THE DISTRIBUTION OF SPINDLE MICROTUBULES DURING MITOSIS IN CULTURED HUMAN CELLS

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ABSTRACT

WI-38 and HeLa cells in mitosis have been selected from fixed monolayer cultures and serially sectioned for electron microscopy. Sections perpendicular to the spindle axis permit counting of the number of microtubules at each position on the spindle axis and hence the preparation of tubule distribution profiles. Errors intrinsic to this method are discussed. The changes in the tubule distributions from one mitotic stage to another provide evidence concerning the behavior of the spindle tubules during mitosis. The ratio of the number of tubules passing the chromosomes on the metaphase plate to the maximum number in each half spindle is about 1/2. This ratio changes little in early anaphase, and then decreases in late anaphase at about the same time that a zone of increased tubule number develops at the middle of the interzone. The region where the stem bodies form contains about 3/2 the number of tubules seen elsewhere in the interzone. This ratio is almost constant as the midbody forms in telophase and then increases to 2/1 in early interphase before the final stages of cytokinesis occur.

The structure and behavior of the mitotic spindle have been investigated by many workers using the complementary morphological techniques of polarization and electron microscopy. Polarization optical studies on living material have demonstrated that the spindle exists as an equilibrium between ordered and disordered states of a fiber subunit (12, 22). Ultraviolet microbeam studies indicate that the equilibrium is under the control of defined sites within the cell, located at the poles, at the kinetochores, and, in plant material, at the phragmoplast (22, 23). Fine structure studies of mitosis have revealed that the principal fibrous constituent of spindle is the microtubule (15, 19, 41). At metaphase there are at least two families of spindle tubules: one set which runs from the kinetochores on the chromosomes to the poles (the chromosome tubules), and another set which starts at the poles, passes through the metaphase plate, and runs an unknown distance toward the opposite pole (the continuous or interpolar tubules) (2, 4, 9, 14, 24, 25, 38, 40, 42). Where the spindle tubules are close to each other, one can sometimes detect intertubule bridges (21, 29, 46).

During anaphase, the motion of the chromosomes may be divided into two components: a chromosome-to-pole motion accompanied by a shortening of the chromosome tubules, and a motion of pole away from pole without change in the chromosome-to-pole distance. In general, one or the other of these motions predominates, but several cells show both motions, often sequentially in time (5, 7, 28, 32, 37, 43). As the anaphase chromosomes separate, microtubules can usually be seen in the interzone (2, 10, 20). These tubules are morphologically indistinguishable from other spindle tubules, and are generally thought to be the continuous tubules which formed during early

mitosis (32). The dark-staining "stem bodies" which appear at the midplane of the interzone during late anaphase (5) are seen in the electron microscope to be short segments of the interzone tubules surrounded by a dense, amorphous material (10). There is some evidence from both plant and animal material that the interzone tubules overlap in the stem bodies (20, 35). As the cleavage furrow pinches in, the stem bodies cluster to form the midbody (*Zwischenkörper*), and the resulting shaft of microtubules interconnects the daughter cells well into interphase (11).

A considerable body of evidence, which has been well summarized in several reviews (13, 32, 43), indicates strongly that during anaphase the chromatids are pulled to the poles by spindle fibers attached to their kinetochores. (The term "spindle fiber" will be used here to mean a cluster of fibrils which is visible in the light microscope.) For an understanding of the mechanistically significant events of karyokinesis, it is therefore necessary to determine the mitotic motions of the spindle fibers themselves. The chromatids mark one end of the chromosome fibers, but other markers are needed to determine the motion of the anaphase chromosomes relative to their own fibers and relative to the fibers of the continuous spindle. Studies of the zones of reduced birefringence which can be produced by irradiation with ultraviolet microbeams (17, 18) and work on spindle fiber–associated "particles or states" (1, 3) are yielding helpful evidence about mitotic motions, but it is not clear at present just how these phenomena are related to the spindle microtubules visible in the electron microscope. On the basis of current knowledge, certain mechanisms for spindle force generation can be ruled unlikely, e.g., the constant diameter of the chromosome tubules throughout anaphase argues against spindle tubule contraction. There is not, however, enough information about spindle behavior to discriminate between several acceptable hypotheses for chromosome motion (16, 23, 31, 32, 34, 45).

In the absence of unambiguous markers to reveal the motions of spindle tubules, we have measured the changes with time in the distribution of microtubules along the spindle axis (30). To determine the total tubule number at a given point on the spindle axis, we have examined sections cut perpendicular to the line running from pole to pole and counted the number of tubules present in the whole cell cross-sections. To graph the tubule distribution along the spindle axis, one can prepare serial sections for electron microscopy, count one out of every 5 or 10 sections, and then express tubule number as a function of position. The time-dependent changes in these distribution profiles provide evidence which can help to determine the behavior of spindle tubules during mitosis.

METHODS

WI-38 cells, obtained from the Cell Culture Collection Committee, American Type Culture Collection, and HeLa cells, strain S-3, were maintained on Culture-Stat Eagle's basal medium, Earle's base, supplemented with 10% fetal calf serum. In preparation for microscopy, cells were plated out on 2-inch square pieces of coverslip plastic (General Biological Supply House, Inc., Chicago, Ill.) in Falcon plastic Petri dishes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). Cells were fixed for 15 min with 3% glutaraldehyde in the culture medium, washed in phosphate-buffered sucrose for 30 min, postfixed in 1% OsO_4 in phosphate buffer for 30 min, dehydrated in ethanol, and infiltrated with Epoxy resin. After polymerization at 60°C the wafer of Epon-embedded cells may be viewed with phase optics, either with the coverslip in place or after the coverslip has been removed. A thin layer of immersion oil may be spread directly on the Epon if the surface is too rough to permit good image formation. Cells of interest were marked with a Leitz slide marker (E. Leitz, Inc., Rockleigh, N.J.), cut out with a razor blade, and fastened to an empty plastic block in any desired orientation with Araldite glue.

Sections were cut on a Porter-Blum-MT 1 ultramicrotome. Three or more ribbons of 8–15 adjacent sections each were placed side by side on one LKB slot grid coated with a Formvar film (Ladd Research Industries, Inc., Burlington, Vt.). The sections necessary to get from one spindle pole to the other, about 250, could thus be collected on 5–10 grids. The specimens were stained with 1% uranyl acetate and Reynolds' lead citrate (36), and examined in a Siemens Elmiskop la or a Philips 300 operated with a beam current of less than 10 μa. Pictures were taken of every fourth or fifth section with an instrument magnification varying from 8000 to 24,000, unless more detailed information was needed. (For example, analysis of tubule distribution in the stem bodies and midbodies required pictures of every other section.) In some cases, however, problems such as stain precipitate, dirt, or a broken support film necessitated a gap in the collection of the data (see, for example, Fig. 7).

For the preparation of distribution profiles, the position of a given section on the pole-to-pole axis (hereafter referred to as the spindle axis) was deter-

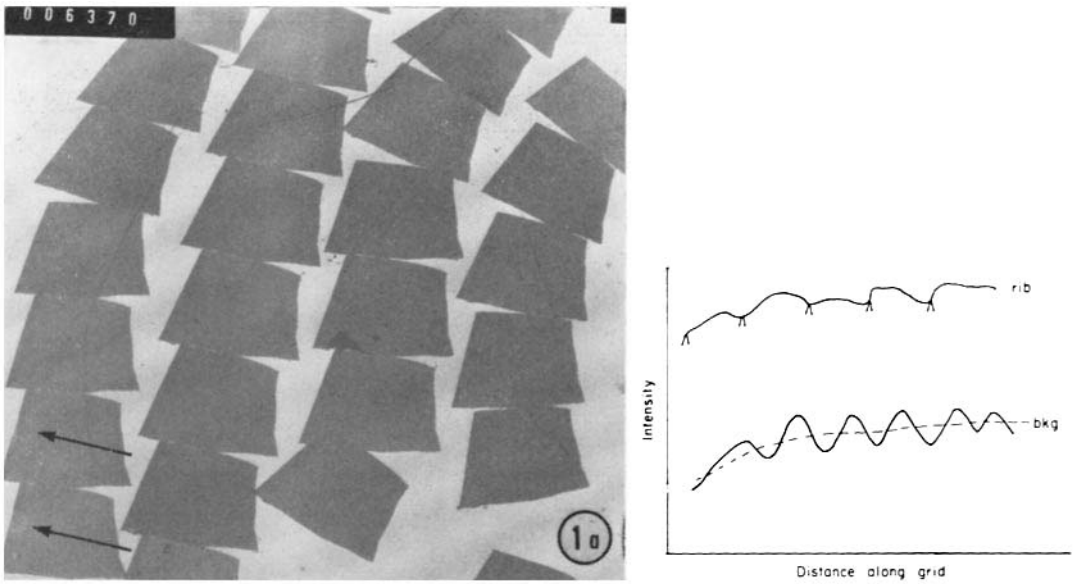


FIGURE 1 Fig. 1 *a* shows sections from a WI-38 cell (arrows) as seen with the scanning mode of the Philips microscope. The cutting sequence of the sections is from bottom to top and from left to right. × 100. Fig. 1 *b* is a graph of the light intensity passing through the photographic plate for the picture shown in Fig. 1 *a*. The background trace (*bkg*) was run on the interribbon region of the Formvar film between the first and second ribbons from the left; the trace showing section thickness (*rib*) is from the five central sections on the second ribbon from the left. The faint ripple in the background is due to a wrinkling of the film as the sections were picked up from the knife boat. Since the ripple is largely absent from the image of the sections themselves, it has been graphically averaged out before the use of the base line for measuring the section thickness. The variation in section thickness has been estimated from the average difference between traces over each section.

mined by counting the number of sections which separated it from certain unambiguous reference points such as the centrioles. With a trapezoidal block face and a convention for laying down ribbons of sections on the coated grids, the order of the sections is unambiguous, but the thickness of the sections and hence the apparent paraxial distance from one structure to another is subject to some variability, even though the sections were cut in a temperature-controlled room. The interference color of adjacent sections within a ribbon is generally without noticeable variation, so the difference in section thickness is probably small. Microdensitometric measurements on EM plates taken in the linear region of the gray scale indicate that the variation in section thickness is generally less than 8% (Fig. 1). At the beginning of each ribbon there is sometimes one thick and one thin section; the optical density measures indicate that the sum of the thicknesses of these two sections is equal to the sum of the thicknesses of two regular sections.

For microscopy of the later stages of mitosis we used a Philips goniometer stage permitting 45° of tilt in any direction. Accurate counting of closely packed microtubules, such as those found in telophase, depends upon a proper alignment of the tubules' axes relative to the axis of the electron beam. The microtubules of the telophase and interphase stem which interconnects the daughter cells in HeLa and WI-38 are often helically arranged around the stem axis, and no single cross-section will reveal them all. Further, we were not always able to orient the specimen correctly for sectioning, so the availability of 45° of stage tilt in any direction was a convenience.

Spindle tubules were counted in three cells each in metaphase, mid-anaphase, late anaphase, telophase, and interphase, using prints at a final magnification of at least 30,000. The entire cross-section of the cell was counted for each data point, since the distribution of the tubules within the dividing cell is far from uniform. In some cases the pictures were ruled off into small squares which could be counted individually, while in other cases the tubules were marked with a felt-tipped pen as they were recorded on a hand counter. Both methods were used on all metaphase and anaphase cells, but only the felt-tipped pen method was used for telophase and inter-

phase cells, since the tubules of the stem are too close together for accurate counting in the small squares.

All sections analyzed were counted at least twice, once by one of us (Dr. McIntosh), and once by one or more other observers who were not biologists and who knew little about mitosis and the problem being studied. These observers were trained to recognize microtubules in cross and oblique section by having them go over sample micrographs until their recognition of tubules on the samples was the same as that of an experienced observer and until their total count for the picture agreed with his count to within 10%. Thereafter, all observers counted independently without consulting each other's data.

DISCUSSION OF TUBULE COUNTING

The success of the method outlined above depends both upon good preservation of tubules within the cells and upon accurate recognition of tubules in the micrographs.

Variability of Tubule Preservation and Total Tubule Number

THE PROBLEM OF UNIFORM FIXATION: The accuracy with which observed distribution profiles reflect conditions in the living cell depends upon the uniformity of tubule fixation. It is possible that the tubule counts contain systematic errors due to gradients in fixation of the spindle tubules. In sea urchin eggs, a conventional glutaraldehyde fixative causes uniform decrease in spindle birefringence, indicating that the order of the spindle subunits is not entirely preserved in this material (23). We have observed that the tubules of the mammalian midbody are more stable than spindle tubules at other mitotic stages when treated with high concentrations of glycerol, which suggests that they may also be more stable during fixation. We are not aware of any studies of mammalian cells which show that glutaraldehyde does not preserve all of the spindle tubules, and direct addition of fixative to the monolayer cultures causes almost instantaneous cessation of all motion visible in the light microscope. It must be remembered, however, that a selective solubilization of certain regions or elements of the spindle would have a marked effect on the observed tubule distributions.

VARIATION IN TOTAL TUBULE NUMBER: Different cells from the same line and at the same stage of mitosis do not necessarily have the same number of microtubules in their fixed, embedded spindles. Whereas two HeLa telophase stems (not in the midbody) contained approximately 1000 and 1100 tubules each, four anaphase WI-38 cells showed maximum interzone tubule counts of about 600, 700, 720, and 800 microtubules each. Even more variation was seen in two metaphase WI-38 cells which contained 1600 and 3400 tubules each at the distribution maximum between the metaphase plate and the poles. The data generally group around values characteristic for each cell type and each stage in mitosis, but there is significant deviation from the group mean. Aneuploidy and variability in fixation effects may contribute to these differences.

The tubule counts presented here may be examined in such a way that variation in total tubule number from one cell to another does not affect the analysis. If we attach no particular significance to direct comparisons of total tubule number from cell to cell, but consider only the ratios of tubule numbers within each cell, then the absolute tubule number cancels out. We have found that tubule distribution profiles show reproducible shape from cell to cell, so it is the ratios which will be compared at different times in mitosis.

Errors of Counting

Accurate tubule counting depends upon reliable tubule recognition. Microtubules in cross-section are easy to identify, but tubules oblique to the plane of section are often difficult to distinguish from tiny vesicles and fibrous precipitates of cytoplasm. Close packing of oblique tubules compounds the problem. The orientation and the packing of spindle tubules change during mitosis, so that the precision of our tubule counts varies both with position on the spindle and with the stage in division.

In the absence of an independent measure of tubule number, such as spindle birefringence, we have used the reproducibility of our results to assess their validity. The reproducibility of multiple counts made on one micrograph by one observer was always better than the agreement between counts made by different observers, so only "agreement" will be discussed. We define agreement as the standard deviation of the several counts of a given picture by different observers, expressed as a per cent of the mean tubule number for the picture. Since only two counts are compared in some cases, the agreement is intended as a

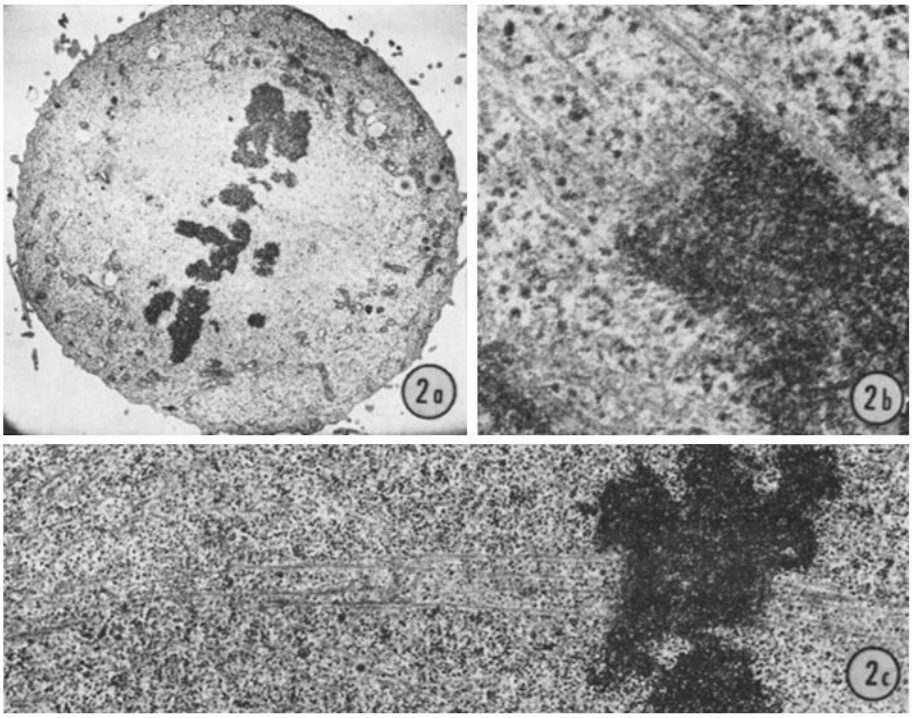


FIGURE 2 Fig. 2 *a* shows a late prometaphase-metaphase WI-38 cell. The spindle is seen at this magnification only as a zone of exclusion for mitochondria and other large cytoplasmic organelles. The spindle, as well as the cell, is approximately spherical. One centriole pair is visible at the upper left, the other appears four sections farther on. × 3000. Fig. 2 *b* shows a metaphase chromosome with one kinetochore evident and a tubule which passes the kinetochore zone. Serial sections demonstrate that this microtubule does not enter any kinetochore, but it has not been possible to follow it very far from the metaphase plate. × 40,000. In Fig. 2 *c*, the metaphase chromosome appears to possess a functional twofold axis perpendicular to the line joining the centers of the sister kinetochores. The same sort of symmetry is seen both in the metaphase plate as a whole and in the spindle-chromosome complex throughout anaphase and telophase. × 13,000.

guideline for the reader rather than as a property or justification of the technique.

QUALITY OF MICROSCOPY AND FIXATION: The clarity of the micrographs had a predictably important effect upon agreement. More difficult to assess, but equally important, was the effect of fixation. The whole cytoplasm of some fixed cells is packed with a finely fibrous feltwork which obscures detail and is difficult to distinguish from oblique microtubules. Agreement in these fibrous cells was very bad, and the data from three such cells have been discarded, even though the shapes of the tubule distributions were generally similar to those seen in the cells which counted with best agreement.

OBLIQUITY OF TUBULES: When tubules are viewed in cross-section, counting agreement is good.[1] Well-oriented sections of the interphase stem or of anaphase stem bodies show agreements better than 1%. The average agreement on all counts from these regions is 5%. Average agreement at the metaphase plate is 8%; in the interzone during late anaphase and telophase it is about 10%. During early anaphase the alignment of tubules is less precise, and interzone agreement

[1] During metaphase and anaphase, spindle cross-sections sometimes reveal tubules with a double image, similar to the outer nine microtubules of cilia and flagella (44). We have investigated these images with the tilting stage and have found that all such images examined (about 20) can be changed into a customary single image by tilting the section in the microscope. Spindle "doublet tubules" have therefore been counted as one single tubule.

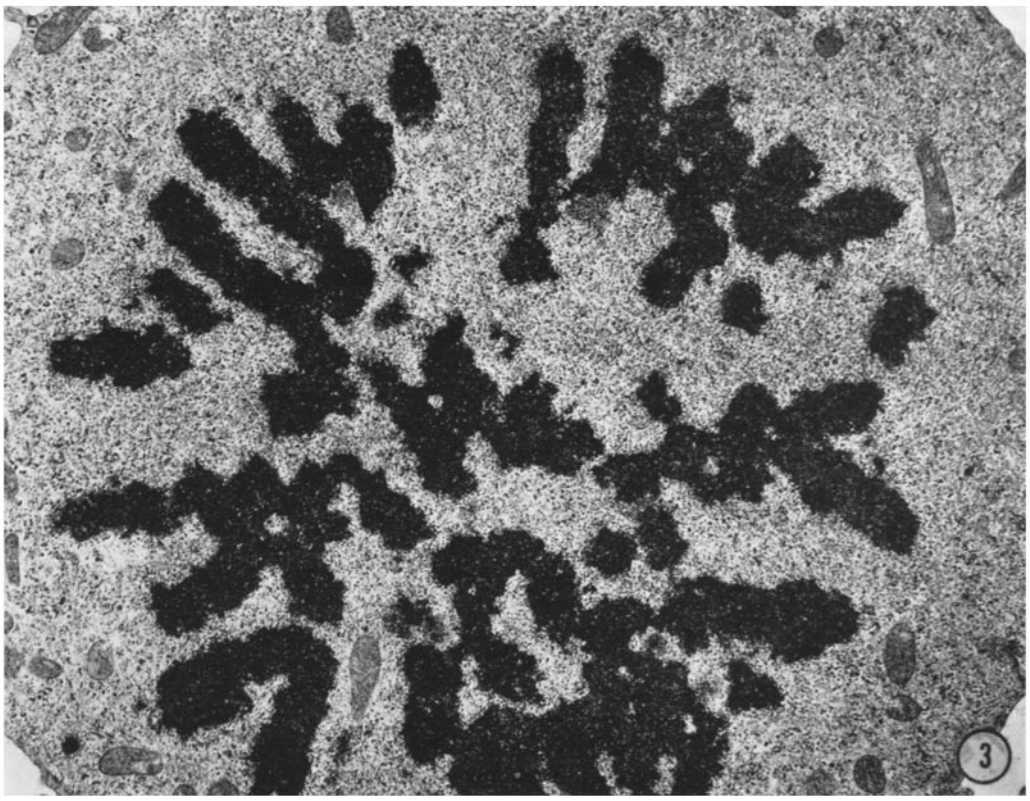


FIGURE 3 The chromosomes are uniformly distributed on the metaphase plate, but long chromosome arms are turned outward from the spindle axis. Note that mitochondria are excluded from the region of the spindle but not from the region of the chromosomes. × 8000.

is no better than 20%. Toward the poles, as the tubules bend and cluster, the agreement becomes even less precise. In the immediate vicinity of the poles, in metaphase and anaphase the agreement is occasionally as bad as 90%. Qualitatively, the standard error of multiple counts is directly related to the percentage of oblique tubules. The agreement percentage will be stated for each datum as it is discussed.

OBSERVATIONS

WI-38, a human lung-derived fibroblastic cell of almost stable diploid karyotype, possesses a spindle similar to those of the other mammalian cells which have been described (9, 15, 24, 25, 38). Fig. 2 *a* shows a WI-38 cell in late prometaphase-metaphase. Figs. 2 *b* and 2 *c* show metaphase chromosomes and microtubules. The shape of the whole metaphase spindle in longitudinal section is approximately circular as judged from both polarization optical and electron microscope observations. The chromosomes on the equator are arranged with sister kinetochores oriented toward opposite poles (Fig. 2 *c*), and both chromosomal tubules and tubules which cross the metaphase plate are seen (Fig. 2 *b*). The radial arrangement of long chromosome arms seen in Fig. 3 provides evidence for an outward-radial force (33), even in this cell which has a uniform distribution of the chromosomes at the metaphase plate rather than the annular distribution sometimes seen in other organisms (28).

The disposition of the spindle tubules at the metaphase plate is shown in Fig. 4. Some tubules pass between chromosomes, and some pass through them (arrow). Some tubules enter the light-staining chromatin characteristic of a kinetochore (K), but one light-staining chromatin zone in this section contains almost no tubules. Tubules can be

seen in this zone in the next serial section on the ribbon.

Fig. 5 shows a spindle cross-section taken at the surface of the metaphase plate. Figs. 4 and 5 are from the same spindle and are presented at a magnification high enough to permit the reader to count the tubules. The area marked off on Fig. 5 is the same fraction of the total spindle cross-section at that position on the spindle axis as the entire area shown in Fig. 4. The authors find 366 ± 8% tubules in Fig. 4 and 505 ± 10% tubules in the area marked on Fig. 5.

Fig. 6 shows the metaphase configuration (a light micrograph showing the stage of mitosis), and Fig. 7 gives the microtubule distribution profile for the cell seen in Figs. 4–6. The sections which correspond to Figs. 4 and 5 are marked with stars on the graph in Fig. 7. The position of the poles and the sections in which chromatin was seen are portrayed on the diagram above the graph. The wide distribution of the chromatin at the equator is attributed to the fact that the plane of section for this particular cell was not perpendicular to the spindle axis. Sections at the surface of the metaphase plate showed few chromosomes, and only 10 sections at the middle of the chromatin region showed the display of chromosomes seen in Fig. 3.

The microtubule distribution has been analyzed for one metaphase HeLa cell, but in less detail than for WI-38 (6 sections instead of 15). The maximum number of tubules found between the metaphase plate and one pole was 2400 ± 20%, the minimum number counted at the plate was 1600 ± 10%, and the maximum on the other side of the plate was 2100 ± 20%. Tubule counts from an additional metaphase WI-38 cell are included in Table I, which presents data from several mitotic cells studied but not explicitly described in this paper.

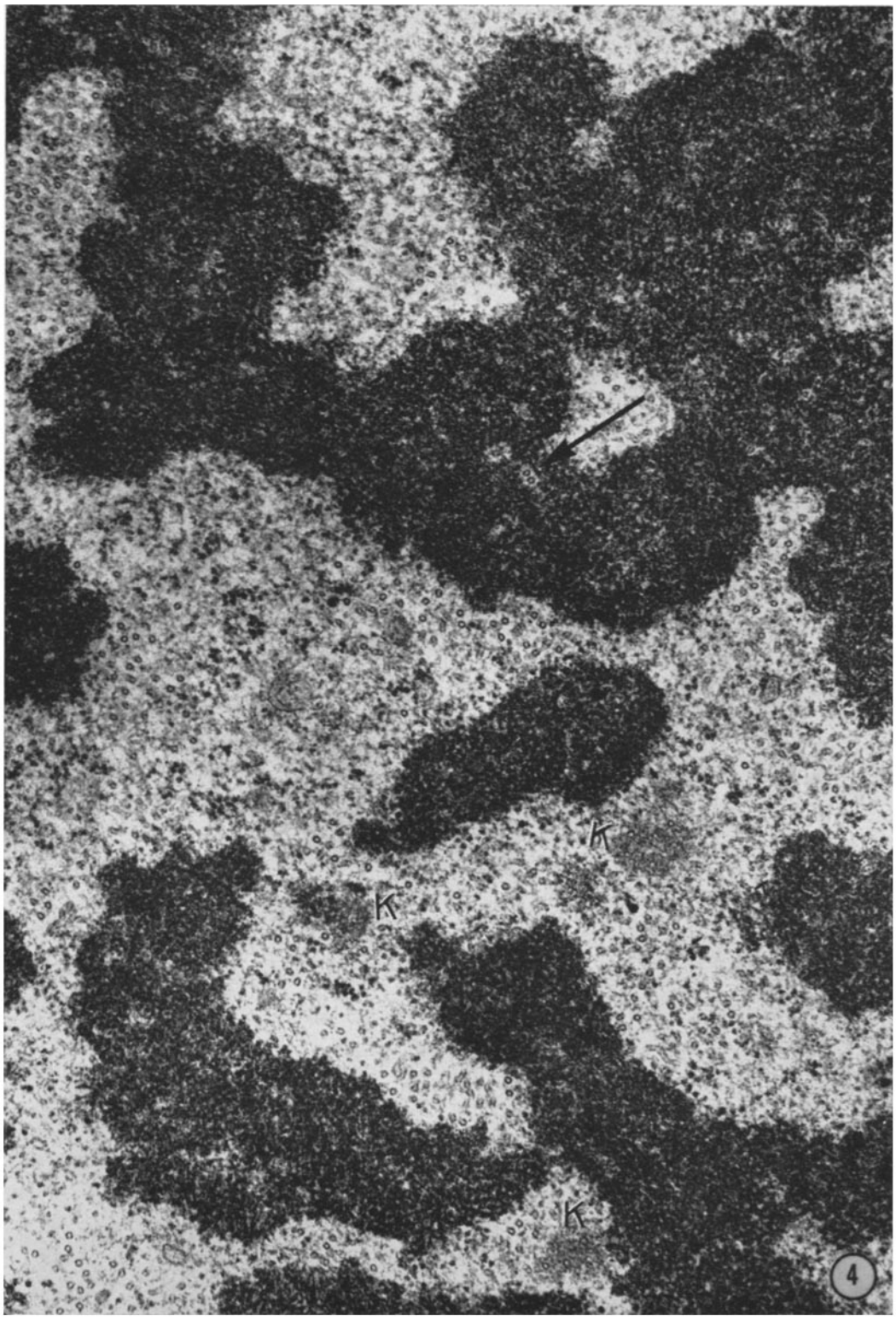
Figs. 8, 9, and 10 are anaphase spindle cross-sections taken near the pole, in the vicinity of one set of chromosomes, and in the interzone. Fig. 11 shows the mid-anaphase configuration, and Fig. 12 is the graph of the microtubule distribution in the cell shown in Figs. 8–11. The sections presented as Figs. 8, 9, and 10 are marked with stars on Fig. 12. We find 360 ± 70% tubules in Fig. 8, 727 ± 15% tubules in Fig. 9, and 303 ± 10% tubules in Fig. 10. The number of tubules counted through the interzone is not quite constant, given the precision of the observations. The sections which include more tubules than the interzone minimum contain the few stem bodies which have already begun to form at this stage of anaphase (Fig. 10). Note the marked clustering of the interzone tubules and the invasion of the spindle by both mitochondria and elements of the endoplasmic reticulum.

We have investigated the astral region of the spindle during both metaphase and anaphase using transverse and longitudinal sections. Meaningful tubule counts in this zone have been impossible, due to the disarray of the spindle fibers. The asters of both HeLa and WI-38 cells are small at metaphase, and they do not appear to increase in size during anaphase. Spindle tubules generally terminate at the poles and only occasionally pass the centrioles to add to the population in the astral region.

Figs. 13 *a*, 13 *b*, 13 *c*, and 13 *d* show a HeLa cell fixed at about the same stage in anaphase as the WI-38 cell of Fig. 11. The dark-staining structures marked *S* are stem bodies beginning to form. We have found that when stem bodies first appear they are elongate, but that as mitosis proceeds, as judged by the reformation of the nuclear envelopes and the progress of the cleavage furrow, the stem bodies become shorter parallel to the spindle axis (Figs. 13 *e*, 13 *f*, and 13 *g*). The longest stem body seen in HeLa was 1.7 μm, whereas mature HeLa stem bodies are less than 0.5 μm.

Fig. 14 shows a late anaphase configuration in WI-38, and Fig. 15 is the microtubule distribution profile for the cell shown in Fig. 14. There is a peak in the tubule number at the middle of the interzone in the place where most of the stem

FIGURE 4 A portion of a WI-38 metaphase plate. Some microtubules pass between chromosomes, some pass through them (arrow), and some enter the light-staining chromatin characteristic of kinetochores (*K*). An occasional profile of endoplasmic reticulum is visible, and some of the membranes appear rough surfaced. The region within the spindle is filled with clusters of dark-staining particles which resemble ribosomes. A zone of low staining intensity is visible around the tubules where they pass through the chromosomes, but it is conspicuously absent from the tubules at the kinetochores. × 40,000.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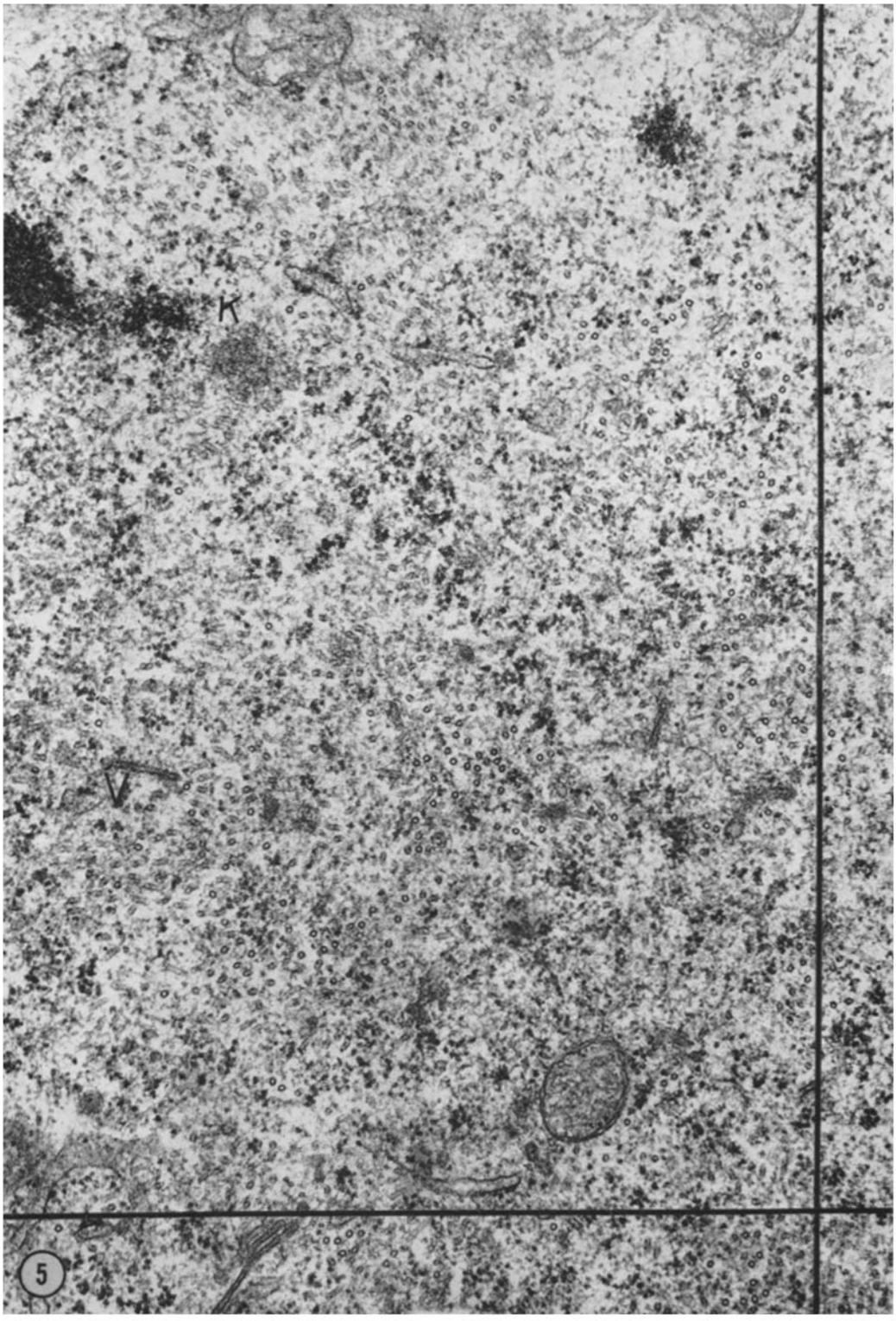
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bodies lie. Individual stem bodies, however, are not always easy to delineate in WI-38. Fig. 16 shows a few clusters of tubules with the amorphous material around them which we have used as the defining property of stem bodies.

HeLa cells have darker stem bodies than WI-38 cells; we have investigated the tubule distribution in individual interzone spindle fibers using this more favorable material. Fig. 17 shows the late anaphase-telophase configuration of a HeLa cell and Figs. 18 *a*, 18 *b*, and 18 *c* are a set of three pictures of sections from the interzone of the cell shown in Fig. 17. The sequence includes one side of several stem bodies, the bodies themselves, and then the other side. It is possible to trace many tubules from the stem bodies out into the clusters of tubules comprising the interzone fibers which are sometimes visible with polarization and Nomarski differential interference contrast optics. Thorough examination has shown that each of the interzone tubules of the HeLa cell shown in Figs. 17 and 18 enters one stem body somewhere along its length.

A comparison of the number of tubules in the stem bodies in HeLa with the number in the adjacent regions of the interzone fibers which run from the stem bodies on either side shows that there are significantly more tubules in the stem bodies than in the neighboring portions of the interzone fibers. In some stem bodies, such as the one labeled *A* on Fig. 18 *a*, the number of tubules in the region of the amorphous, dark-staining material is approximately twice the number on either side; in other stem bodies, the difference is less. Table II includes the data collected from 14 of the stem bodies in the HeLa cell shown in Figs. 17 and 18. The average of the ratios of the tubule number in the stem body to the mean tubule number in the fiber connecting to the stem body on either side is 1.5.

The total microtubule distribution in the HeLa

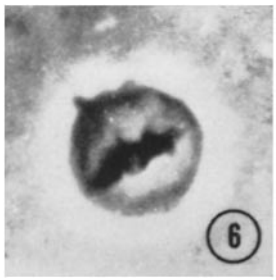

FIGURE 6 A fixed, plastic-embedded WI-38 cell in metaphase, photographed with Zernicke phase optics on a Zeiss microscope before orientation and sectioning for electron microscopy. × 850.

cell shown in Figs. 17 and 18 contains a peak similar to the one seen in Fig. 15 for WI-38. The whole cell cross-sections corresponding to the regions shown in Figs. 18 *a*, 18 *b*, and 18 *c* contain 1225 ± 10%, 1421 ± 10%, and 1201 ± 10% microtubules, respectively. Six sections farther along in either direction from the midzone, there are no stem bodies remaining, and the sections contain 1125 ± 10% and 1092 ± 10% tubules each.

The timing of the onset of cytokinesis is not tightly coupled to the events of karyokinesis: cells with a given development of the cleavage furrow will not necessarily show the same chromatin condition. However, by the time the cleavage furrow confines the stem bodies to a single unit, they are generally well formed, even though they have not necessarily come into perfect registration. Fig. 19 *a* shows a WI-38 cell in telophase with a completed cleavage furrow. Clustered in the stem with the stem bodies and the microtubules are many fine filaments (Fig. 19 *b*). Such fibers are generally a feature of the cell cortex only; we have been unable to detect any similar fibers in the metaphase or anaphase spindle.

Figs. 20 (WI-38) and 21 (HeLa) show sets of pictures taken from sections before the telophase

FIGURE 5 A WI-38 spindle cross-section at the surface of the metaphase plate, showing one kinetochore (*K*). There are no more chromosomes between here and the pole. Numerous small vesicles pervade the spindle at this and subsequent stages of mitosis (see Figures 8–10, 13, 18, 21, 25, and 26); the concept of organelle exclusion from the spindle might well be modified to include only those structures which are greater than a certain size. Note the flattened vesicles (*V*), visible in numerous places on this plate and in Figs. 9 and 10, which are a persistent feature of the metaphase and early anaphase spindle (39). This picture is from the same sequence of sections as Fig. 4 and comes from the same cross-sectional quadrant. The area marked off by black lines is approximately the same fraction of the total spindle cross-sectional area as the entirety of Fig. 4. × 40,000.

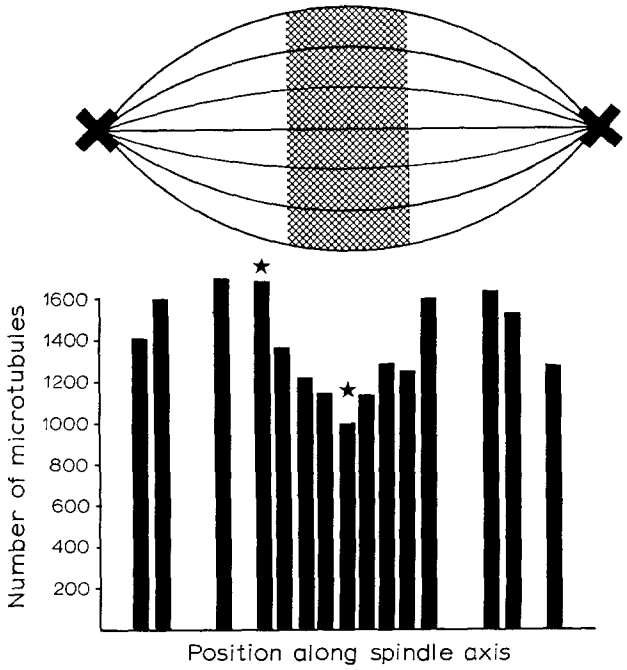

FIGURE 7 The microtubule distribution profile for the cell shown in Figs. 4–6. The schematic spindle above the graph shows the relation of the graph to the spindle from which it was obtained. The black crosses mark the position of the centrioles; the cross-hatching marks the chromosomes. The stars on the bar graph mark the location of the sections shown in Figs. 4 and 5. The estimated uncertainty of the bar height in the region of the chromosomes is about 8%, in the first two sections outside the plate about 15%, and in the two sections nearest the poles on either side about 40%.

TABLE 1

Microtubule Counts from Cells not Otherwise Described in the Paper*

Cell type	Division stage	Tubule counts versus position
WI-38	Metaphase	*pole* 950, 1150, 1100 *chromosomes* 800 1175, 1190, 1000 *pole*
WI-38	Early anaphases	*pole* 425, 600, 900, 800 700 *chromosomes* 800, 850 *chromosomes* 500 *pole* 700, 950, 850, 800, 700 600 750, 825
WI-38	Late anaphase	*pole* 550, 700, 1100 500 *chromosomes* 720 450 *chromosomes* 1000, 850, 600 *pole*
WI-38	Telophase	*pole nucleus* *midbody*‡ *nucleus pole* 250, 260, 290, 310, 340, 320, 290, 240, 210
HeLa	Interphase	*cell stem* *midbody*‡ *stem cell* 1050, 1100, 1200, 1400, 1500, 1550, 1550, 1400, 1250, 1200

* Some of these cells have been counted by only one observer (Dr. McIntosh). For probable errors of the various values, see the error discussion in Discussion of Tubule Counting. All numbers have been rounded off. Position on the table is indicative of position in the cell, but the representation is schematic, not graphic.

‡ Both of the midbodies presented here were sectioned obliquely, and the graphical integration technique described in the Discussion is necessary for proper interpretation.

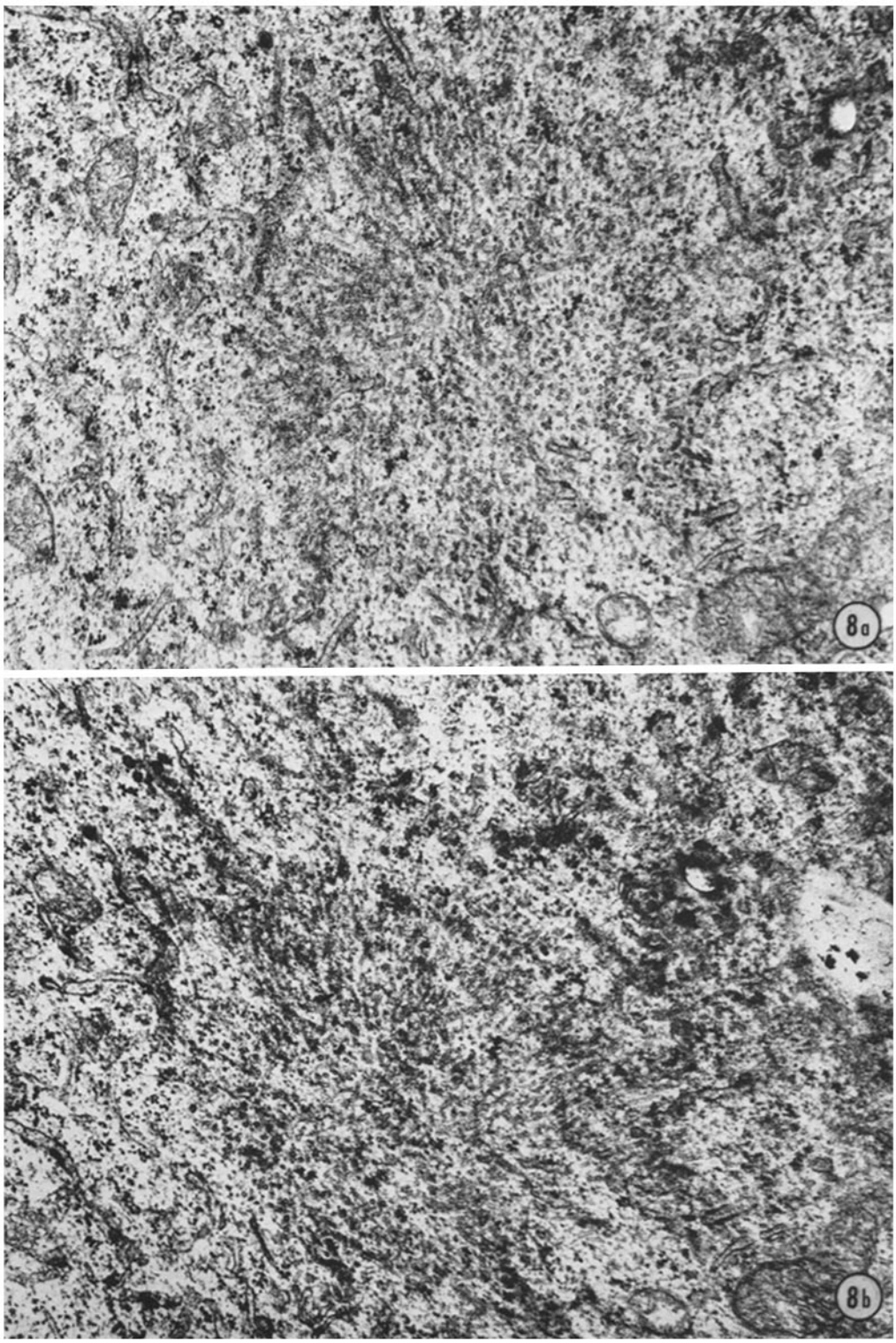

FIGURE 8 Two pictures of one section near an anaphase pole of a WI-38 cell. In Fig. 8 *a* the section was tilted 45° about an axis approximately parallel to the long edge of the page, and in Fig. 8 *b* the tilt was 45° the other way. Note the obliquity and clustering of the tubules and the accumulation of vesicles at the poles. × 30,000.

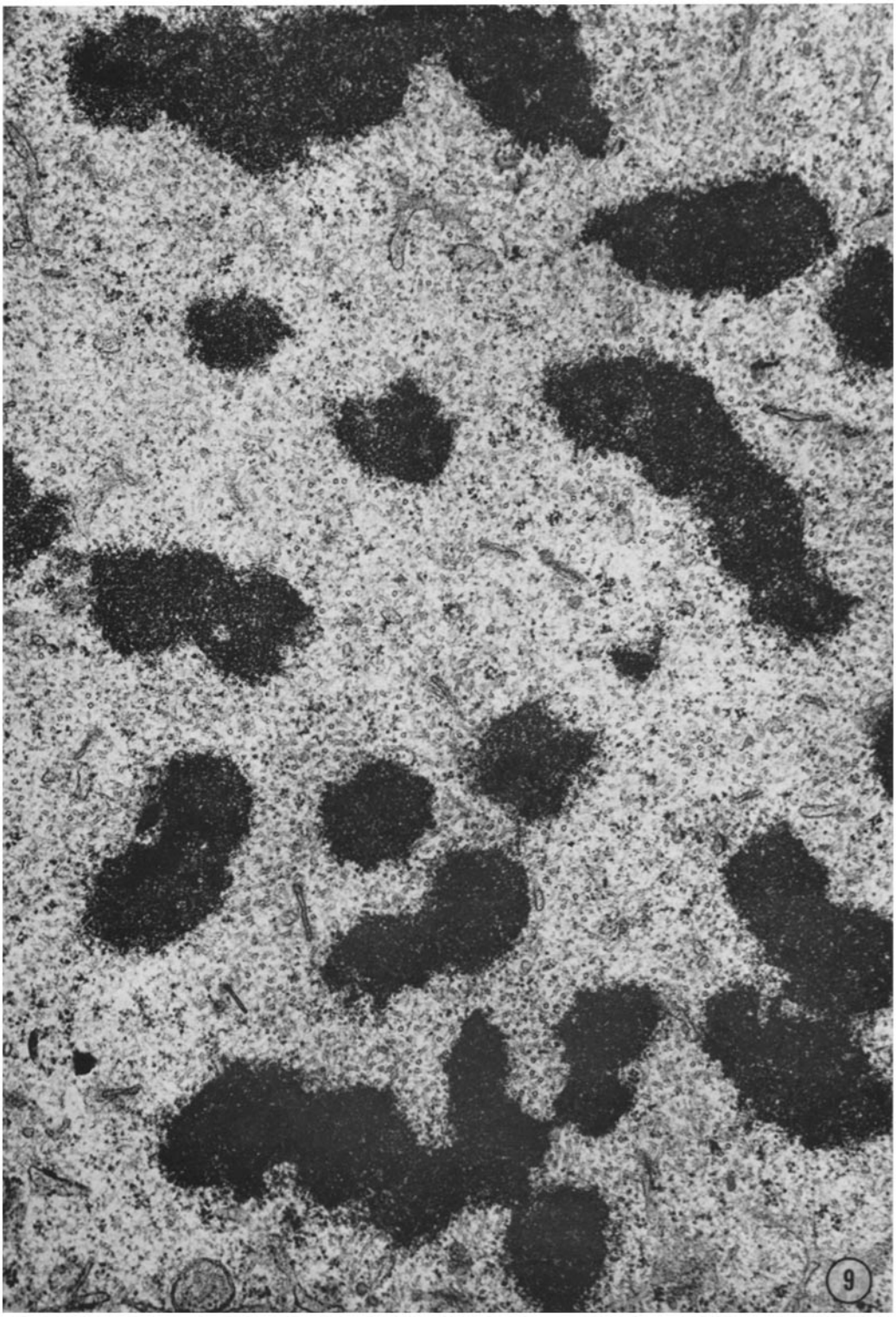

FIGURE 9 A section through one set of anaphase chromosomes from the same cell as shown in Fig. 8. Virtually the whole spindle cross-section is shown. × 30,000.

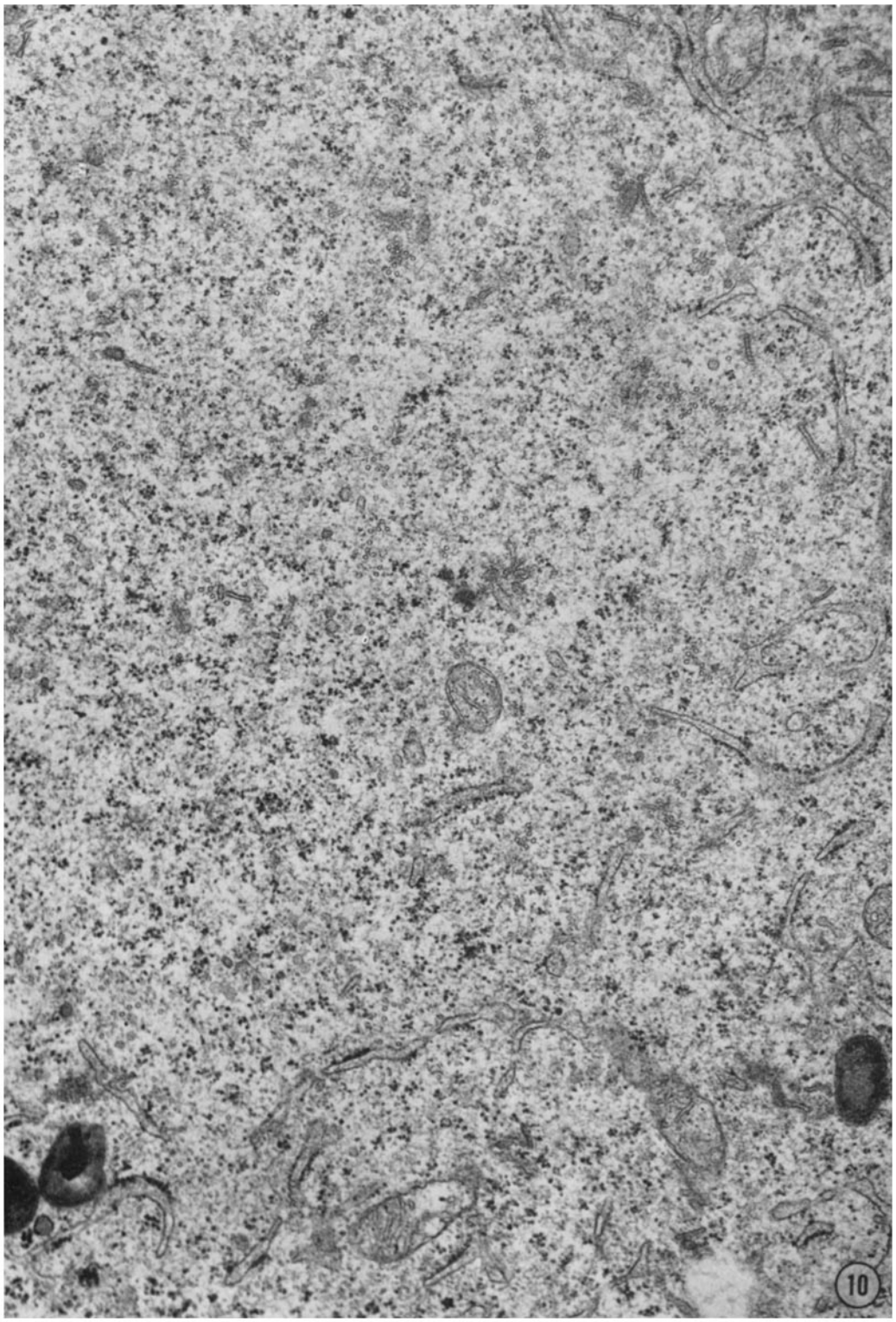

FIGURE 10 A section in the interzone from the same cell as Figs. 8 and 9. Only a portion of the interzone cross-section is shown. There are many ribosome-like particles and rough-surfaced membranes but only an occasional polysome at this and previous stages of mitosis. The obvious clustering of microtubules suggests the existence of a lateral attraction between the tubules. Such clustering is seen in WI-38 cells only at the poles, at the kinetochores, and in the interzone during anaphase. ×30,000.

midbody, in the midbody, and after the midbody. Fig. 22 shows the WI-38 telophase configuration for the cell shown in Fig. 20. Fig. 23 presents the data from counts made on the cell shown in Figs. 20 and 22, and Fig. 24 presents corresponding data for the cell shown in Fig. 21. Those sections which contain more tubules than the number found at the sides of the curves in Figs. 23 and 24 all contain dark-staining midbody material. The number of sections displaying the dark-staining material is greater than expected from the paraxial length of the midbodies, but the discrepancy can be accounted for by the tilt of the plane of section relative to the axis of the stem and by imprecise registration of the stem bodies in the midbody (Fig. 19). Tilt and imprecise registration can also account for the absence of a single section which shows midbody material across the entire stem. The telophase tubule distributions have not been followed far into the daughter cells, because the tubule disorder found there both in very late anaphase and in telophase precludes meaningful counting.

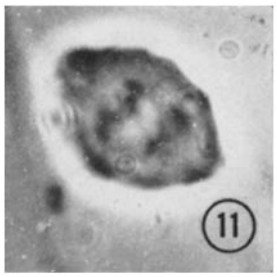

FIGURE 11 A WI-38 cell in mid-anaphase, photographed before orientation and sectioning for electron microscopy. × 850.

After a variable period of time in interphase, the daughter cells round up again and the final stages of cytokinesis begin (11). This phase may be distinguished unambiguously from telophase by the interphase condition of the chromatin and by the length of the stem. Figs. 25 (WI-38) and 26 (HeLa) show sets of pictures of sections taken before the midbody, in the midbody, and after the midbody from cells in interphase. Fig. 27 shows the interphase configuration for the WI-38 cell shown in Fig. 25, and Fig. 28 presents the tubule distribution profile for the cell shown in Figs. 25

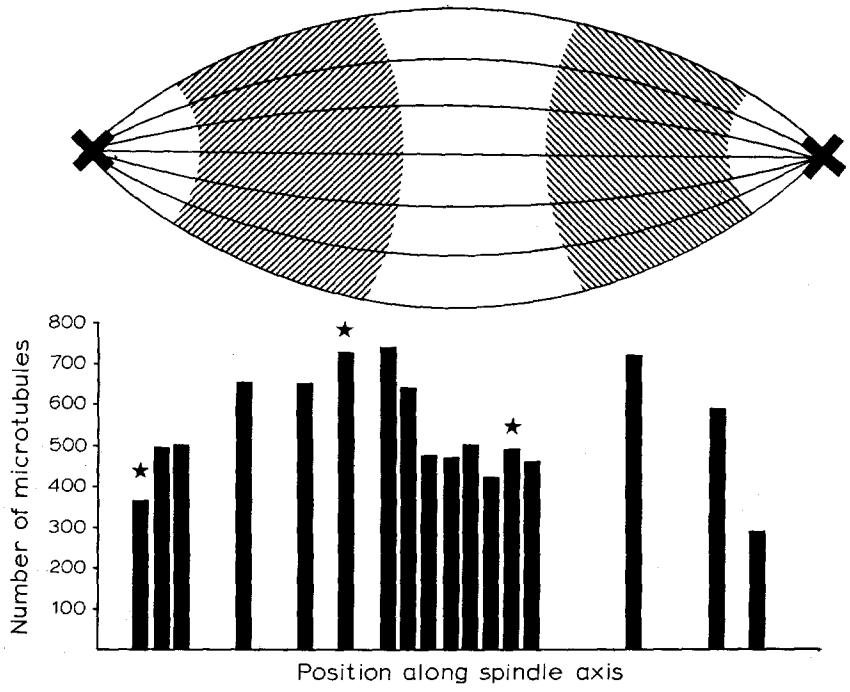

FIGURE 12 The microtubule distribution profile for the WI-38 cell shown in Figs. 8–11. The stars mark the location of the sections shown in Figs. 8–10.

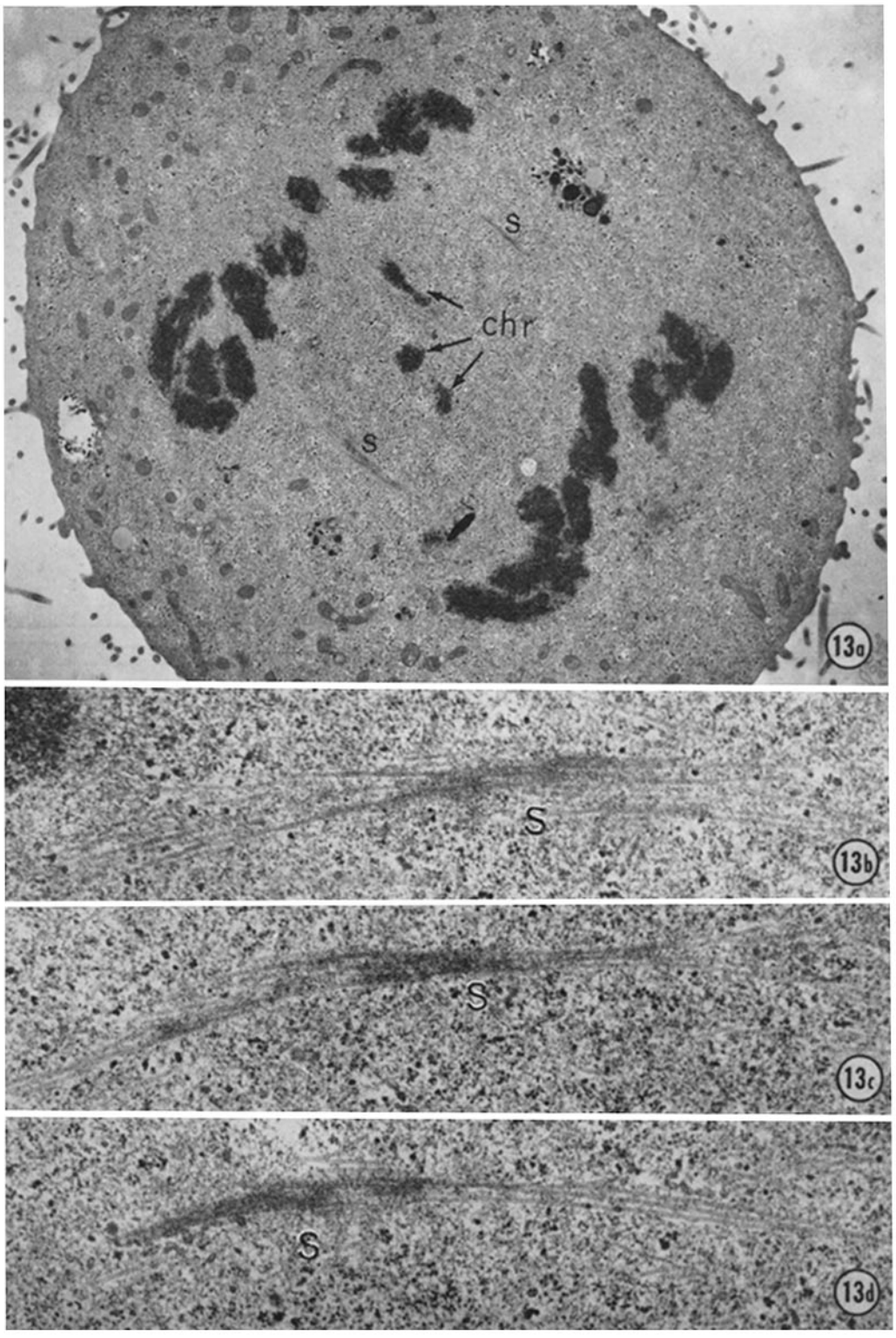

FIGURE 13 Fig. 13 *a* shows an anaphase HeLa cell. Note the lagging chromosomes (*chr*) and the forming stem bodies (*S*) in the interzone. × 5000. Figs. 13 *b*, 13 *c*, and 13 *d* show elongate stem bodies (*S*) from different sections through the cell of Fig. 13 *a*. × 30,000.

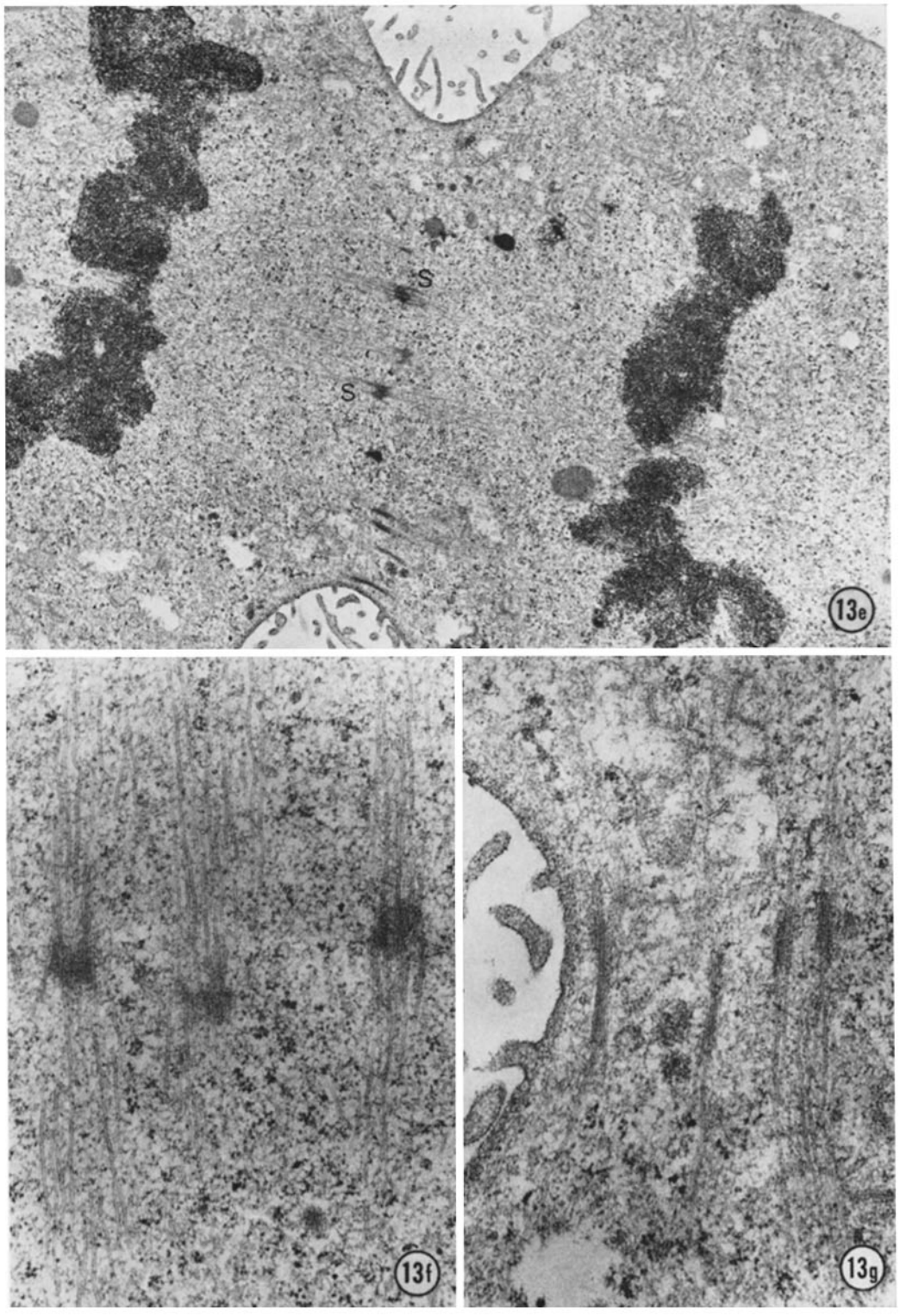

FIGURE 13 Fig. 13 *e* shows a HeLa cell in late anaphase-telophase when most of the stem bodies (*S*) have shortened. × 6500. Figs. 13 *f* and 13 *g* are from the section shown in Fig. 13 *e* and may be compared with Figs. 13 *b*, 13 *c*, and 13 *d*. × 30,000. (Figs. 13 *e*, 13 *f*, and 13 *g* were taken by Dr. P. K. Hepler.)

and 27. Fig. 29 shows the tubule distribution profile for the HeLa cell shown in Fig. 26. There are generally fewer microtubules in the stem during interphase than during telophase, although two exceptions have been found. The ratio of the number of tubules in the interphase midbody to the number of tubules on either side is almost precisely 2/1.

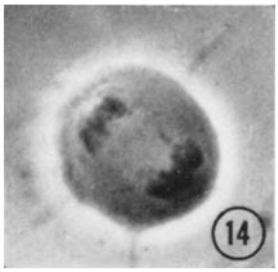

FIGURE 14 A WI-38 cell in late anaphase, photographed before orientation and sectioning for electron microscopy. × 1000.

FIGURE 17 A HeLa cell in late anaphase-telophase, photographed before orientation and sectioning for electron microscopy. The granularity of the picture results from the absence of immersion oil from the surface of the plastic. × 850.

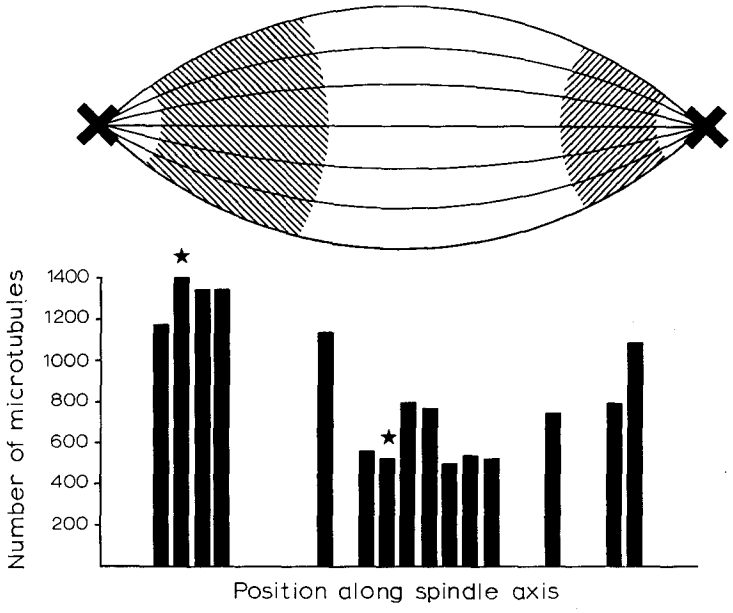

FIGURE 15 The microtubule distribution profile of the WI-38 cell shown in Fig. 14. The stars mark the location of sections used for the computation of tubule count ratios in the Discussion.

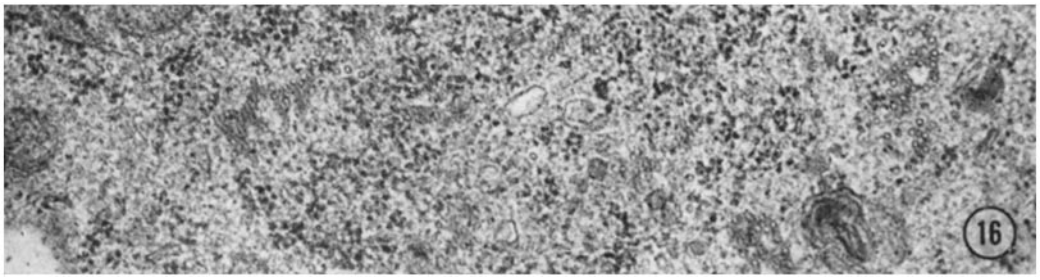

FIGURE 16 A section from the interzone of the WI-38 cell shown in Figs. 14 and 15. The stem bodies can be distinguished as clusters of microtubules with dark-staining material around them. × 40,000.

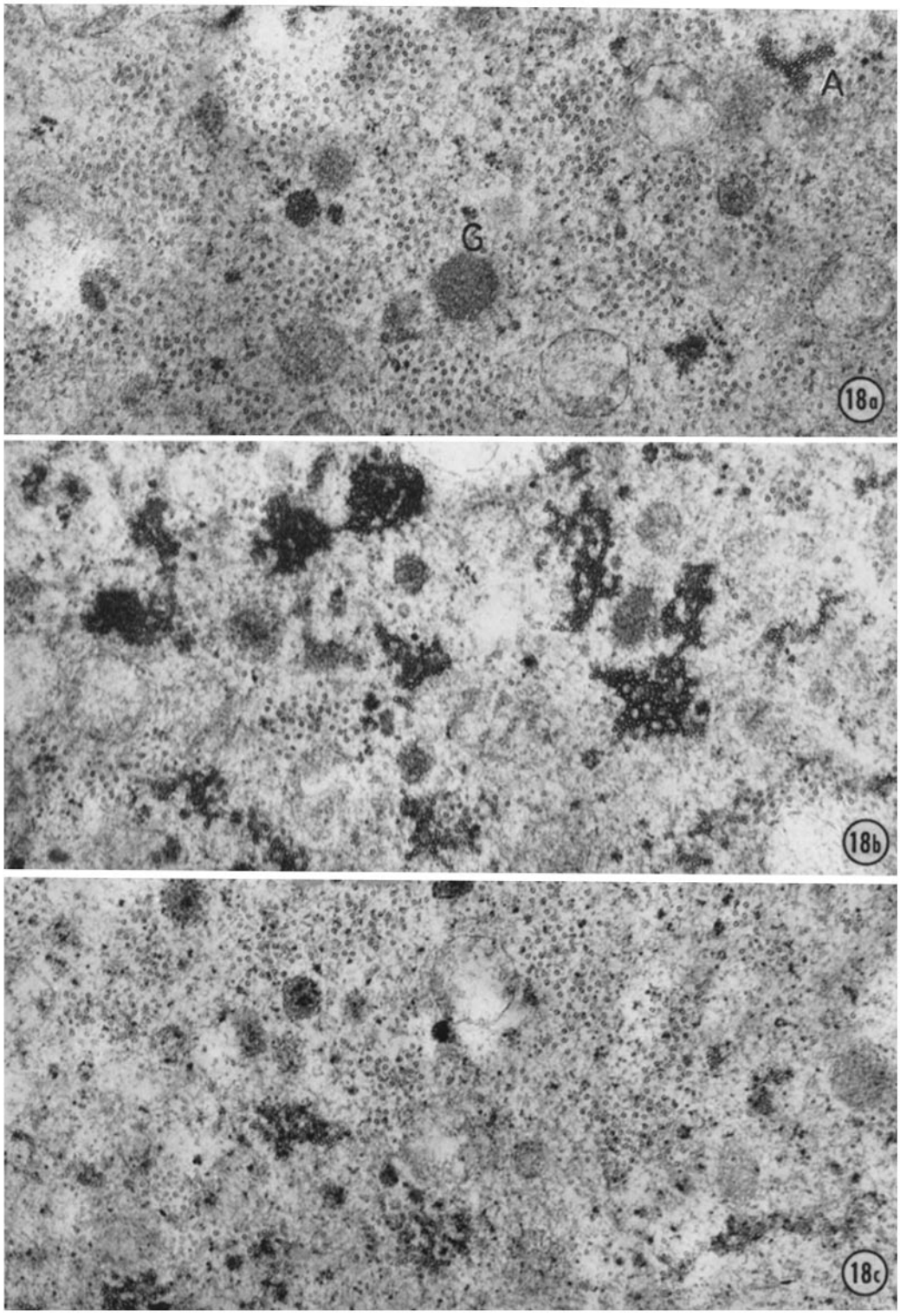

FIGURE 18 Three sections from the interzone of the HeLa cell shown in Fig. 17. Stem bodies are obvious in Fig. 18 *b*, but some are visible in Figs. 18 *a* and 18 *c* as well. In several cases one can follow a cluster of tubules from the sections containing the stem body into the subsequent sections where the tubules are devoid of dark-staining material. It is then possible to compare the number of tubules in the different regions of a single interzone fiber. The stem body labeled *A* in Fig. 18 *a* shows an approximate doubling of the tubule number. The circular profiles of amorphous, granular material (*G*) seen in these sections and in Fig. 13 *e* are characteristic of anaphase and prometaphase in HeLa cells. These granules show a tendency to congregate at the middle of the interzone during late anaphase and are generally gone by telophase. (The small, dark dots on Fig. 18 *c* are stain precipitate.) × 30,000.

TABLE II

Tubule Counts on Interzone Fibers from the HeLa Cell Seen in Figs. 17 and 18

I Tubule no. in stem body	II Mean tubule no. on either side	III Ratio of column I to column II
27	14	1.9
65	45	1.4
55	30	1.8
64	43	1.5
32	26	1.2
51	44	1.2
113	71	1.6
6	3	2.0
28	19	1.5
90	62	1.5
63	39	1.6
101	72	1.4
64	48	1.3
35	27	1.3

Mean ratio of I/II is 1.5 ± 0.24.
Experimental uncertainty of the ratios is about 6%.

DISCUSSION

The observations presented above permit us to describe certain time-dependent changes in the distribution of spindle tubules during mitosis. Due to limitations in the quality of the data, as characterized in the Discussion of Tubule Counting, we think it appropriate to confine this discussion primarily to an interpretation of the location of spindle tubules at different stages of mitosis. The mechanisms of tubule action and the relation between our distribution profiles and existing models for mitotic mechanism will not be considered in any detail.

The metaphase tubule distribution profiles show a dip at the spindle equator, and then fall off at the polar regions of each half spindle. Apparently some of the continuous spindle tubules run from one pole to within a few micrometers of the other, but others end after crossing the metaphase plate. Given the uncertainty of the tubule counts in the polar regions of these cells, this is a point which merits further investigation. The careful studies by Manton et al. (26, 27) of spindle structure in the diatom *Lithodesmium undulatum* show that cells exist in which tubule distribution may be measured with uniform accuracy from one end of the spindle to the other. In the diatom the microtubule counts indicate that about 10% of all continuous tubules run from one pole to the other, while the rest of the continuous tubules start at one pole and terminate somewhere between the spindle equator and the opposite pole. Our results are thus consistent with those of Manton et al. An independent investigation of mammalian spindle structure by Brinkley and his coworkers (8; Brinkley, B. R., and J. Cartwright. 1971. Ultrastructural analysis of mitotic cells in vitro. Direct microtubule counts. *J. Cell Biol.* In press.), will help to elucidate this and other points at question in the research reported here. It must be noted, however, that comparison of the tubule distribution profiles from our study with profiles measured elsewhere will require precise determination of stage within anaphase. Our terms "mid-anaphase" and "late anaphase" are convenient descriptions but are not sufficiently exact to serve as the basis for quantitative comparison. A more precise method of specifiying anaphase stages would be to measure the fraction of the total metaphase half-spindle distance which the chromosomes have moved. For the "mid-anaphase" cell shown in Figs. 8–12 this fraction is about ½, and for the "late anaphase" cell in Figs. 14–16, about 1.

The dip in the number of tubules at the metaphase plate can be interpreted as the result of cutting sections which pass between the kinetochores of some sister chromatids, and thus contain no microtubules from these particular chromosomes. The height of the distribution curve at its minimum should be related to the number of continuous tubules in the spindle (the interzone tubules of metaphase). If a perfect metaphase plate were sectioned exactly perpendicular to the spindle axis, we would expect to see a square well whose height at the bottom would be a direct measure of the total number of tubules crossing the metaphase plate. The shape of the well actually observed in any given cell will be a function of the orientation of the plane of section, but the area of the well should not vary with changes in the angle of sectioning since the area under the curve in a given region of the tubule distribution graph is, in effect, a measure of the volume of the microtubules contained in that region. By graphical integration of the tubule distribution curve over the region of the observed well, we can determine how deep the well would have been in perfectly oriented sections, and hence we can determine the total decrease in the number of tubules at the metaphase plate due to the absence of chromosome tubules between

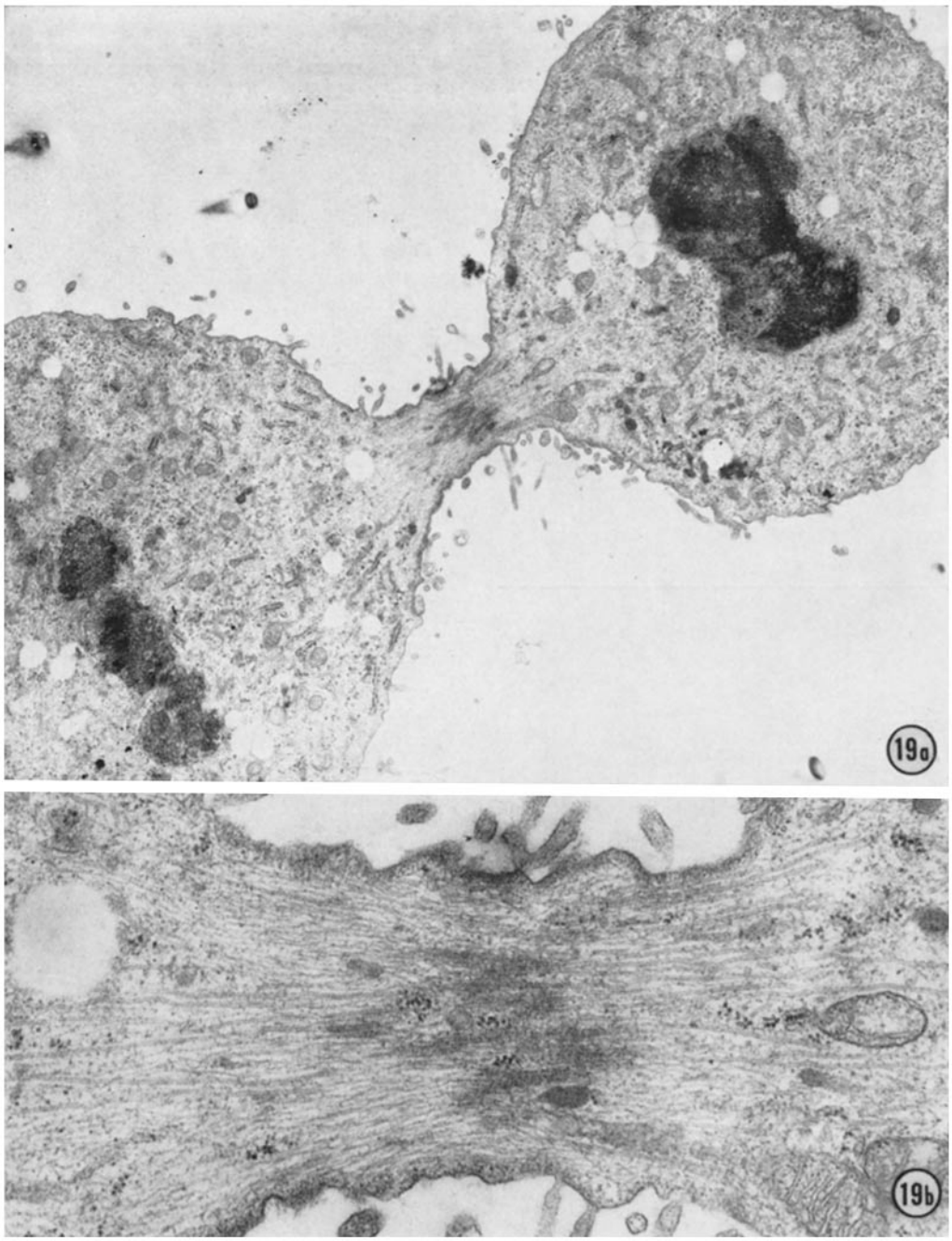

FIGURE 19 Fig. 19 *a* shows a WI-38 cell in telophase. The completed cleavage furrow has formed a narrow stem, and the stem bodies have clustered to form the midbody, although not all stem bodies are in register. The nuclear envelope has largely reformed by this stage, but the chromatin is still in a condensed form. × 5000. Fig. 19 *b* shows the stem at higher magnification and reveals that both microtubules and fibers lie side by side in this particular section. Other mitotic stages from the same fixation have not shown equivalent fibers, and this image may result from the close apposition of the interzone spindle and the cell cortex which occurs at cytokinesis. × 30,000. (These pictures were taken by Mr. Dennis Landis.)

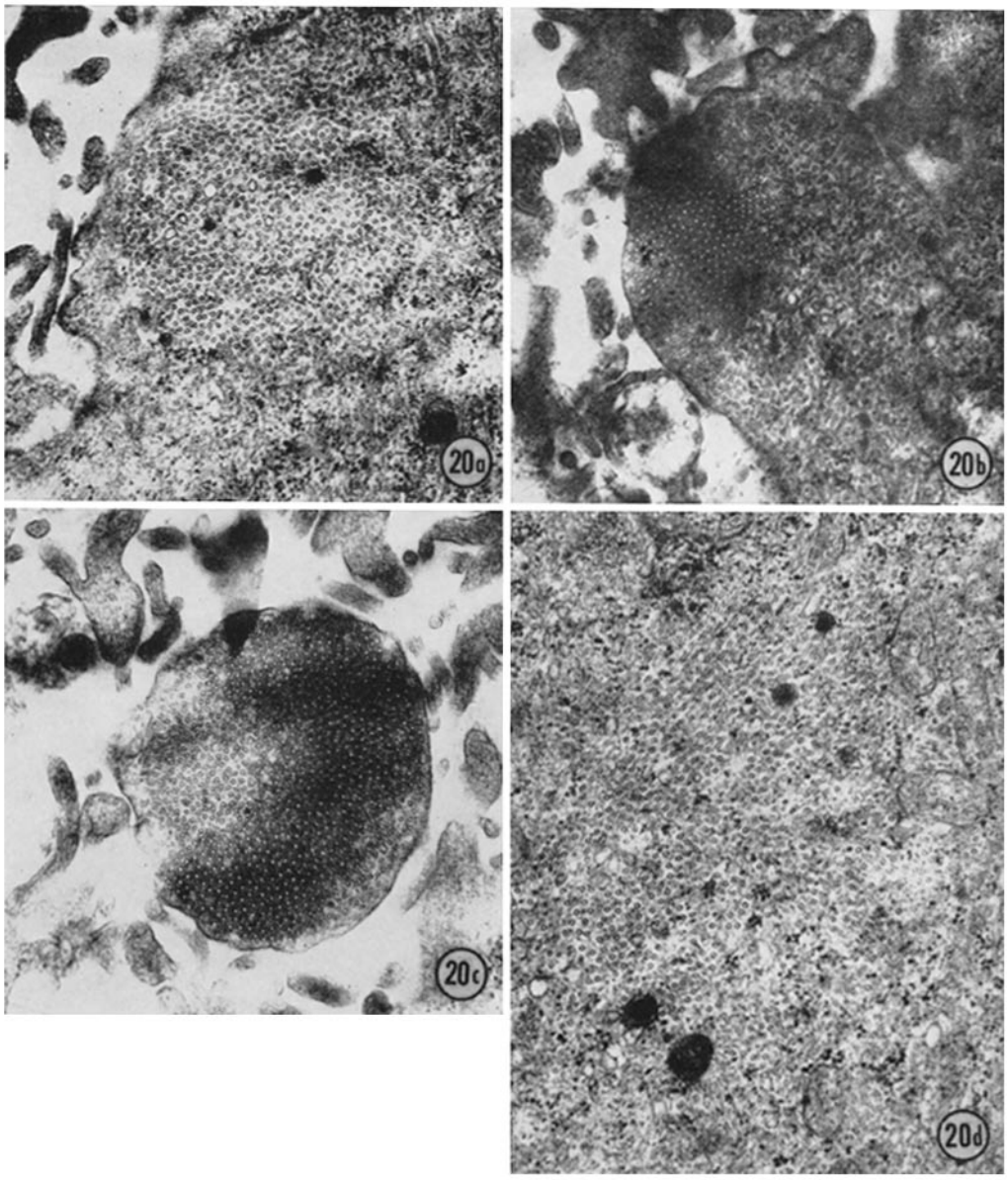

FIGURE 20 A sequence of sections from the stem of a telophase WI-38 cell. The counts corresponding to these sections are the starred bars from left to right on the distribution profile shown in Fig. 23. All four pictures were taken with the sections tilted 30° from the horizontal. No one picture contains all of the dark-staining midbody material, but a complete serial sequence reveals that all tubules in the stem are surrounded by the midbody matrix somewhere along their length. Note that there is a suggestion of structure in the matrix: each tubule is surrounded by a dark polygonal line which is closely applied to the polygonal lines of all of the neighbor tubules. × 25,000.

sister kinetochores (Fig. 30). The width of an ideal well would be the average distance between sister kinetochores, a distance which has been measured on four chromosomes in each of three WI-38 cells and is used in Fig. 30 to define the width of the ideal well for the cell graphed in Fig. 7. Based on this calculation, the WI-38 spindle shown in Fig. 7 has about 800 continuous and 800 chromosome

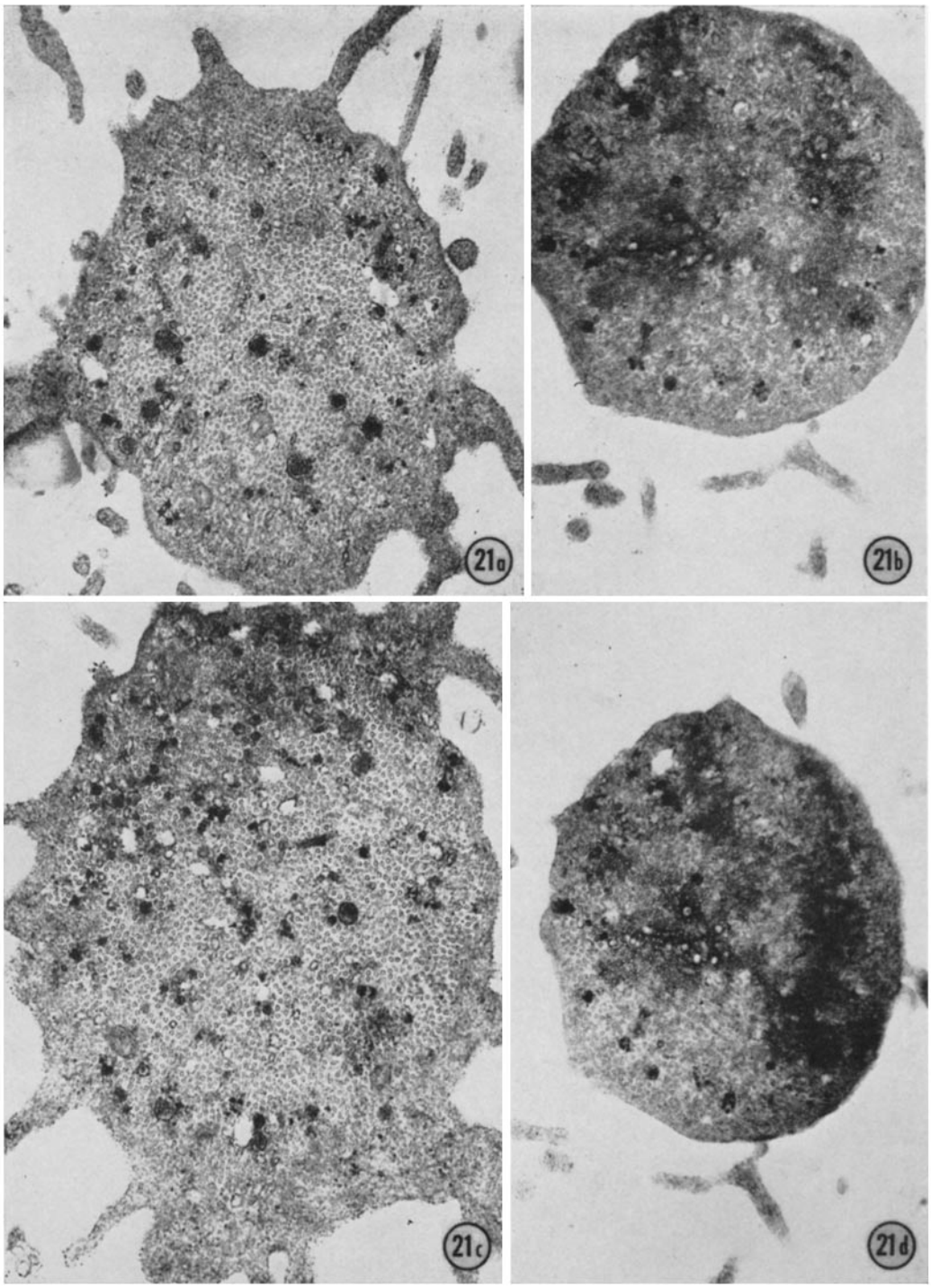

FIGURE 21 A serial sequence through the midbody of a telophase HeLa cell. Figs. 21 *a*, 21 *b*, and 21 *c* are sections cut before, in, and after the midbody, micrographed with a tilt of 25°. Fig. 21 *d* is the same section as Fig. 21 *b*, but tilted 15° on the other side of horizontal. In some cases a given angle of tilt does not reveal all of the tubules in the section. Note the large number of membranous vesicles which are among the tubules of the stem, both here and in interphase as seen in Figs. 25 and 26. The counts corresponding to Figs. 21 *a*, 21 *b*, and 21 *c* are the starred bars in Fig. 24. × 25,000.

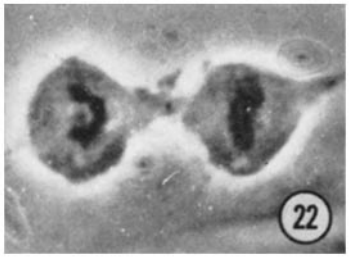

FIGURE 22 A WI-38 cell in telophase, photographed before orientation and sectioning for electron microscopy. The cup-shaped arrangement of the chromatin in the left daughter is common. The midbody is the small, dark granule in the middle of the stem. × 850.

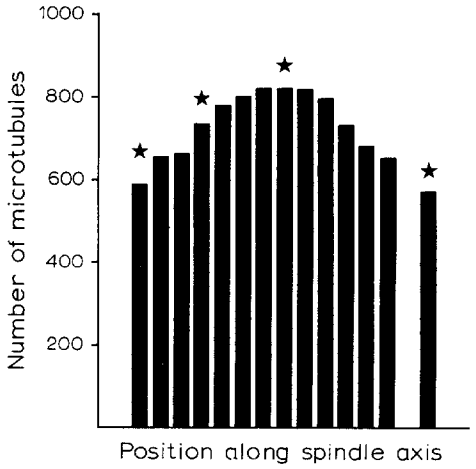

FIGURE 23 The microtubule distribution profile presenting counts over a distance of 1.5 μm on either side of the center section through the midbody of the telophase WI-38 cell shown in Figs. 20 and 22. The sections marked with stars correspond to the pictures in Fig. 20.

tubules in each half spindle, yielding the observed total of 1600 on either side of the metaphase plate. The ratio of the number of continuous tubules at metaphase to the total number of spindle tubules is thus 0.5 ± 13%. (For ease of comparison, all ratios in this discussion are expressed as decimal fractions with an unwritten denominator of 1. The standard errors are calculated from the agreement of multiple counts as defined in the Discussion of Tubule Counting above.)

In the mid-anaphase microtubule distribution profile shown in Fig. 12, the ratio of the number of tubules in the interzone to the number of tubules

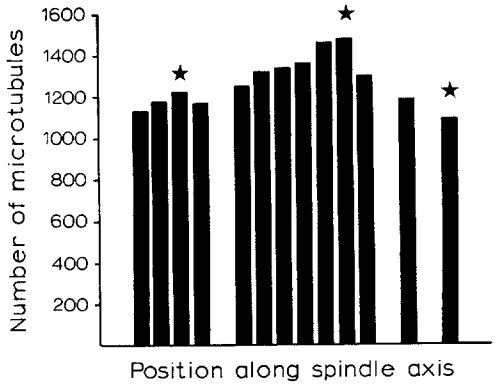

FIGURE 24 The microtubule distribution profile presenting counts over a distance of 1.6 μm on either side of the center section through the midbody of a HeLa cell in telophase. The sections marked with stars correspond to the pictures in Fig. 21.

in the region of the chromosomes (as marked by the middle and right-hand stars on Fig. 12) is 0.6 ± 22%. The number of tubules near the poles in mid-anaphase is less than the number near the chromosomes. If the reproducibility of the counts is an adequate estimate of the precision of measurement, the ratio of the minimum number of interzone tubules to the maximum tubule number between the chromosomes and the poles is approximately constant from metaphase to mid-anaphase, and there is a decrease in the ratio of the number of tubules near the poles to the number immediately poleward from the chromosomes.

The constancy from metaphase to mid-anaphase of the ratio of the minimum number of tubules between the chromatids to the maximum tubule number between the chromatids and the poles supports the interpretation that the interzone tubules of anaphase are the continuous tubules of earlier mitosis. If this is true, then it follows that during anaphase the chromosome tubules either depolymerize at the kinetochores or slide past at least some of the continuous tubules. The motions of microbeam-induced zones of reduced birefringence during anaphase (17, 18) and the anaphase migration of spindle "particles or states" (1, 3) imply that the kinetochore does not move relative to its own fiber during anaphase. It therefore appears probable that chromosome tubules slide past the continuous spindle tubules as the chromatids move to the poles.

The decrease during early anaphase in the tubule number near the poles relative to the tubule

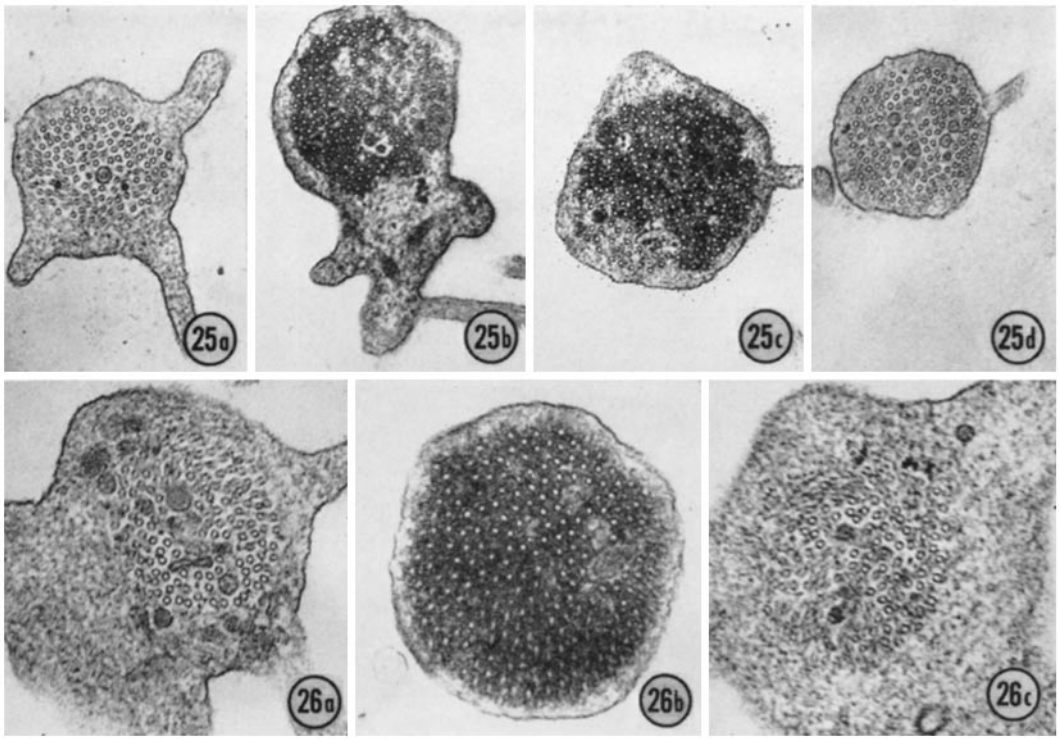

FIGURE 25 A series of sections from the stem of an interphase WI-38 cell. The stem of this cell (see Fig. 27) is about four times as long as the WI-38 telophase stem (Fig. 22). Before this elongation there seems to have been a loss in the number of stem microtubules. All pictures were taken at a tilt of 36°. × 30,000.

FIGURE 26 A sequence of sections from the stem of a HeLa cell in interphase. Figs. 26 *a* and 26 *c* are untilted; Fig. 26 *b* is tilted by 23°. The image of the tubules in the stem suggests strongly that they are helically arranged around the stem axis. × 40,000.

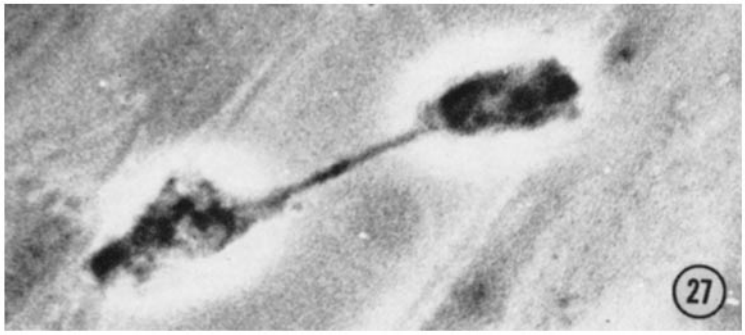

FIGURE 27 A WI-38 cell in interphase during the elongation of the stem, photographed before orientation and sectioning for electron microscopy. × 850.

number immediately poleward from the chromosomes suggests either that some spindle tubules are broken down several micrometers from the poles or that some spindle tubules are pulled away from the poles during early anaphase. The uncertainty of the counts from this region of the spindle leaves the point in considerable question.

During late anaphase in WI-38 the ratio of the

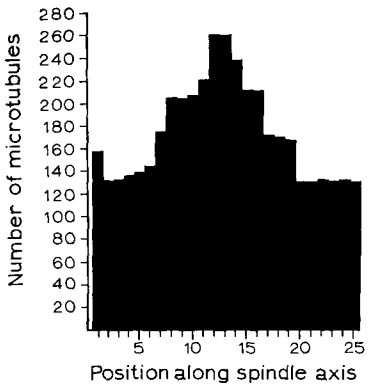

FIGURE 28 The microtubule distribution profile presenting counts over a distance of 1.2 μm on either side of the center section through the midbody of the WI-38 cell shown in Figs. 25 and 27. Every section was counted in this case.

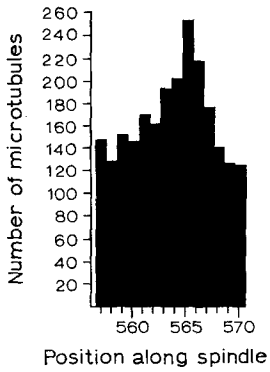

FIGURE 29 The microtubule distribution profile presenting counts over a distance of 1.4 μm on either side of the center section through the midbody of HeLa cell in interphase. This is the cell shown in Fig. 26. Counts were made on every other section.

minimum number of tubules seen in the interzone to the maximum number of tubules on the poleward edge of the chromosomes (as marked by stars in Fig. 15) decreases to 0.36 ± 15%. The ratio of the number of tubules seen in the middle of the interzone to the minimum number of interzone tubules on either side along the spindle axis is 1.5 ± 12%. The ratio of the number of tubules in the middle of the interzone to the maximum number on the poleward edge of the chromosomes is 0.7 ± 15%.

The peak in tubule number at the midregion of the interzone can be interpreted as a result either of tubule overlap or of new tubule growth. Paweletz (35) has examined this region of HeLa cells using longitudinal sections, and has shown that the stem bodies contain more tubules than the regions of the interzone fibers on either side. He concluded from his paraxial images that tubules enter a stem body from one side and terminate at its far side; he accounts for the increase in tubule number within the stem bodies by overlap. Another possible interpretation of his pictures and of our data is that the stem bodies contain short segments of microtubules which do not protrude from the region defined by the dark-staining matrix. For the latter interpretation to be correct, however, one must argue that section artifact accounts for the images shown by Pawelitz and by Hepler and Jackson (20) in which tubules appear to begin on one side of the stem body and to extend beyond it on the other side. We have obtained pictures like those published by Pawelitz and by Hepler and Jackson with sufficient frequency to believe that it is unlikely that they are artifact. Our complete serial sections parallel to the spindle axis also support the view that the tubules of the stem bodies overlap, although the evidence is not unequivocal.

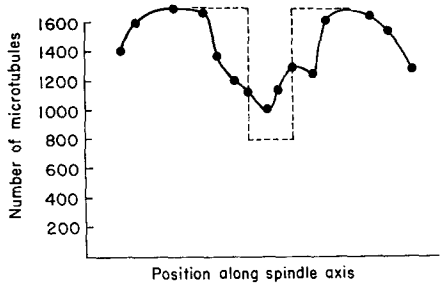

FIGURE 30 The data points show the height of the bars on the microtubule distribution profile for the metaphase cell graphed in Fig. 7. The dotted line marks off a square well those area is equal to the area of the observed well. The depth of the square well should be a measure of the difference between the number of microtubules in the continuous spindle and the total number in each half spindle (see Discussion).

The data on tubule distribution in the interzone show, however, that a simple overlap of all tubules cannot account for the observations. If all stem body tubules overlapped, the ratio of the maximum number of tubules found in the stem body-containing region of the interzone to the number in a region devoid of stem bodies should be 2. The ratio observed in our material is 1.3 ± 10% for HeLa and 1.5 ± 12% for WI-38. The discrepancy

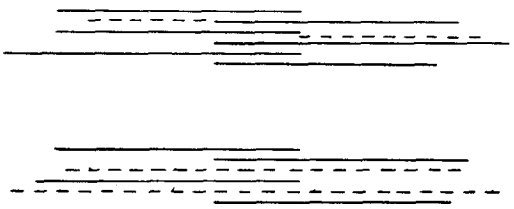

FIGURE 31 The drawing shows two possible arrangements of microtubules which would give rise to the anaphase and telophase distribution profiles shown in Figs. 15, 23, and 24. The solid and dotted lines represent two classes of interzone tubules, one of which overlaps in the stem bodies, the other of which does not. Either of the arrangements shown could be achieved in at least two ways: the two classes of tubules could have formed earlier in mitosis and then rearranged themselves to form the structure shown, or during anaphse one or more of the classes could have formed directly in the arrangement shown.

between the observed ratios and that to be expected for perfect overlap may be due in part to incomplete registration of the stem bodies, but the average of 14 ratios comparing tubule number in individual stem bodies to the tubule number in the interzone fibers on either side is only 1.5. It thus appears that if overlap is the correct interpretation of the counts, then not all interzone tubules overlap in stem bodies. Since every HeLa interzone tubule in late anaphase is associated with a stem body somewhere along its length, it follows that some interzone tubules either pass through the stem bodies or terminate on the side at which they enter the stem body (Fig. 31). Our distribution profiles cannot discriminate between the two possibilities.

The shortening of the stem bodies along the spindle axis results from the collecting of amorphous dark-staining material during late anaphase at the midplane of the cell. The origin and significance of this material is unknown, but it is morphologically similar to the electron-opaque matrix frequently associated with the ends of microtubules.

The tubule distributions in metaphase and anaphase are in large part consistent with several existing models for mitosis, including one based upon the postulate of mechanochemical cross-bridges between spindle tubules (31). Bridge action could account for nearly all of the observations reported here: for the motion of the chromosome tubules toward the poles, for the decrease during anaphase in the number of tubules near the poles, for the decrease during late anaphase in the ratio of the minimum number of tubules in the interzone to the maximum number in the region of the chromosomes, for the development of a zone of tubule overlap at the midplane of the interzone, and for the shortening of the stem bodies. Since, however, the ratio of the number of tubules in the middle of the anaphase interzone to the number on either side is about 1.5, not 2.0, it is necessary to introduce some additional feature into the sliding model to interpret the late anaphase tubule distributions. Either of the arrangements shown in Fig. 31 could account for the departure from doubling predicted by simple tubule sliding (see legend, Fig. 31).

The telophase distribution profiles in WI-38 show that the ratio of the number of tubules in the middle of the midbody to the number a few micrometers along the stem axis on either side, as marked by stars in Fig. 23, is less than the equivalent ratio at late anaphase. In HeLa (Fig. 24) the discrepancy between the anaphase and telophase ratios is of the same sign and greater. Since the stem body and midbody portions of the interzone are regions of defined paraxial length which are seen to contain more microtubules than the zones a few micrometers away along the spindle axis, the technique of graphical integration may again be applied to determine the tubule count increase which would have been observed in the ideal case of a single section which passed through all of the dark-staining material at once. The paraxial length of the stem body or midbody need not be known in absolute units, because one can determine directly from the pictures on which the counts are made how many sections are necessary to pass from one side of a dark-staining region to the other, and use this number of sections on the graph as the width for a square peak. The area under the peak should again be independent of peak shape and can be taken from the empirical curve (Figs. 32 *a* and 32 *b*).

With graphical integration the ratio of the number of tubules in the middle of the WI-38 midbody to the number on either side of the dark-staining material is about the same as it was in late anaphase: 1.6 compared with 1.5. Apparently there is little or no initiation of new tubules in the interzone of this cell from late anaphase to telophase. In HeLa, on the other hand, the average ratio of the number of tubules in the stem bodies to the number in the connecting interzone fibers on either side is 1.5, while in telophase the ratio of the

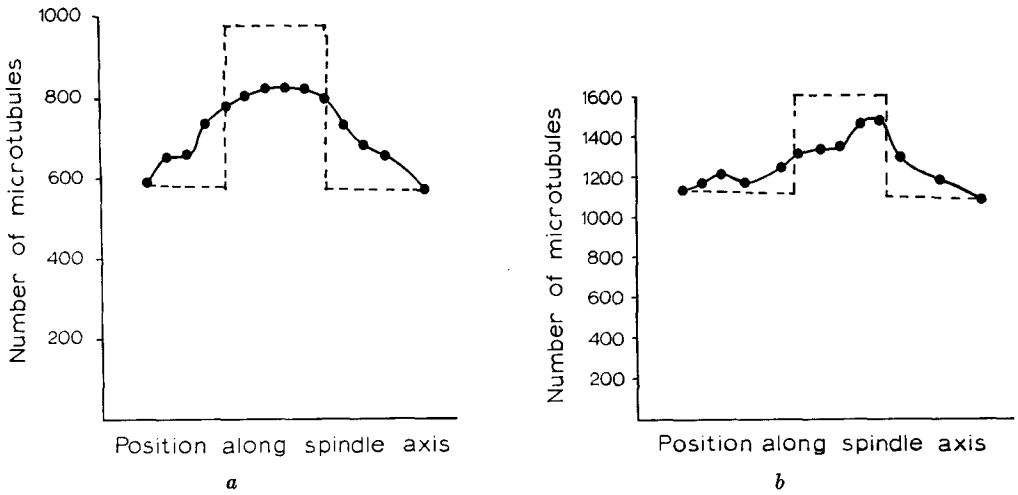

FIGURE 32 The data points on Fig. 32 *a* show the height of the bars in the microtubule distribution profile for the telophase WI-38 cell shown in Fig. 23; Fig. 32 *b* corresponds to Fig. 24. The dotted lines mark off square peaks whose areas are the same as the areas of the curved peaks observed. The height of each square peak should represent the number of microtubules which would have been observed in a perfectly registered midbody displayed in a single section.

number of tubules in the integrated midbody to the average number of tubules on either side is 1.3. In this cell there are three possible interpretations of the counts: the number of tubules in the midbody has decreased from the sum of all stem bodies, the number of tubules within the stem but outside the midbody has increased relative to its anaphase equivalent, or both processes have occurred.

From several lines of evidence, we favor the view that the number of tubules on either side of the midbody has increased. First, we have observed that the tubules in the midbody are the most stable of all interzone tubules under conditions of glycerination, and hence a decrease in the number of these tubules is not be expected. Second, the absolute number of tubules in the graphically integrated midbody is often close to the absolute number of continuous tubules observed at metaphase and to the number of interzone tubules observed in early anaphase. Third, the number of tubules on the sides of the telophase midbody is in general higher than the number of tubules found there in late anaphase. Fourth, there is compelling evidence from several systems (20, 22, 42) that during telophase the microtubules of the interzone can grow in number, in length, or both. Thus we feel that the microtubule counts from late anaphase and telophase in HeLa cells can best be interpreted as a result of new microtubule growth from the stem bodies and/or the midbody.

Microtubule growth from the stem bodies is consistent with the ideas of Bělař (5, 6) as to the "pushing body action" of these structures. Perhaps, in the tubule counts from late anaphase and telophase, we are looking at the fine structure of the cellular mechanism whereby the spindle poles are pushed apart in late anaphase and telophase. The work of Ris (37), showing a difference in drug susceptibility between the decrease in chromosome-to-pole distance and the increase in pole-to-pole distance, supports the idea that the two motions are mechanistically distinct.

The microtubule counts on the interphase stem show that the midbody persists as a zone containing more tubules than the neighboring portions of the stem. The change during early interphase in the ratio of the number of tubules in the midbody to the number on either side, from about 1.5 to the 2.0 expected from precise tubule overlap, is an unexplained phenomenon. We are currently investigating this and other problems discussed above by studying microtubule distributions during mitosis in drug-treated cells.

Regardless of interpretation, however, we can now identify certain characteristics of human spindle tubule distribution. In HeLa and WI-38 cells there is a continuous spindle at metaphase which contains about the same number of tubules as the interzone spindle of early anaphase. There are about 1.5 times more tubules in the stem bodies and midbody than in the adjacent regions of the interzone. All interzone tubules enter one stem

body, and some of the stem body tubules probably overlap. The ratio of tubule counts in the midbody to tubule counts elsewhere on the stem increases during early interphase while the total number of stem tubules usually decreases. These observations may be added to the large body of information available about mitosis which serves as the basis for any thinking about spindle mechanisms.

The authors regret that, due to financial considerations, reprints can be sent only when the *Journal* is not available in a local library.

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BIBLIOGRAPHY

1. ALLEN, R. D., A. BAJER, and J. LA FOUNTAIN. 1969. Poleward migration of particles or states in spindle fiber filaments during mitosis in *Haemanthus*. *J. Cell Biol.* **43**(2, Pt. 2):4 a. (Abstr.)
2. ALLENSPACH, A. L., and L. E. ROTH. 1967. Structural variation during mitosis in the chick embryo. *J. Cell Biol.* **33**:179.
3. BAJER, A. 1967. Notes on ultrastructure and some properties of transport within the living mitotic spindle. *J. Cell Biol.* **33**:713.
4. BAJER, A. 1968. Chromosome movement and fine structure of the mitotic spindle. *Symp. Soc. Exp. Biol.* **22**:285.
5. BĚLAŘ, K. 1927. Beitrag zur Kenntnis des Mechanismus der indirekten Kernteilung. *Naturwissenschaften.* **15**:725.
6. BĚLAŘ, K. 1929. Beitraege zur Kausalanalyse der Mitose. Untersuchungen an den Spermatocyten von *Charthippus lineatus*. *Wilhelm Roux Arch. Entwicklungsmech. Organismen.* **118**: 359.
7. BOSS, J. 1954. Mitosis in cultures of newt tissue. *Exp. Cell Res.* **7**:443.
8. BRINKLEY, B. R., J. CARTWRIGHT, and P. MURPHY. 1969. Ultrastructural analysis of spindle elongation in mammalian cells in vitro. *J. Cell Biol.* **43**(2, Pt. 2):169 a. (Abstr.)
9. BRINKLEY, B. R., and E. STUBBLEFIELD. 1966. The fine structure of the kinetochore of a mammalian cell *in vitro*. *Chromosoma.* **19**:28.
10. BUCK, R. C., and J. M. TISDALE. 1962. The fine structure of the midbody of the rat erythroblast. *J. Cell Biol.* **13**:109.
11. BYERS, B., and D. H. ABRAMSON. 1968. Cytokinesis in HeLa: post-telophase delay and microtubule-associated motility. *Protoplasma.* **66**:413.
12. CAROLAN, R. M., H. SATO, and S. INOUÉ. 1965. A thermodynamic analysis of the effect of D_2O and H_2O on the mitotic spindle. *Biol. Bull.* **129**:402 a. (Abstr.)
13. CORNMAN, I. 1944. A summary of evidence in favor of the traction fiber in mitosis. *Amer. Natur.* **78**:410.
14. DE HARVEN, E. 1968. The centriole and the mitotic spindle. *In* Ultrastructure in Biological Systems. A. J. Dalton and F. Haguenau, editors. Academic Press Inc., New York. **3**.
15. DE HARVEN, E., and W. BERNHARD. 1956. Étude au microscope electronique de l'ultrastructure du centriole chez les vertébrés. *Z. Zellforsch. Mikrosk. Anat.* **45**:378.
16. DIETZ, R. 1969. Bau und Funktion des Spindelapparats. *Naturwissenschaften.* **56**:237.
17. FORER, A. 1965. Local reduction of spindle fiber birefringence in living *Nephrotoma suturalis* (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol.* **25**(1, Pt. 2):95.
18. FORER, A. 1966. Characterization of the mitotic traction system and evidence that birefringent spindle fibers neither produce nor transmit force for chromosome movement. *Chromosoma.* **19**:44.
19. HARRIS, P. 1961. Electron microscope studies of mitosis in sea urchin blastomeres. *J. Cell Biol.* **11**:419.
20. HEPLER, P. K., and W. T. JACKSON. 1968. Microtubules and early stages of cell-plate formation in the endosperm of *Haemanthus katherinae*. *J. Cell Biol.* **38**:437.
21. HEPLER, P. K., J. R. MCINTOSH, and S. CLELAND. 1970. Intermicrotubule bridges in the mitotic spindle apparatus. *J. Cell Biol.* **45**:438.
22. INOUÉ, S. 1964. Organization and function of the mitotic spindle. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press Inc., New York.

23. 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**:259.

24. JOKELAINEN, P. T. 1967. The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. *J. Ultrastruct. Res.* **19**:19.

25. KRISHAN, A., AND R. C. BUCK. 1965. Structure of the mitotic spindle in L strain fibroblasts. *J. Cell Biol.* **24**:433.

26. MANTON, I., K. KOWALLIK, and H. A. VON STOSCH. 1969. Observations on the fine structure and development of the spindle at mitosis and meiosis in a marine centric diatom (*Lithodesmium undulatum*). I. Preliminary survey of mitosis in spermatogenesis. *J. Microsc.* **89**:295.

27. MANTON, I., K. KOWALLIK, and H. A. VON STOSCH. 1969. Observations on the fine structure and development of the spindle at mitosis and meiosis in a marine centric diatom (*Lithodesmium undulatum*). II. The early meiotic stages in male gametogenesis. *J. Cell Sci.* **5**:271.

28. MAZIA, D. 1961. Mitosis and the physiology of cell division. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press Inc., New York. **3**.

29. MCINTOSH, J. R. 1968. Periodic projections from the surface of microtubules in developing chicken sperm cells. *J. Cell Biol.* **39**(2, Pt. 2): 89 a. (Abstr.)

30. MCINTOSH, J. R., and S. CLELAND. 1969. Anaphase sliding of spindle microtubules. *J. Cell Biol.* **43**(2, Pt. 2):89 a. (Abstr.)

31. MCINTOSH, J. R., P. K. HEPLER, and D. G. VAN WIE. 1969. Model for mitosis. *Nature (London).* **224**:659.

32. NICKLAS, R. B. 1970. Mitosis. *In* Advances in Cell Biology. D. M. Prescott, L. Goldstein, and E. McConkey, editors. Appleton-Century-Crofts, New York. **2**. In press.

33. ÖSTERGREN, G. 1951. The mechanism of co-örientation in bivalents and multivalents: the theory of orientation by pulling. *Hereditas.* **37**:85.

34. ÖSTERGREN, G., J. MOLÈ-BAJER, and A. BAJER. 1960. An interpretation of transport phenomena at mitosis. *Ann. N.Y. Acad. Sci.* **90**:381.

35. PAWELETZ, N. 1967. Zur Funktion des "Flemming-korpus" bei der Teilung tierisches Zellen. *Naturwissenschaften.* **20**:533.

36. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.

37. RIS, H. 1949. The anaphase movement of chromosomes in the spermatocytes of the grasshopper. *Biol. Bull.* **96**:90.

38. ROBBINS, E., and N. K. GONATAS. 1964. The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* **21**:429.

39. ROBBINS, E., and G. JENTZSCH. 1969. Ultrastructural changes in the mitotic apparatus at the metaphase-to-anaphase transition. *J. Cell Biol.* **40**:678.

40. ROTH, L. E. 1964. Motile systems with continuous filaments. *In* Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya, editors. Academic Press Inc., New York.

41. ROTH, L. E., and E. W. DANIELS. 1962. Electron microscopic studies of mitosis in amebae. *J. Cell Biol* **12**:57.

42. ROTH, L. E., H. J. WILSON, and J. CHAKRABORTY. 1966. Anaphase structure in mitotic cells typified by spindle elongation. *J. Ultrastruct. Res.* **14**:460.

43. SCHRADER, F. 1953. Mitosis. Columbia University Press, New York, 2nd edition.

44. STUBBLEFIELD, E., and B. R. BRINKLEY. 1967. Architecture and function of the mammalian centriole. *Symp. Int. Soc. Cell Biol.* **6**:175.

45. SUBIRANA, J. A. 1968. Role of spindle microtubules in mitosis. *J. Theor. Biol.* **20**:117.

46. WILSON, H. J. 1969. Arms and bridges on microtubules in the mitotic apparatus. *J. Cell Biol.* **40**:854.