INTERMICROTUBULE BRIDGES IN MITOTIC SPINDLE APPARATUS

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INTRODUCTION

Fine interconnections or "cross-bridges" have been observed between cytoplasmic microtubules in many different organisms (1, 3, 7, 9, 10, 12, 13). Recently, similar bridges have been noted between the microtubules of the spindle apparatus in dividing plant and animal cells (4, 6, 7, 11, 14). Increasing attention is being directed toward intertubule bridges, since they may play an important role in microtubule-mediated motility (1, 8, 9).

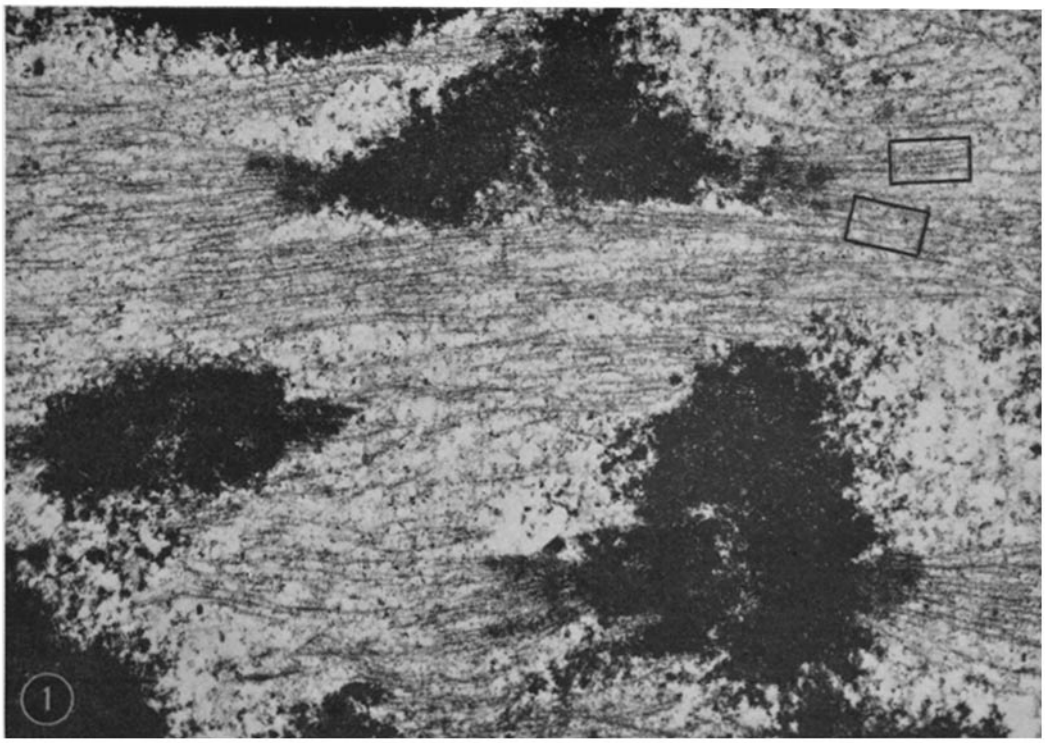
In this brief note we present our observations on the structure of arms and bridges on the microtubules of the spindle apparatus in cultured human cells (HeLa and WI-38) and in the endosperm of the African blood lily, *Haemanthus katherinae*. We demonstrate the presence of bridges between kinetochore tubules, between continuous tubules, and, during later stages of mitosis, between the tubules of the interzone. We also show bridges between tubules and vesicles in the postmitotic stem of HeLa cells and in the phragmoplast of *Haemanthus*.

MATERIALS AND METHODS

Cultured human cells were obtained from Baltimore Biological Laboratory (HeLa) and from the American Type Cell Culture Collection, Rockville, Md. (WI-38). They were grown on plastic cover slips in Falcon plastic Petri dishes in a CulturSTAT medium consisting of Eagle's basal medium, with 10% calf serum (BBL). Cells adhering to the cover slips were fixed with 3% glutaraldehyde in the culture medium or in 0.05 M phosphate buffer for 15 min, washed in phosphate buffered sucrose for 30 min, and postfixed in 1% OsO_4 in phosphate buffer for 30 min.

Cells of the liquid endosperm of *Haemanthus katherinae* were expressed from immature seeds and plated on plastic cover slips that had been coated with a 5–10 μ thick layer of 0.5% agar containing 3.5% sucrose. The endosperm preparations were examined in the light microscope with phase-contrast optics, and those with cells in division were fixed in the vapor above a 25% solution of glutaraldehyde, placed in an aqueous atmosphere to remove residual glutaraldehyde, and postfixed in the vapor above a 2% solution of OsO_4 (4).

After fixation, both the cultured human cells and the *Haemanthus* endosperm cells were dehydrated in a graded ethanol series, infiltrated with a few drops of an Epon embedding medium, and polymerized in a 60°C oven for 24–48 hr. Following polymerization, wafers of Epon containing the cells were cleaved from the plastic cover slips. The fixed-embedded cells were then examined in the light microscope, and individual cells in the desired stages of division were isolated and mounted, either flat or on-end in order to permit sectioning parallel or normal to the axis of the spindle apparatus. Sections were cut with diamond knives on a Porter-Blum MT-1 or MT-2 ultra-microtome (Ivan Sorvall Inc., Norwalk, Conn.), mounted on coated grids, counterstained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I. (For details in the embedding procedure, see Hepler and Jackson [4].)



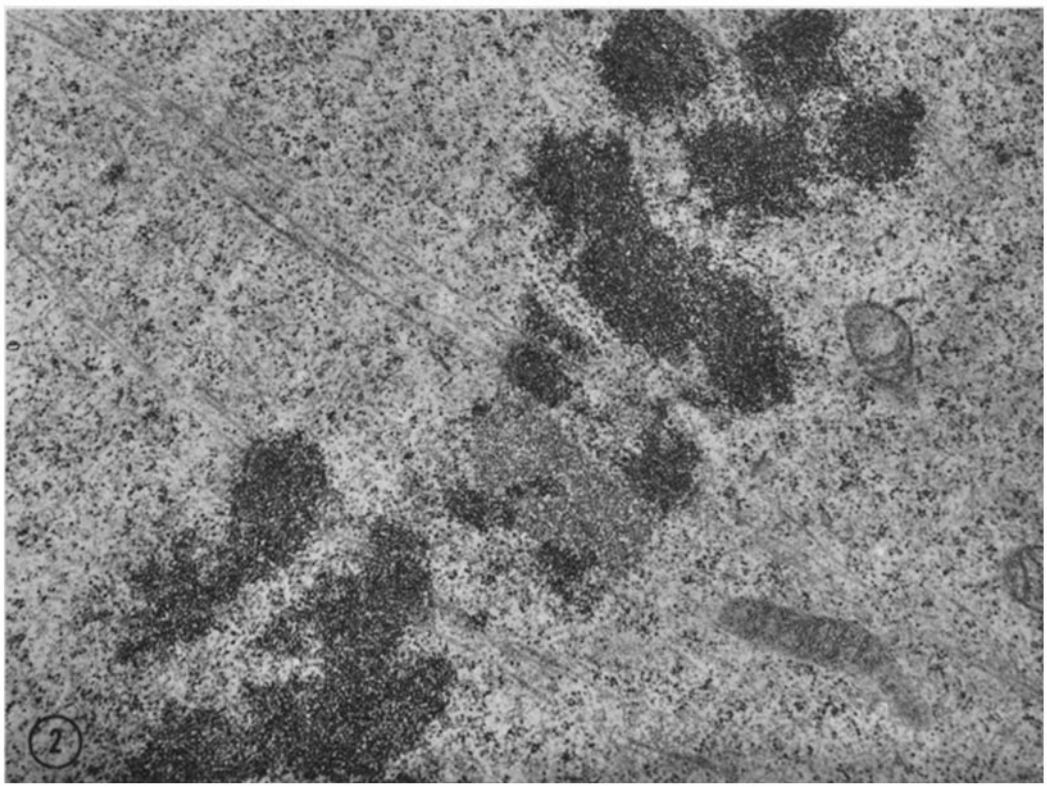
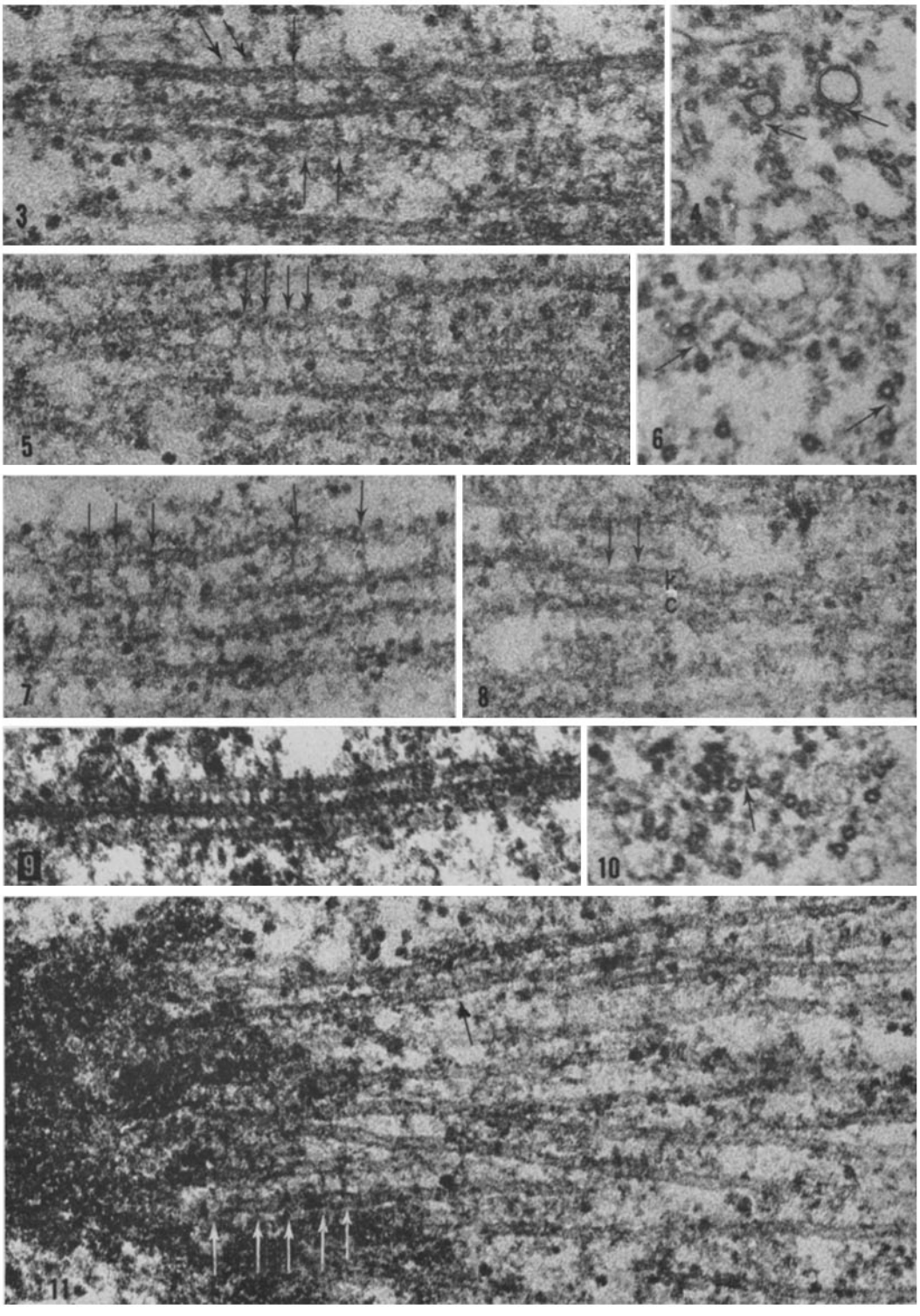


FIGURE 1 Late prometaphase in *Haemanthus*. Chromosomal tubules which emanate from oppositely directed kinetochores on sister chromatids, fan out and intermingle with the tubules of the continuous spindle. Two outlined areas are shown at higher magnification in Figs. 7 and 8. × 12,000.

FIGURE 2 The onset of anaphase in HeLa cell. Kinetochore and continuous tubules may be distinguished and are seen to intermingle. × 12,000.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RESULTS

Electron micrographs of longitudinal and cross-sections of spindle microtubules show that the bridges are faintly stained, fine threads projecting from the surface of the tubules (Figs. 1–26). In both *Haemanthus* and cultured human cells the bridges are of similar appearance, measuring about 20–50 A in width and 100–400 A in length. In HeLa and WI-38 cells they are most frequently about 200 A long, whereas in *Haemanthus* the length is more variable. Arms that are morphologically similar to the bridges can occasionally be seen studding the surface of tubules that lack near neighbors (Figs. 6, 16–18). Arms and bridges are both straight (Figs. 3, 5, 7, 9, 11, 12–19) and curved (Figs. 6, 12, 13, 20, 21, 23, 26), and they may occur at various angles of tilt relative to the tubule surface (Figs. 3, 12, 17). In addition it is observed that within the thickness of any transverse section two or more arms or bridges may project in different directions from the surface of a single microtubule (Figs. 20, 25).

The irregular packing of the microtubules in the spindle apparatus and the variable morphology and tilt of the bridges themselves have made it difficult to study the arrangement and spacing of the arms and bridges along the tubule surface. It has, however, been possible in a number of instances to measure interbridge spacing and look for a periodicity. We find that over most of the length of the spindle tubules the bridges are far apart, sometimes as much as 1 μ. When several bridges are clustered together, the smallest paraxial distance between two bridges is approximately 100 A, but most frequently they are spaced at about 200, 300, and 400 A.

Microtubule arms and bridges occur throughout the spindle apparatus in cultured human cells and in the *Haemanthus* endosperm, and they are present from prometaphase through telophase (the prophase spindle has not been investigated). Kinetochore tubules, identified by their characteristic connections with chromosomes, may be distinguished, in low magnification micrographs, from the tubules of the continuous spindle that pass through the metaphase plate (Figs. 1, 2). Examination of selected areas at high magnifica-

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FIGS. 3–11 *Haemanthus* in various mitotic stages showing intermicrotubule bridges × 100,000.

FIGURE 3 Prometaphase. Bridges between continuous tubules. Some of the bridges are straight, (vertical arrows), but two (slanted arrows) are tilted.

FIGURE 4 Telophase. Transverse section through the phragmoplast shows bridges between microtubules and vesicles (arrows).

FIGURE 5 Metaphase. A cluster of cross-bridges along continuous tubules (arrows).

FIGURE 6 Late anaphase. Transverse section of interzone tubules showing a slightly curved bridge and a free arm (arrows).

FIGURE 7 A high magnification view of the upper outlined area in Fig. 1. Bridges occur between kinetochore tubules (arrows).

FIGURE 8 The lower outlined area of Fig. 1 shows a continuous tubule (*c*) and a kinetochore tubule (*k*) in close apposition. Short filaments, interpreted as bridges, are indicated by arrows.

FIGURE 9 Anaphase. Closely spaced bridges on interzone tubules.

FIGURE 10 Telophase. Bridged pairs of tubules in the phragmoplast (arrow).

FIGURE 11 Metaphase. A kinetochore and its attached tubules. Bridges can be seen between tubules in the dense staining region of the kinetochore (arrows).

tion reveals bridges both between kinetochore tubules (Figs. 7, 11, 12, 19) and between the continuous tubules (Figs. 3, 5, 14, 20). In addition it appears that the kinetochore tubules bridge to continuous tubules (Fig. 8), although an unequivocal demonstration has not yet been possible due to uncertainty of microtubule origin in the region in which kinetochore and continuous tubules intermingle. Interzone tubules of cells in anaphase and telophase are also found to be interconnected by bridges (Figs. 6, 10, 22, 23, 26).

Microtubules bridge with membranous elements, as well as with other tubules. In a telophase cell of *Haemanthus* undergoing cell plate formation, bridges are seen between the phragmoplast tubules and the vesicles that eventually become part of the growing plate (Fig. 4). Tubule-vesicle bridges are also observed in HeLa cells during the elongation of the stem that interconnects the daughter cells of the previous division (Fig. 24).

DISCUSSION

Our findings indicate that microtubule arms and bridges are persistent spindle components distributed throughout the mitotic apparatus of the three cell types examined. It seems likely that all of these fine projections (the free arms, the tubule-tubule bridges, and the tubule-vesicle bridges) are the same basic unit that is able to bind to different cytoplasmic components. The arms and bridges observed in the spindle apparatus of cultured human cells and of *Haemanthus* endosperm are similar in structure, not only to one another but also to the microtubule arms and bridges reported in several cytoplasmic systems (1, 3, 9, 10, 12, 13) and to those seen on the interzone tubules of the

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FIGS. 12–26 HeLa and WI-38 cells in various mitotic stages showing intermicrotubule bridges. × 100,000.

FIGURE 12 Early anaphase in HeLa cell. Bridges run both straight (horizontal from arrows) from one tubule to another and at an angle (slanted arrows) relative to the tubule surface.

FIGURE 13 Metaphase in WI-38 cell. A pair of tubules with bridges (arrows).

FIGURE 14 Metaphase in HeLa cell. A cluster of tubules with bridges and arms (arrows).

FIGURE 15 Metaphase in WI-38 cell. Unevenly spaced bridges between tubules (arrows).

FIGURE 16 Metaphase in HeLa cell, showing closely spaced arms on a tubule near the centriole (arrow).

FIGURE 17 Metaphase in HeLa cell. Arms at different angles of tilt (arrows).

FIGURE 18 Metaphase in HeLa cell. Microtubules near the pole showing arms at various spacings and tilts (arrows).

FIGURE 19 Metaphase in WI-38 cell. A cluster of kinetochore tubules linked by bridges (arrows).

FIGURE 20 Metaphase in WI-38 cell. Transverse section of bridged tubules (arrows). One tubule shows an arm as well.

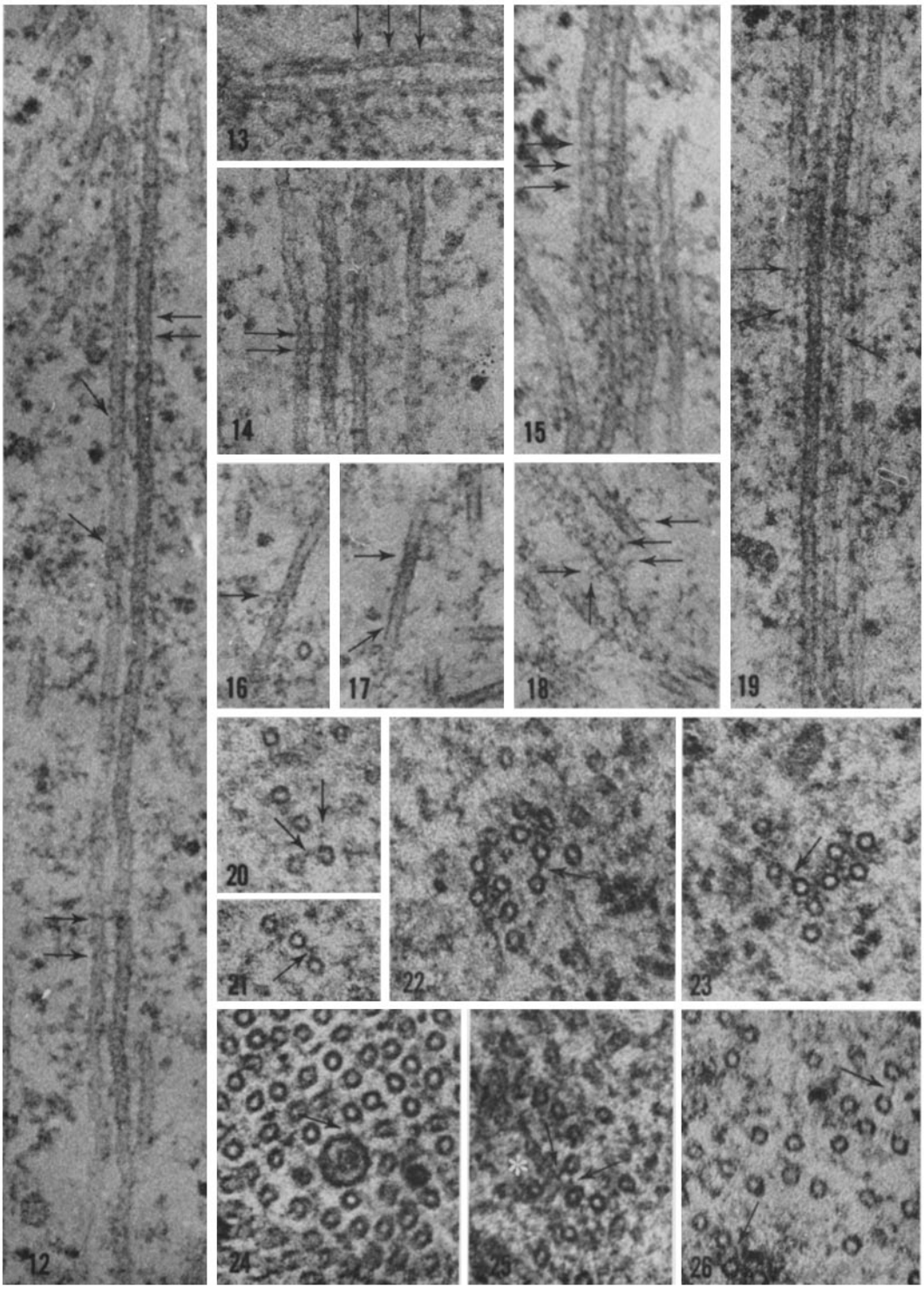
FIGURE 21 Anaphase in WI-38 cell. A curved bridge is evident (arrow).

FIGURES 22 and 23 Anaphase in WI-38 cell. Bridges between tubules in the interzone (arrows).

FIGURE 24 Late telophase in WI-38 cell. A bridge can be seen between a microtubule of the stem and a membrane-bounded vesicle (arrow).

FIGURE 25 Anaphase in WI-38 cell at the polar region. The plane of the picture is at 45° to the pole-to-pole axis. A tubule is bridged to two neighbors (arrows), one of which is in oblique section (*).

FIGURE 26 Anaphase in HeLa cell. Bridges between interzone tubules (arrows).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spindle apparatus in a dividing alga (14). The presence of arms and bridges on microtubules from such diverse species suggests that they may be structures commonly associated with microtubules.

Of particular interest is the modal distribution of arms and bridges along the axis of the tubule. In the helical array of microtubules surrounding the nucleus of the developing chicken spermatid, arms and bridges occur at intervals of 105 A, 207 A, 304 A, and 424 A along the length of the tubule (J. R. McIntosh, unpublished observations). It has been suggested that binding sites for bridges are located in a regular array on the microtubule surface, and that bridge units are present in an equilibrium between a bound and an unbound state, filling only a fraction of the periodic sites along the tubule wall (7). The ordered arrangement of the microtubules in the perinuclear helix has made feasible a more detailed analysis of bridge periodicity and morphology than is possible in the spindle apparatus. Nevertheless, our measurements of interbridge spacing on the spindle tubules, which show that bridges occur at approximately 100 A, 200 A, 300 A, and 400 A along the tubule, agree closely with those found in the chicken spermatid helix.

The possible functional analogy of intertubule bridges to the interfilament bridges in muscle has prompted speculation about microtubule cross-bridge function. In two nonspindle microtubule systems, the tubule helix in developing chicken spermatids (9), and the rows of microtubules associated with kinetosomes (km fibers) of *Stentor* (1), evidence has been presented to show that cross-bridged microtubules slide relative to one another. It has been suggested in each case that the intertubule bridges serve as the force-generating mechanism for tubule sliding.

Wilson (14), in a study of the interzone tubules of the spindle apparatus of the coenocytic alga *Blastophysa*, emphasizes the structural similarity of the tubule arms and bridges to dynein, the ATPase of ciliary microtubules (2). He mentions that there might be a functional analogy of the tubule cross-bridges to the interfilament bridges in muscle, but he favors the suggestion that the tubule arms and bridges are involved in the growth of microtubules during spindle elongation in anaphase, serving as loci for the addition of new microtubule subunits (14).

In a theoretical paper on the mechanism of chromosome motion (8) we have postulated that the intermicrotubule bridges of the spindle apparatus are active, mechanochemical units capable of functioning in a fashion analogous to muscle interfilament bridges (5) to push material with which they bind along the microtubule surface. This postulate may be combined with known facts about the mitotic spindle to account for the principal events of mitosis.

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