gradually depleted of MTs. Simultaneously, new MTs appear between the trailing chromosome arms (10, 18). These new MTs, which also form at the edges of each half-spindle, give them a "bushy" appearance; new MTs radiate from the poles outward toward the cell periphery (Fig. 1 *e* and *f*). It has been proposed that these new MTs anchor each half-spindle during the latter part of anaphase, assuring their independent motile autonomy (10).

Spontaneous aberrations such as dicentric bridges are rare (<3%) but can be easily produced, e.g., by ionizing radiation (6). Such bridges become progressively thinner at the equator, and often break.

Telophase

The onset of telophase is marked by the contraction of the chromosomes, most easily observed in the long trailing arms. MTs radiating from the remnants of half-spindles, i.e., from the polar regions, continue to grow in all directions, but predominantly toward the equator (Fig. 1 *g*). This leads to the formation of "asterlike" and "double domelike" structures instrumental in the development of phragmoplasts and phragmosomes (46 and Fig. 1 *h*). MTs radiating toward the cell periphery become gradually less dense (disassemble) close to the nucleus, with simultaneous accumulation of MTs at the cell periphery. These short MTs, still pointing toward the nucleus, may form a ring around the cell border. Thus, the cytoplasmic MT network formed in telophase (Fig. 1 *h*) differs somewhat from that in interphase cells.

It should be stressed that MTs forming "double-domes" form predominantly outside the spindle. These MTs grow toward the equator where they interdigitate ("double-dome" fuse) and form a ring-shaped phragmoplast and cell plate. The latter usually does not intersect completely the whole cell (10, 18). This supports the notion (10) that interzonal MTs contribute little, at least in the initial stages, to the formation of phragmoplasts and cell plates. Due to the density and perhaps coating of the cell plates, MTs within remain unstained (Fig. 1 *g* and *h*; cf. also references 10, 18).

EFFECTS OF TAXOL

General Effects

The initial effects of taxol on spindle organization are detected in many cells within 3 to 5 min at concentrations of 5 $\times$ 10^{-5}–10^{-6} M and in about 10 min at 10^{-7}–10^{-8} M. Longer exposures (30 min) and higher concentrations often produce very similar effects. Dose/time dependence is clearest at lower concentrations (10^{-7}–10^{-8} M). The rapid action of taxol, combined with long-lasting mitosis in *Haemanthus*, where control anaphase lasts 25–40 min at 21°–23°C, permits one to follow the effects of taxol on any stage and distinguish between the "primary" direct effects on spindle structure and the "secondary" or prolonged ones. In the latter, the effects of taxol are somewhat reversed, especially at lower concentrations, and a spindle assumes a more normal appearance. Endosperm cells show, therefore, taxol adaptation, and mitosis progresses even through several disturbances. Only primary effects will be discussed here.

The following effects of taxol on *Haemanthus* endosperm can be noticed even with little familiarity with the material; some are stage dependent: (*a*) An increase of packing between MTs reflected in density of IGS stain and interpreted as enhancement of lateral associations, especially at their distal ends. This leads to the rapid disappearance of MT splaying within MT arrays and is detected in all stages of mitosis. (*b*) A change in the shape of the spindle poles which is seen from prometaphase through mid-anaphase. The shape of the chromosomal fibers changes, and they may bend and curve. (*c*) Formation of additional arrays of MTs in the cytoplasm often observed in all stages of mitosis. (*d*) Multipolar clear zones in prophase found occasionally with as many as six poles. (*e*) The equal length of polar MTs observed most commonly in telophase. (*f*) Disruption of spindle integrity due to local depletion of MTs. This is seen as "holes" in the half-spindle and as "hinging" effects combined with an absence of MTs at least in part of the interzone. (*g*) Excessive stretching of trailing chromosome arms and the formation of bridges and fragments in anaphase-telophase.

These initial "shock"-type effects which change the microtubular organization of the spindle appear within 10 min or less and persist for at least 30 min at concentrations of 10^{-4} (saturated solution) to 10^{-7} M.

Interphase

Cells with a single nucleus have a more pronounced MT network but generally with a normal pattern of distribution.

Striking effects are occasionally seen in multinucleated cells at high concentrations (5 $\times$ 10^{-6} M and higher). In such syncytia, containing both prophase and interphase cells (Fig. 2 b), MTs form multiple converging centers reminiscent of short fir trees. They are scattered throughout the cytoplasm and radiate from the periphery of the nuclei. Such a distribution of MTs is never observed in controls. However, it does resemble MT arrangements in some multinucleate untreated cells at low temperature, as well as those formed at the very beginning of recovery from cold treatment (Fig. 2 *a*).

Prophase and Breaking of the Nuclear Envelope

At concentrations of 10^{-5} M and higher (10–30 min of action), a spindle-shaped clear zone (3) is often no longer observed; instead, interconnected bundles of MTs appear in the cytoplasm. MTs within these arrays are often bent and parallel (Fig. 3 *a*, *b*, and *c*). A clear zone similar in appearance to that in controls was observed, however, at concentrations of 10^{-7} to 10^{-8} M in cells treated for as long as 1 h (Fig. 3 *d*). The initial 30-min period of taxol action discussed here is too short to follow further transformation of the clear zone into the spindle, and this aspect of taxol action will be discussed elsewhere.

Late Prometaphase and Metaphase

The effects of taxol on early prometaphase are intermediate between those on prophase and late prometaphase.

During late prometaphase and metaphase at concentrations lower than 10^{-6} M, the reorganization of the spindle proceeds slower than at higher concentrations (e.g., 10^{-5} M) and therefore can be followed step by step. The first observed effect is an increase of packing (see p. 5) (associations between) of MTs within individual chromosomal fibers. MTs become more parallel and closely associated. This initial effect is very short (<5 min) at concentrations 10^{-6} M and higher but is very clear after 10 min or less taxol action at 10^{-7} M. It is accompanied by a sideways tilting of entire chromosomal fibers, especially pronounced at the edges of the spindle, toward the center of the polar region (cf. p. 5 - control) where occasionally a short protruding pole remains. Consequently, a barrel-shaped spindle is transformed into a shorter and flat structure. MTs at the polar regions are predominantly arranged perpendicular to the spindle axis and the spindle becomes often more wide than long. Due to the bending of the chromosomal fibers toward the center of the polar region, spindle fibers are at different focal levels, even in flattened cells (Fig. 4 *a*). During this rearrangement of MTs, some regions of the spindle become depleted of MTs and the spindle may seem to have "holes" (Fig. 4 *a*). The density of chromosomal fibers increases considerably, and they become often much thicker than in controls (Fig. 4 *a*).

Another effect, especially at concentration 10^{-6} M and

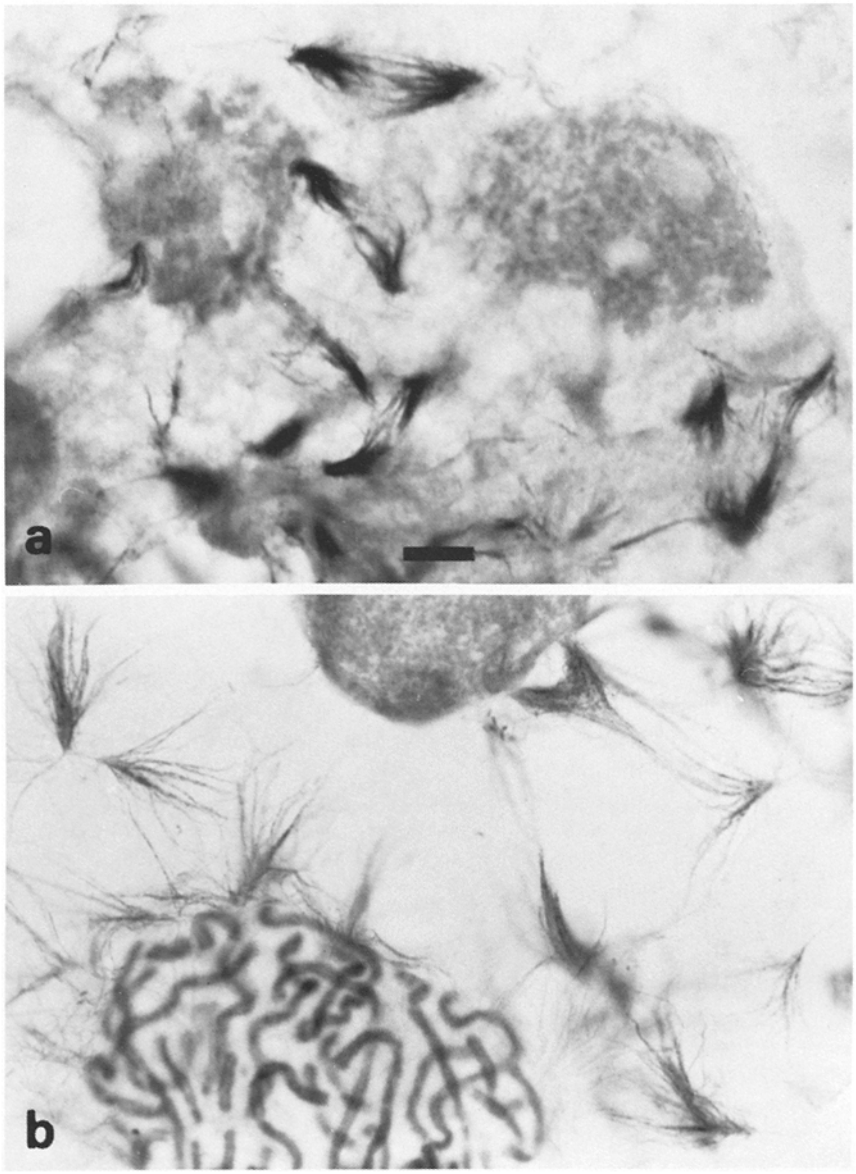


FIGURE 2 "Explosive" formation of MTs. (*a*) Multiple MT nucleation centers in the cytoplasm containing interphase and late telophase nuclei. Cells were exposed to 4°C for 3 h followed by 5-min recovery at room temperature. (*b*) The formation of similar structures is stimulated by taxol treatment (10^{-5} M for 35 min) in the cytoplasm containing interphase and prophase nuclei. G-B Filter. Bar, 10 μm. × 1,000.

higher, is the formation of irregular MT arrays in the cytoplasm during prophase-metaphase. Such MT arrays are usually not pronounced but are rarely observed in controls after breaking of the nuclear envelope.

Anaphase

The most characteristic features in early and mid-anaphase are: a change of MT arrangement in the half-spindles (Fig. 4*b* and *c*), the formation of dicentric bridges (Fig. 4*d*), and the breaking of the whole spindle (Fig. 4*c*, *d*, and *f*). "Hinging" of the half-spindles followed by a tendency to form "star configurations"[1] (Figs. 4*e*, *f*) is often observed in late anaphase.

In early anaphase the spindle often splits parallel to the long axis. This leads to the formation of several "subpoles" each containing a few chromosomes, and larger regions than in metaphase may be depleted of MTs. The splitting of the spindle is most pronounced at higher concentrations (10^{-5}–5×10^{-6} M for 10 min) and is seldom seen at 10^{-8} M. It should be stressed that "subpoles" are still connected at the polar regions (Fig. 4*b*) and, in spite of this highly abnormal morphology, multipolar anaphases have not been observed.

Large regions of interzone, often at the edges of the spindle, may become completely devoid of MTs. If chromosomal bridges retarding the migration of kinetochores are formed and kinetochores at the edges continue migration to the poles, then the two sister chromosome groups "hinge" (Fig. 4*e* and *f*), i.e., execute half-circular movements resulting in the formation of star configuration in late anaphase and telophase.

In early and mid-anaphase when trailing chromosome arms still interdigitate, some chromosome arms bend sharply at the equatorial plane. Consequently, segments of arms which normally would be parallel to the axis of the spindle become somewhat curved and are not straight as they are in control cells. Their distal segments at the equator may bend even farther and formation of bridges may follow (see next section).

[1] A star configuration is an arrangement in which the chromosomes form a circle with kinetochores pointing to the center, as in a monopolar spindle (4, 9). During this process, some anaphase chromosomes swing around the pole and may finally face and migrate to the pole from a direction differing by 180° from that at the beginning of anaphase (Fig. 5*a*). The hinging effect is a normal process observed in several types of normal mitoses (4, 36, 38) including *Haemanthus* endosperm, which is greatly amplified by taxol.

As this occurs within the first 10–20 min of taxol's action at concentrations of 10^{-7} M and higher, such arrangements correspond, in time, to the period when chromosomes move backwards as shown in observations in vivo (11 and C. Cypher and J. Molè-Bajer. Manuscript in preparation.). Another occasionally observed effect within 10 min at concentrations up to 10^{-6} M is a pronounced desynchronization of mid-anaphase discussed previously (11).

Late Anaphase-Telophase

In late anaphase, i.e., in the stage when trailing chromosome arms in the interzone are straight, most of these trailing arms,

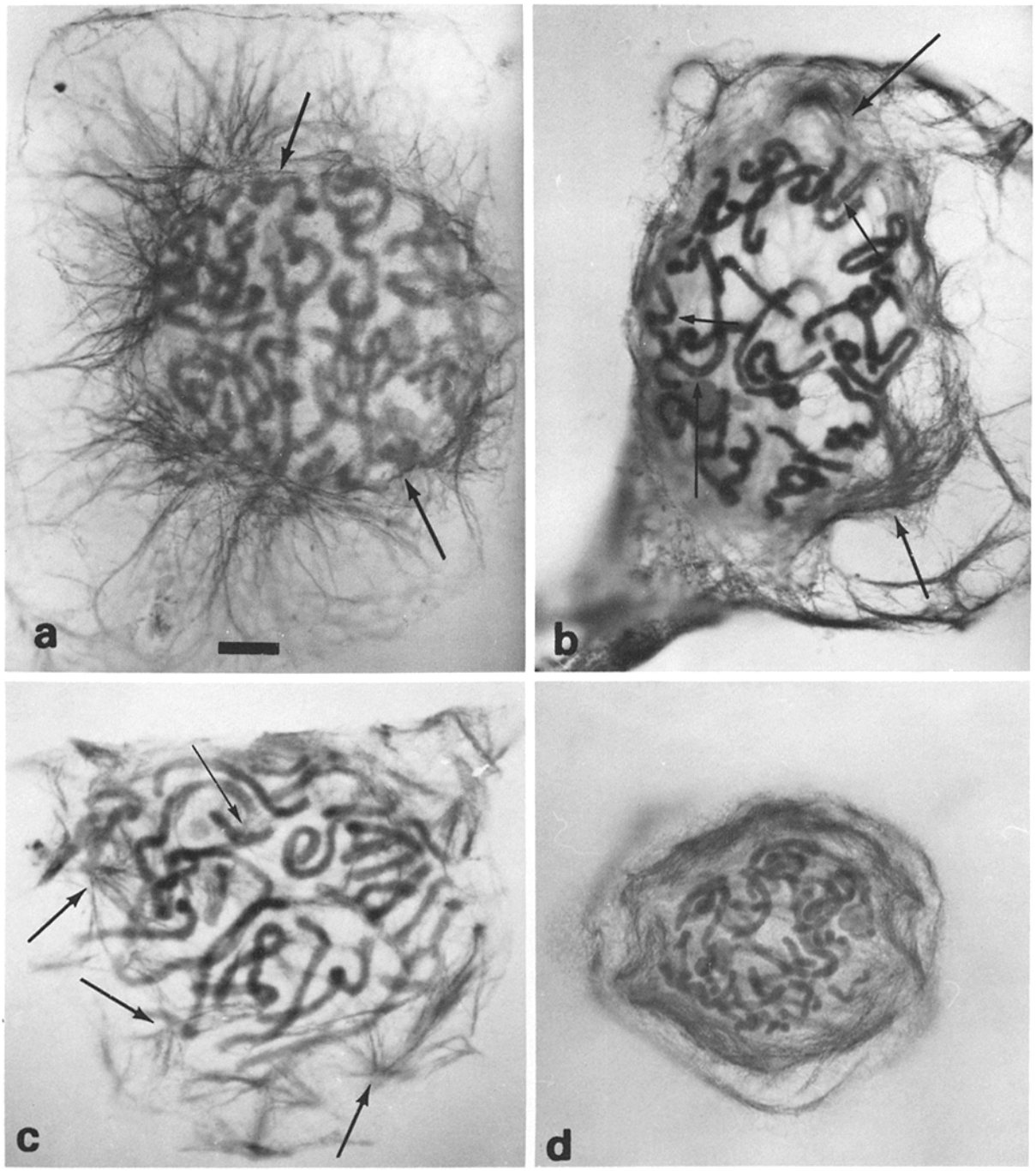


FIGURE 3 Primary effects of taxol at room temperature. (*a*) Late prophase. This stage corresponds to the late prophase in control cells. The nuclear envelope is marked by arrows. A clear zone is not formed. Multiple MTs array concentrated around the nucleus remind one of the beginning of clear zone formation in control cells at a much earlier stage (compare Fig. 1 *a* and *b*). Action of 5 × 10^{-6} M taxol for 20 min. (*b*) Onset of prometaphase. This stage corresponds to the late prophase in control cells, but with an already broken nuclear envelope. Action of 5 × 10^{-6} M taxol for 20 min. Interconnected arrays of MTs do not form a clear zone and are persistently laterally associated (arrows). No kinetochore MTs are detected at the kinetochores (thin arrows). (*c*) Early prometaphase. Action of 5 × 10^{-6} M taxol for 20 min. Multiple nucleating centers of MTs are present both in the nuclear area (arrows) and in the cytoplasm. Some kinetochores (thin arrows) are associated with MTs. Photograph taken with O filter. (*d*) Late prophase at 10^{-7} M concentration of taxol for 20 min. Clear zone is similar in appearance to that in the control cells but contains at least four poles (compare Fig. 1 *d*). Such a multipolar clear zone was never observed in controls. Bar, 10 μm. × 1,000.

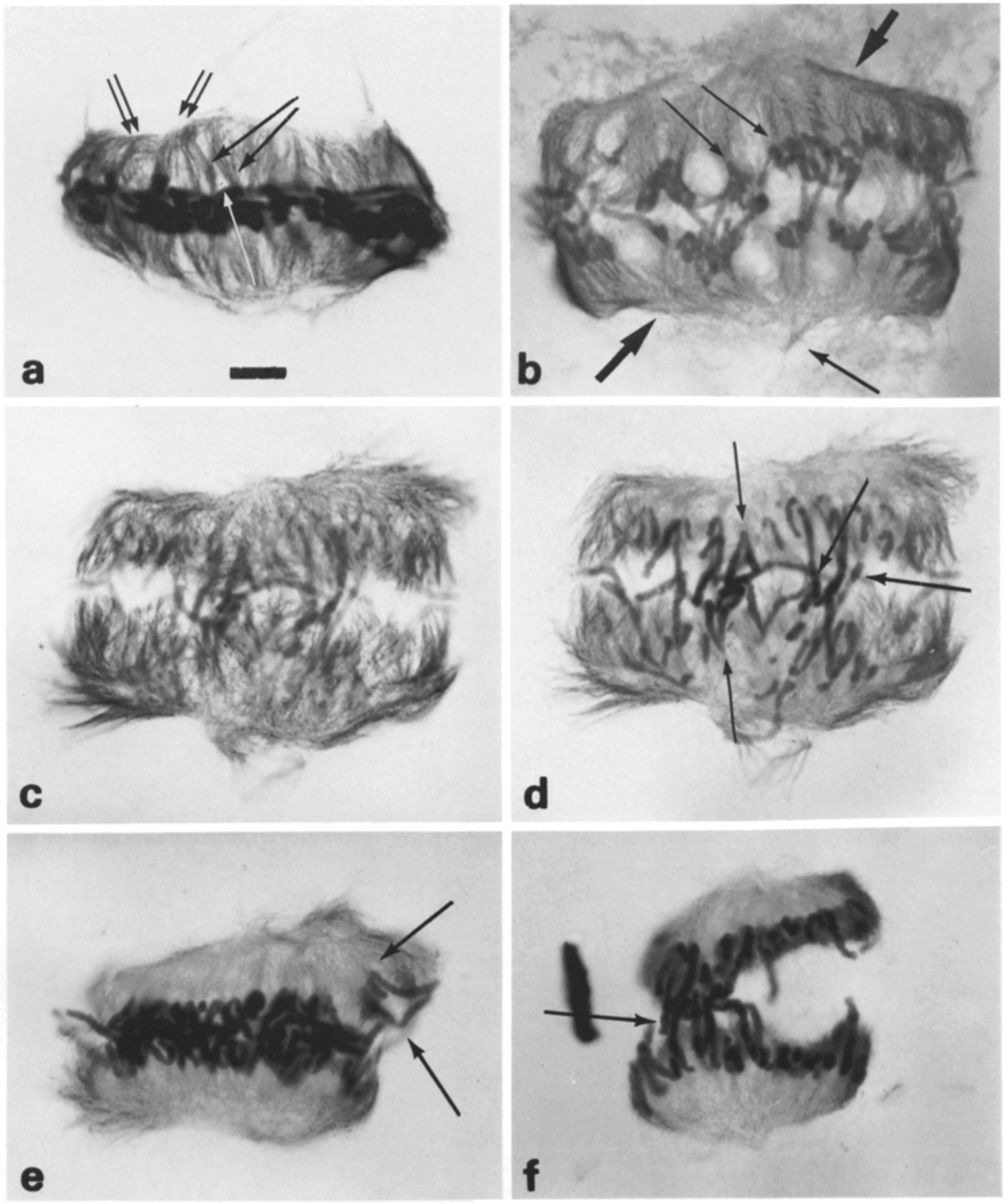


FIGURE 4 Primary effects of taxol at room temperature. (*a*) Metaphase. Action of 10^{-5} M taxol for 5 min. Flattened cell (cf. Fig. 1 *d*). The density of MTs seems to be increased, and the reorganization of the polar regions results in the formation of a "MT cage" enclosing the spindle. Kinetochore fibers are present (arrows) although not so distinctly as in control cells. MTs tilt and laterally associate at the polar regions (thin double arrows). Chromosome arms also tilt and are arranged parallel to the equatorial plane. Regions depleted of MTs—"holes"—marked with thin arrows. (*b*) Anaphase. Action of 10^{-4} M (saturated solution) of taxol for 35 min. Modified spindle similar to the one in (*c*). The half-spindles assume a characteristic flat shape with a protruding pole (arrow) and a pronounced lateral association of MTs in the polar region (thick arrow). Chromosome movements are desynchronized, and some regions of the spindle are deprived of MTs, leading to partial disruption of the spindle. Some kinetochore fibers are marked by thin arrows. (*c* and *d*) Mid-anaphase. 10^{-5} M taxol for 5 min. The density of MTs in both half-spindles is increased. MTs in the interzone are depleted and in some regions they are absent. Two half-spindles form nearly separate entities characterized by abundant polar MTs which give the spindle a "bushy" appearance. The spindle is flat with somewhat protruding poles. Desynchronization of chromosome movements is pronounced and some kinetochore regions (thin arrows) and chromosome arms (arrows) are stretched. (*e* and *f*) Anaphase. (*e*) Early stages of development of the "hinging effect." Anaphase movement of the majority of the chromosomes is retarded while chromosomes on the right edge of the spindle (arrows) continue to migrate. This leads to the "tearing apart" of part of the interzone. The more advanced stage is seen on *f*. Action of 10^{-5} M taxol for 5 min. (*f*) "Hinging effect" which disrupts mid-anaphase spindle. Both half-spindles are kept together by remnant connections at the left side of the interzone (arrow). Action of 10^{-4} M taxol (saturated solution) for 35 min. The early stage of the formation of a "star" (lower half-spindle), more advanced in the upper one of the cell shown in Fig. 6 *a* (action of 10^{-5} M taxol for 5 min) and less in (*b*). Micrographs *a*–*c* taken with G-B and all others with Y filter. Bar, 10 μm. × 1,000.

or their distal ends only, are stretched and bridges are often formed. Distal segments of stretched arms often become arranged parallel to the equatorial plane (Fig. 5 *a*, *b*, and *c*) and are then invariably caught by the cell plate. Therefore, most bridges are not of dicentric type. Chromatid fusion and dicentric bridges are frequent if taxol action begins in early anaphase when the distal portions of sister chromatids are still not completely separated. These bridges are detected, however, only in mid- or late anaphase. Fusion may also occur in telophase during the formation of the cell plate. In several of the included micrographs, chromosome ends are close to each other but are not fused.

Chromosome contraction marking the beginning of telophase (Fig. 5 *c* and *d*) contributes to excessive stretching and often the breaking of chromosome arms. Fragments are seldom transported in the polar direction as expected (6) and, instead, are caught by the cell plate; those located at the edges of the spindle may be transported away from the spindle pole (Fig. 5 *d*), resembling the behavior of exceptional acentric fragments (5).

In telophase, polar MTs form abundantly and are of remarkably uniform length (Fig. 5 *b* and *c*). The ends of MTs distal from the chromosome groups do not splay as in controls but converge gently and associate laterally. This is one of the most characteristic features of taxol action which gradually disappears during recovery (see p. 5). At very low concentrations of taxol (10^{-8}–10^{10}), this feature is not very pronounced although indications of such a trend exist (Fig. 5*f*).

The formation of the cell plate is often delated, although lateral movements (6) which mark the onset of phragmoplast formation seem to be accelerated. The effects of taxol on late telophase when the cell plate is already advanced are not pronounced and, in many cases, the cells resemble controls.

Effects of Taxol at Low Temperatures

Two types of experiments were performed: (*a*) taxol-treated cells were subjected to low temperatures, and (*b*) cells which had been kept at a low temperatures for a certain period of time were treated with taxol at the same temperature.

Low temperature (4°–6°C) arrests mitosis in control cells and disrupts the mitotic spindle (10, 43). Taxol treatment (10^{-5} M and 5×10^{-6} M) during exposure to low temperature restores a normal appearance of the spindle even in some cells exposed to 0°C for 30–60 min and in over 90% of cells kept for 3–4 h at 6°C (Fig. 6 *a* and *b*). Similarly, cells which are exposed to taxol (10^{-5} M and 5×10^{-6} M for 10–20 min) have a normal appearing spindle when exposed to low temperature (4°) for 30 min. Our technique does not permit us to state, however, whether chromosomes move in these conditions.

Recovery

The action of taxol is reversible, although the extent of recovery is variable and depends upon the concentration of taxol, the duration of its action, and the way the cells are treated, i.e., peryfused vs. floated on a large volume of basic medium. Cells recovering from higher concentrations, such as 5×10^{-5} M (for 10 min), after 2–4 peryfusions resemble cells exposed to a lower concentration (10^{-7} M for 10 min) of taxol. The degree of recovery is easiest to estimate in anaphase where the absence of taxol-induced bridges points to cell normality. Another marker is a gradual decrease of lateral associations between the ends of MTs (Fig. 7 *a*); MTs gradually splay and are again of different lengths (Fig. 7 *b*). Recovery also resembles taxol adaptation characteristics for the long action of this drug. These will be discussed elsewhere (C. Cypher and J. Molè-Bajer. Manuscript in preparation.)

DISCUSSION

Taxol in an exceptionally wide range of concentrations (10^{-4}[2]–10^{-8} M) as compared to other inhibitors (6) prolongs, but does not block, mitosis in *Haemanthus* endosperm. Mitosis in progress is completed with a remarkably modified spindle, although with many disturbances. The effect of taxol is detected in some cells even at nanomolar range after 30 min of action. Therefore, concentrations higher by 10^3 or more produce similar effects that seem to be less time/concentration dependent. Such dependence is evident, however, during recovery, especially at lower concentrations (below 10^{-6} M). However, as our observations are made only during the course of mitosis, it is not known whether mitosis is finally stopped, as reported for PtK_2 cells (15, 16).

The effect of taxol on endosperm is consistent with data in vitro (1, 2, 45, 52, 59, 63, 65) and expected on the basis of MT properties (20, 24, 25, 28, 54). We believe that our experiments provide evidence for two major effects of taxol: enhancement of lateral associations between MTs and a rapid shift of MT-tubulin equilibrium toward polymerization. This shift is comparable to the "explosive" assembly of MTs occurring during the initial stages of recovery from cold treatment (42). After this rapid shift in taxol-treated endosperm cells, the spindle gradually recovers (taxol adaptation—C. Cypher and J. Molè-Bajer. Manuscript in preparation.) and mitosis progresses in cells with a largely depleted tubulin pool in the cytoplasm. Thus the conditions are reversed but comparable to those reported by Stephens (64) for sea urchin eggs.

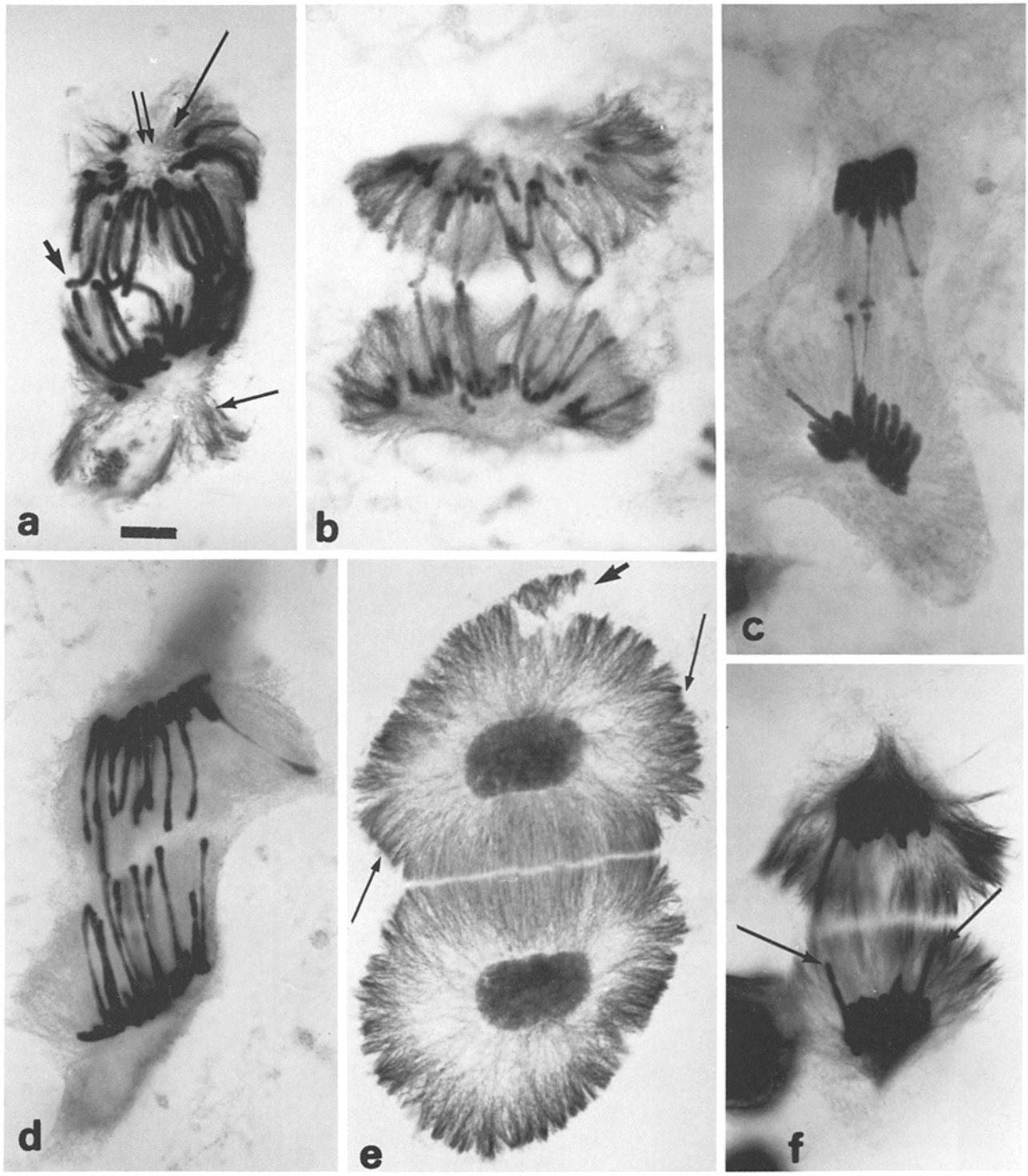
MT Nucleation and Elongation

Changes of the equilibrium between tubulin and MTs toward polymerization have been reported in the spindle as a result of D_2O (37, 55–57) and glycols (50, 53). The action of glycols on *Haemanthus* endosperm is complex although somewhat similar to that of taxol (reference 50 and J. Molè-Bajer, unpublished observations). The action of D_2O on oocytes of *Pectinaria gouldi* is reversible and results in an eightfold increase of the spindle volume (37). The spindle, however, usually retains its normal shape. D_2O causes an elevation in the concentration of polymerizable tubulin which results in both the nucleation and the elongation of MTs (57).

Taxol action on *Haemanthus* presents a somewhat different picture. Additional arrays of MTs, which are seldom found in the cytoplasm after the onset of prometaphase in controls, are common in taxol-treated cells. This indicates that, in controls, either most of the tubulin is incorporated into the spindle MTs or tubulin in the cytoplasm is not able to polymerize; we consider the latter possibility unlikely. Our data suggest that the conditions for MT nucleation and/or the properties of tubulin change during the transition from interphase to mitosis. Heideman and Gallas (29) came to the same conclusion after injecting taxol into the eggs of *Xenopus laevis*. Assembly of MTs was promoted only in unfertilized eggs, while the abundant tubulin in mature oocytes was unable to polymerize.

Multiple new nucleating centers in *Haemanthus* endosperm,

[2] Taxol does not dissolve at 10^{-4} M. This concentration was used to study the effect of saturated solution. The actual concentration is between 10^{-4} and 5×10^{-5} M.


a
b
c
d
e
f

have the shape characteristic for plant cells (28). Their appearance, formation of additional MT arrays in the cytoplasm of prometaphase-metaphase cells, and increased resistance of MTs to low temperature in taxol-treated cells all point to an abrupt decrease of the critical concentration of free tubulin needed for polymerization of MTs. We believe that this increase in MT number[3] occurs not only at low but also room temperature—but is obscured by enhancement of the lateral interaction between MTs which results in rapid tilt of chromosomal fibers. Consequently, the spindle shortens and does not increase in size. Such rearrangement is also detected with the polarizing microscope (C. Cypher and J. Molè-Bajer. Manuscript in preparation.), but due to the variability of the angle at which MTs tilt no conclusions concerning their arrangement could be made. Thus, our data are at variance with those reported by Wolniak et al. (69). Our attempts to measure the change of the length of chromosomal fibers at concentrations 10^{-7} M and higher are not conclusive. At lower concentrations (10^{-8}–10^{-9} M), not analyzed here in detail (see Fig. 5*f*), the spindle may also elongate.

Cold Resistance

MTs in taxol-treated endosperm cells are more resistant to low temperature than in untreated ones. Low temperature, as judged from IGS stain, either completely disassembles MTs or leaves a few long, scattered ones, as predicted from studies in vitro (24, 25); mitotic cells treated with taxol either retain (if they are exposed to the low temperature in taxol solution) or regain (if taxol is added after they have been exposed to the low temperature in the basic medium) most of their original MT network. MT organization of the spindle corresponds to the minimum spindle (10) which may support chromosome movements. We do not know at present whether mitosis in taxol-treated cells progresses at temperatures which normally arrest chromosome movements (43). The ability of taxol to prevent the disassembly of MTs in *Haemanthus* and to restore nearly normal spindle organization at low temperature (0°–4°C) draws attention to the close similarity of the effects of taxol in vivo and in vitro (24, 25, 65).

Lateral Association, Elongation, and MT "Pushing"

The primary change in spindle shape involves the reorganization of the spindle pole due to the tilt of spindle fibers attributed to the increased lateral association (interaction) between MTs. This is the first detectable effect of taxol. It leads to the formation of a dense microtubular "cage" around the spindle. The presence of such a "cage," as well as the polarity of MT growth (12, 13, 23, 30, 31, 47, 48), may prevent the elongation of MTs away from the pole toward the cell periphery. Regardless of the mechanism, the spindle does not increase in size and therefore new MTs must grow toward the equatorial plane of the spindle and/or on its surface. While the spindle fibers of each half-spindle bend and anaphase becomes desynchronized, i.e., the two main features of spindle reorganization, kinetochore fibers remain quite pronounced. Therefore, during these two processes, an additional factor, a pushing force, must be involved. We infer, therefore, that taxol induces the formation and elongation of new MTs, which push the chromosome arms toward equatorial plane in metaphase and whole chromosomes toward the cell plate in anaphase and telophase. Desynchronization of anaphase accompanied by stretching even of short chromosome arms cannot be explained without this assumption (11). Such pushing also prevents the separation of the distal segments of sister chromatids and results in their fusion in early anaphase. We do not find, however, any evidence of an increase in chromosome stickiness which might be suggested on the basis of superficial examination.

[3] A critical evaluation of the IGS method is beyond the scope of this paper. This is a new technique (17) providing a permanent label which permits prolonged observations; due to the optical clarity of the *Haemanthus* spindle, the morphology of a single chromosomal fiber can be often followed. We do not know, however, whether we detect a single MT or an MT bundle. We cannot measure single MT length, but we can estimate the length of MT bundles. The IGS method does not allow quantitative conclusions concerning MT number. We believe, however, that the density of IGS stain within thin MT arrays reflects both the change of MT packing and their number. EM studies (C. Jensen and J. Molè-Bajer. Manuscript in preparation.), although not yet conclusive, are consistent with these assumptions.

FIGURE 5 Primary effects of taxol at room temperature. (*a*) Late anaphase. Action of 10^{-5} M taxol for 5 min. Well-developed polar MTs form pronounced "asterlike" structures (arrows). "Star" formation in the upper half-spindle is marked by a double arrow. Some chromosome arms are stretched and their distal tips are parallel to the equatorial plane (short, thick arrow). (*b*) Late anaphase. Action of 10^{-4} M taxol (saturated solution) for 35 min. Chromosomes are arranged in characteristic semi-circles. Some arms are stretched and their distal tips are parallel to the equatorial plane. This is also seen in *a* and is most advanced in *c* (action of 10^{-5} M taxol for 5 min). The tips are not sticky and do not fuse as seen on different focal levels (not included). Abundant polar MTs tend to be of uniform length and their polar ends are laterally associated. They become of more uniform length in later stages (*e*). (*c*) Early telophase. Action of 10^{-4} M taxol (saturated solution) for 35 min. Chromosome arms have shrunk considerably. The distal ends of some trailing chromosome arms are at the equatorial plane and the arms break. The cell plate has not yet formed. This shows that this arrangement precedes the formation of the cell plate. (*d*) Beginning of telophase. Chromosome contraction leads to an excessive stretching of chromosome arms. As a result, some of them break and form fragments or their distal ends are connected with the main body of the chromosome by only a thin "thread." In both cases, such fragments are transported away from the pole. Action of 10^{-4} M taxol (saturated solution) for 35 min. (*e*) Late telophase. Action of 10^{-5} taxol for 5 min. This cell was affected by taxol after the chromosomes had reached the poles, as is shown by the absence of chromosome fragments and a formed cell plate (white line). Well-developed "asterlike" structures are differentiated: MT distribution is denser at the periphery of the asters. "Astral" (polar) MTs are of uniform length (cf. Fig. 1*g* and *h*). Their ends converge gently as in *c* and associate laterally (thin arrows). MTs in the outer region of the "aster" are strongly connected as is demonstrated by the mechanical breakage (thick arrow) of the astral arrays (during preparation mounting). (*f*) Telophase. Action of a low concentration of taxol (10^{-6} M for 30 min). Some characteristic features of taxol action are still present. The contracting chromosome arms are stretched toward the cell plate (arrows). There is an increased lateral association of MTs at the polar region. The remnants of half-spindles are longer as compared to the same stage in untreated cells. Polar MTs, however, are not of a uniform length. Micrographs a–d were taken with G-B, *e* with W-G, and *f* with Y filter. Bar, 10 μm. × 1,000.

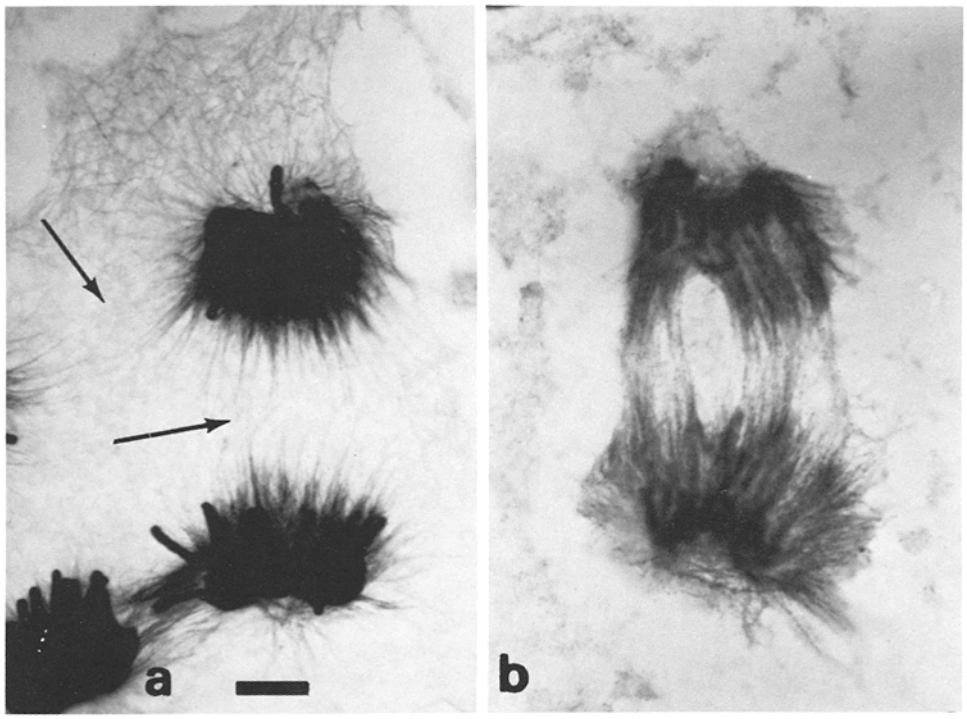


FIGURE 6 Stabilizing and induction of MTs at low temperature. (*a*) Telophase at 4°C for 3 h and 15 min. No normal MT organization is detected. Only very thin and long MTs (arrows) are present in the interzone. (*b*) Telophase at low temperature (3 h at 4°C) followed by treatment with taxol (10^{-5} M for 20 mins) at the same temperature for 8 min. MT organization is similar to that in control cells at room temperature. Micrograph *a* was taken with G-B filter and *b* with W-G filter. Bar, 10 μm. × 1,000.

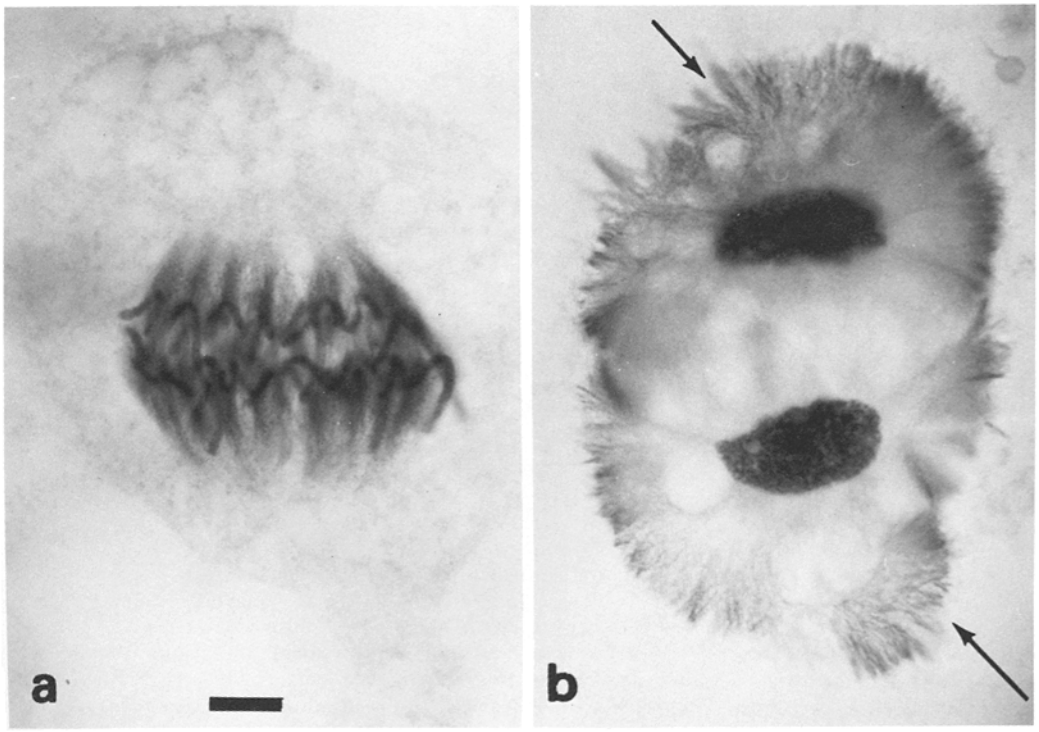


FIGURE 7 Recovery. (*a*) Early anaphase. Action of 10^{-5} M taxol for 20 min is followed by washing out and 30-min recovery at room temperature. A typical taxol-induced compact spindle with a flat polar region is no longer present. Movement of chromosomes is synchronous. (*b*) Late telophase. Action of 5×10^{-6} M taxol for 20 min is followed by washing out and 40-min recovery at room temperature. The distal ends of "astral" MTs begin to splay (arrows) and differ in length. The cell plate is formed but at a different focal level and therefore is not seen. Micrographs taken with a G-B filter. Bar, 10 μm. × 1,000.

The newly formed polar MTs in control cells in late anaphase and telophase are of different lengths, and MTs within arrays splay distally from the nucleus. Their length becomes remarkably uniform in taxol-treated cells. This suggests that the elongation of closely packed MTs is interdependent. Furthermore, MTs converge gently toward their distal, presumably fast-growing ends which become laterally more closely associated. Such association or interaction between MTs and/or spindle fibers (MT arrays) may be an important factor in the mechanism of chromosome transport and spindle integrity (7). Thus, taxol modifies MT association perhaps through HMW and MAPs the same as in vitro (32).

Elongating polar MTs which contribute to the formation of the phragmoplast in control cells (10, 18) stretch and often break trailing chromosome arms in cells exposed to taxol. These fragments located occasionally at the ends of MTs (11) move away from the poles. In contrast, fragments observed after ionizing irradiation in untreated cells are, as a rule (5), transported toward the poles. Thus, the action of taxol provides the first case known to us where pushing by growing MTs is

directly demonstrated in higher plant spindles. The existence of a pushing force exerted by elongating MTs has been predicted by Hill (33) and Hill and Kirschner (34). We believe (10) that such pushing, enhanced by taxol, occurs also during the normal process of mitosis and contributes to pole separation, anchorage, and functional autonomy of each half-spindle in the anastral anaphase. Thus, newly formed polar MTs (10, 62), and not interzonal ones, contribute to the spindle elongation.

We would like to stress in conclusion that while the two most easily recognized effects of taxol on MTs are the enhancement of lateral associations and the promotion of their assembly, we believe that indirectly a variety of other tubulin-dependent processes are triggered, although they are not detectable with our technique. Variations of taxol's effects are to be expected in different cell types, and caution in making generalizations is required.

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