ULTRASTRUCTURAL CHANGES IN THE MITOTIC APPARATUS AT THE METAPHASE-TO-ANAPHASE TRANSITION

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ABSTRACT

As the metaphase HeLa cell approaches anaphase, pericentriolar spindle tubules fragment and become encapsulated by a unit membrane. By early anaphase, the encapsulated forms appear to have expanded, giving rise to polar spherical aggregates. Some of these elements show ribosomes on their bounding membrane, and some of them localize on the condensed chromatin during reformation of the nuclear membrane. It thus is suggested that these elements are newly derived cisternae of the endoplasmic reticulum (ER). Similar transformations are seen in later anaphase in the interzonal region, and it may be that the ER serves as a storage depot for some fraction of depolymerized microtubules. The time and location of the pericentriolar transitions are consistent with their being intimately involved in the mechanics of chromosome separation.

The universal occurrence of spindle tubules in mitotically dividing cells leaves little doubt of their importance, but how they carry out their functions or even precisely what their functions are remains to be clarified. This is especially true regarding the contribution of spindle tubules to chromosome separation at anaphase. The suggestion by 19th century cytologists that spindle tubule (fiber) contraction was responsible for chromosome movement during anaphase has been questioned because spindle tubules do not increase their diameter upon shortening as might be expected from any contractile system of constant mass (see Schrader [1] for review). The disposition of spindle tubules admirably suits them to the task of effecting chromosome separation, and Forer (2) has been able to show that destruction of spindle tubule birefringence by ultraviolet microbeam can, indeed, inhibit chromosome movement once it has begun; however, Swann (3) has noted that, shortly after the start of anaphase, spindle tubule birefringence—and presumably spindle tubule ultrastructure—could be disrupted with colchicine without impeding chromosome movement. In any case, the integrity of the spindle was necessary for initiation of anaphase since disruption in metaphase prevented further progress of the cycle. It thus appears that some interaction between the spindle tubules and the chromosomes is a necessary stimulus for karyokinesis.

In a previous paper, we described some of the centriolar, pericentriolar, and associated spindle tubule transitions that occur as the mammalian HeLa cell passes through its life cycle (4). It was suggested that certain pericentriolar electron opacities and vesicles accumulating during G_2 and prophase have a role in the formation of the mitotic spindle, since they appear during spindle proliferation and disappear when the spindle is completed. Furthermore, a direct physical continuity between the vesicles and newly forming tubules can often be demonstrated. The present report suggests that a series of vesicular transformations may also play some role in the spindle dispersion that follows metaphase. In this regard, it is of interest that Sandborn et al. (5) have suggested a general

relationship between microtubules and vesicles, while Mazia (6) has presented evidence indicating that microtubules, membranes, and other structural cell proteins have very similar amino acid composition. Mazia has suggested that these may represent a broad class of proteins which he calls tectins. While the results presented here are not applicable to all types of cells examined, they bear note as to what may be one of several mechanisms open to the cell for dispensing with spindle tubules as anaphase proceeds.

MATERIALS AND METHODS

Monolayers of HeLa cells (S_3) and diploid fibroblasts cultured on carbon-coated slides (7) were used in all studies. Cells in different phases of the mitotic cycle were selected in the light microscope for subsequent electron microscopic examination as reported earlier (7). Although it was a simple matter to recognize and select cells in various stages of prophase or anaphase, this was not true of metaphase which at the light microscope level looks the same immediately after prophase as it does just before anaphase. For obtaining metaphase cells at known points, cells were located in prophase with phase-contrast microscopy and observed at 37°C until they reached metaphase. They were then fixed at 5-, 10-, 20- or 30-min intervals subsequent to entry into metaphase. Since metaphase is, on average, 35 min long, the choice of intervals provided cells in early, mid-, and late metaphase. Cell encirclement, fixation with glutaraldehyde and OsO_4, rapid embedding, and serial sectioning were then carried out as previously described (7, 8).

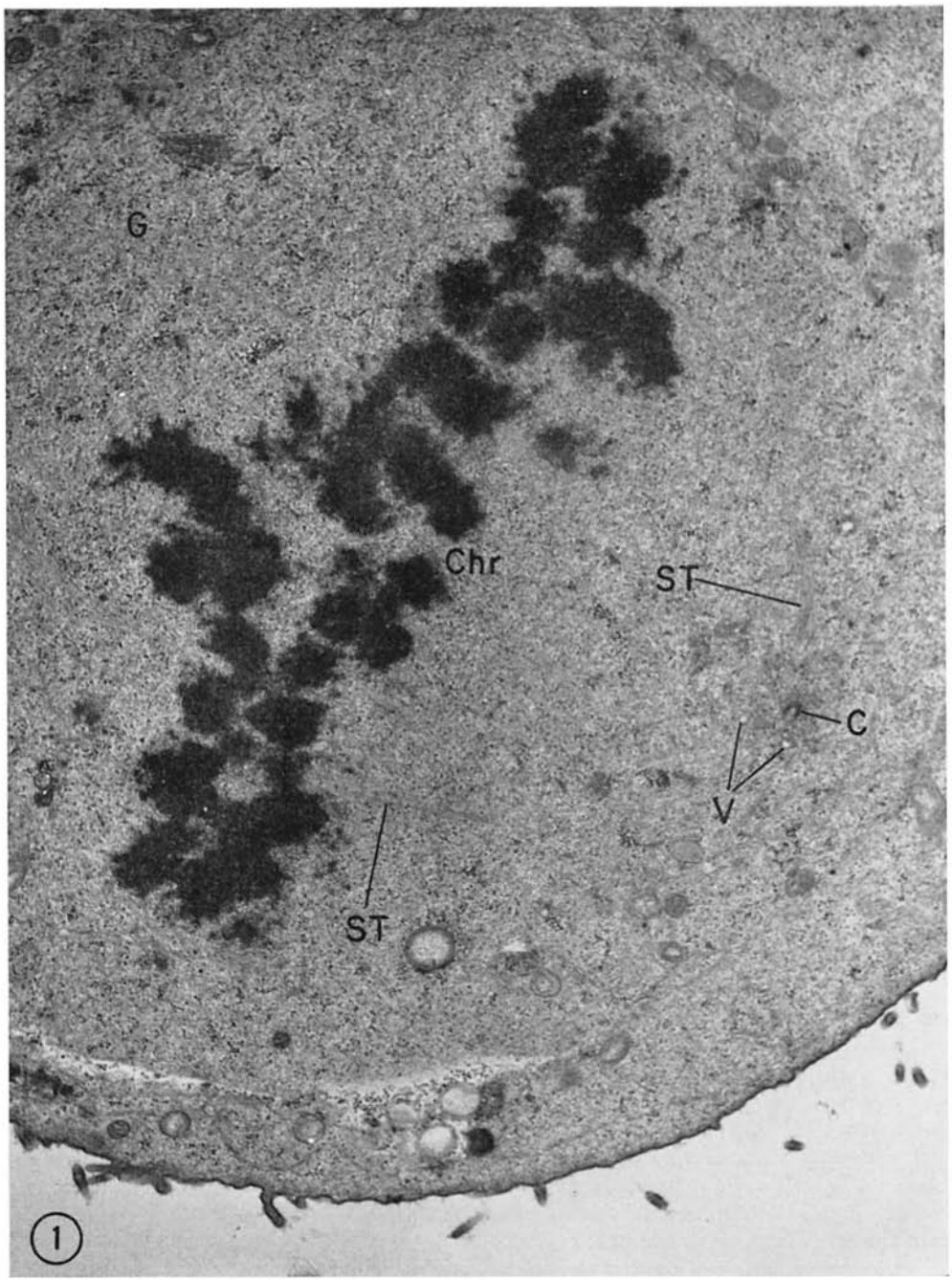
RESULTS

The only visible change in the dividing HeLa cell as it passes from early to late metaphase takes place in the pericentriolar region. The electron-opaque halo which is so prominent during prophase spindle tubule formation (4, 9) remains through early metaphase, but fades by 20 min. Pericentriolar spindle tubule morphology is unaltered during this time. However, by late metaphase a transition in these structures is evident. Fig. 1 is a low magnification of a section through a late metaphase cell; pericentriolar vesicles, essentially absent in mid-metaphase, can be seen. The tracts of spindle tubules passing from the chromosomes and curving towards the centriolar complex terminate at a variable but appreciable distance from the centriole itself, as has been frequently reported. Higher magnification (Fig. 2) reveals that the pericentriolar complex contains spherical and elongated vesicles as well as short, rodlike segments with electron-opaque walls. At × 80,000, the dimensions of these segments (Fig. 3) are assessed as identical to those of spindle tubules, and the electron opacity of the segment wall is seen as caused by the juxtaposition of a unit membrane (*2*). Some of the elongated vesicles in the figure are completely surrounded by a unit membrane, but still maintain the same width as spindle tubules (*3*). Spindle fragments (*1*), dumbbell-shaped vesicles (*4*), and spheroids (*5*) are also seen in the field. Flat cisternae cut in their short axis could conceivably give this picture; there are, however, several factors which mediate against this possibility. These factors include the delimited localization of the structures under consideration, the specific time of their appearance (i.e. late metaphase), and their consistent measurements from one cell to another. In addition, since all preparations are made from serial sections it has often been possible to determine that sectioning of flat cisternae was not involved.

Sections taken from cells in early anaphase, just a few minutes subsequent to the stage of the cycle shown in Fig. 3, show the accumulation of extensive pericentriolar vesicular aggregates. The elements of these are irregularly spherical and their content is slightly electron opaque (Fig. 4). At low magnification, they bear resemblance to the previously described, transformed lysosomal aggregates of mitosis (9); however, the individual spheroids of these aggregates are smaller than lysosomes, are less intensely stained with heavy metals, and are negative for acid phosphatase reaction product. Higher magnification of the polar region (Fig. 5) shows that these irregular membrane-bounded spheroids are intimately admixed with elements of the endoplasmic reticulum, and that some of them even possess membrane-bounded ribosomes (*R*, Fig. 5). Fig. 6 shows several spheroids, possibly derived from the polar vesicular aggregates, taking their place on the condensed chromatin mass, presumably contributing to the reformation of the nuclear envelope.

As the cell progresses further into anaphase and the nuclear envelope is reconstituted, the pericentriolar activity we have described subsides (Fig. 7); however, the time in mitosis at which this occurs is variable, and instances of elongated vesicles in the pericentriolar sphere have been seen as late as telophase.

At the stage of anaphase pictured in Fig. 7 we



Abbreviations

Chr, chromosomes
ST, spindle tubules
C, centriole
V, vesicle
MBF, membrane-bounded spindle tubule fragment
STF, spindle tubule fragment
VA, vesicle aggregate
ER, endoplasmic reticulum
R, ribosome
EV, elongated vesicle
NM, nuclear membrane
MB, mid-body
CV, coated vesicle
um, unit membrane
G, Golgi apparatus

FIGURE 1 Low magnification of late metaphase cell. Centriolar complex and pericentriolar vesicles are indicated. Although the Golgi apparatus usually disappears during metaphase, isolated fragments are occasionally seen (*G*). × 10,500.

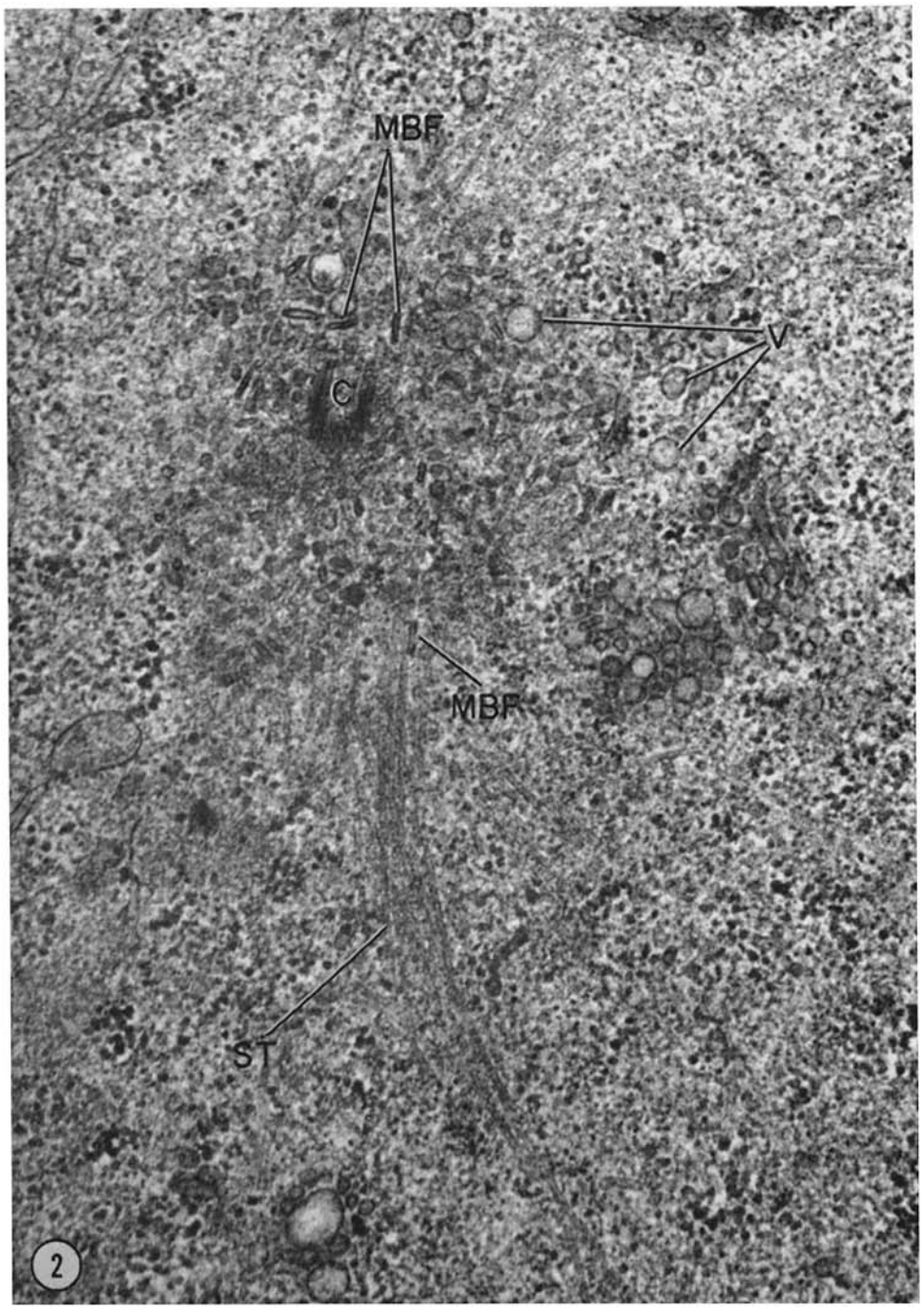


FIGURE 2 Higher magnification of centriolar complex from the same cell as in Fig. 1. Note fragments of spindle tubules and rodlike segments with electron-opaque wall (*MBF*). Numerous vesicles are indicated (*V*). × 35,000.

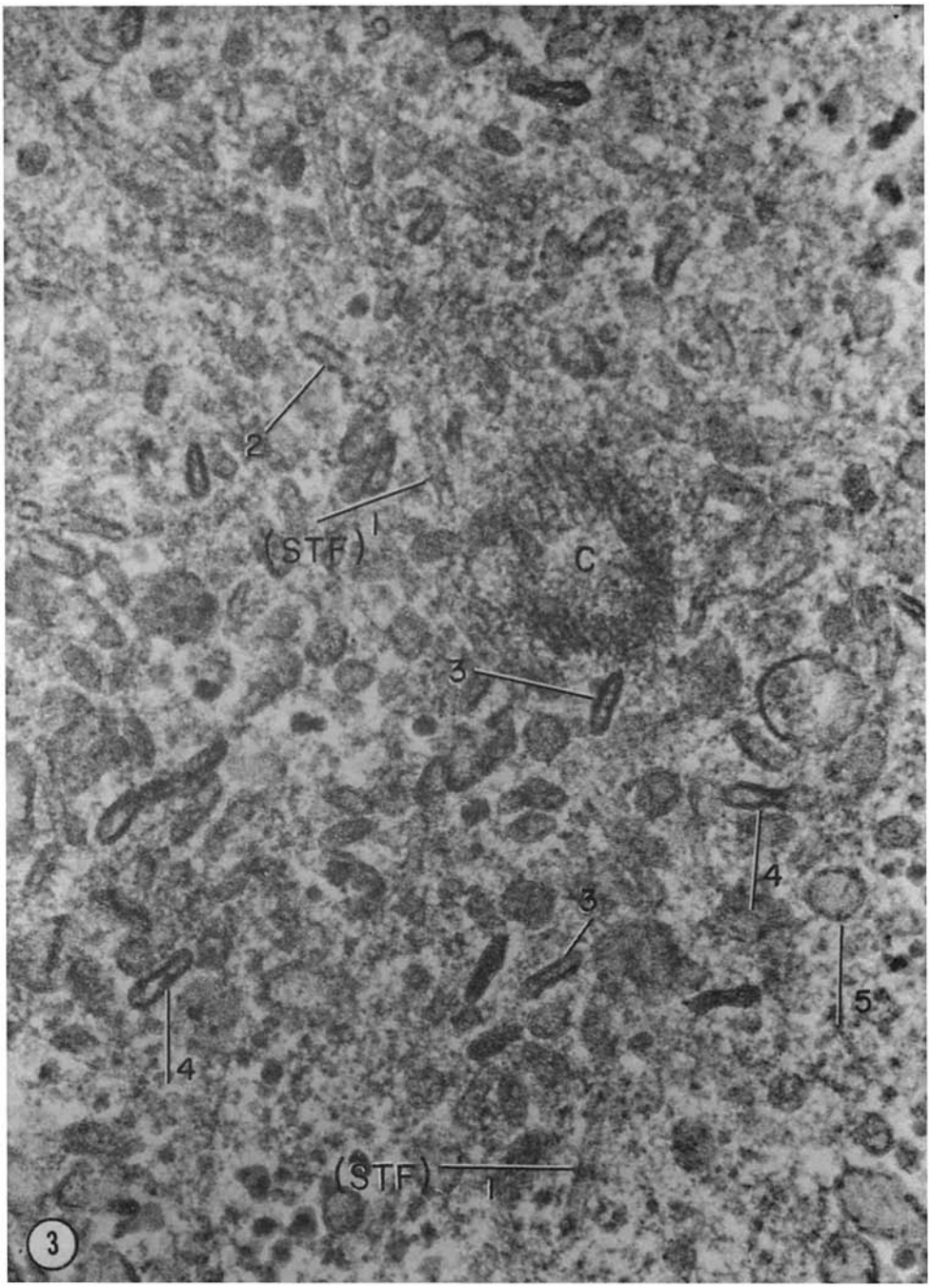


FIGURE 3 High magnification of a section through a late metaphase cell showing possible sequence of fragmentation and encapsulation of spindle tubules. See text for details. × 87,000.

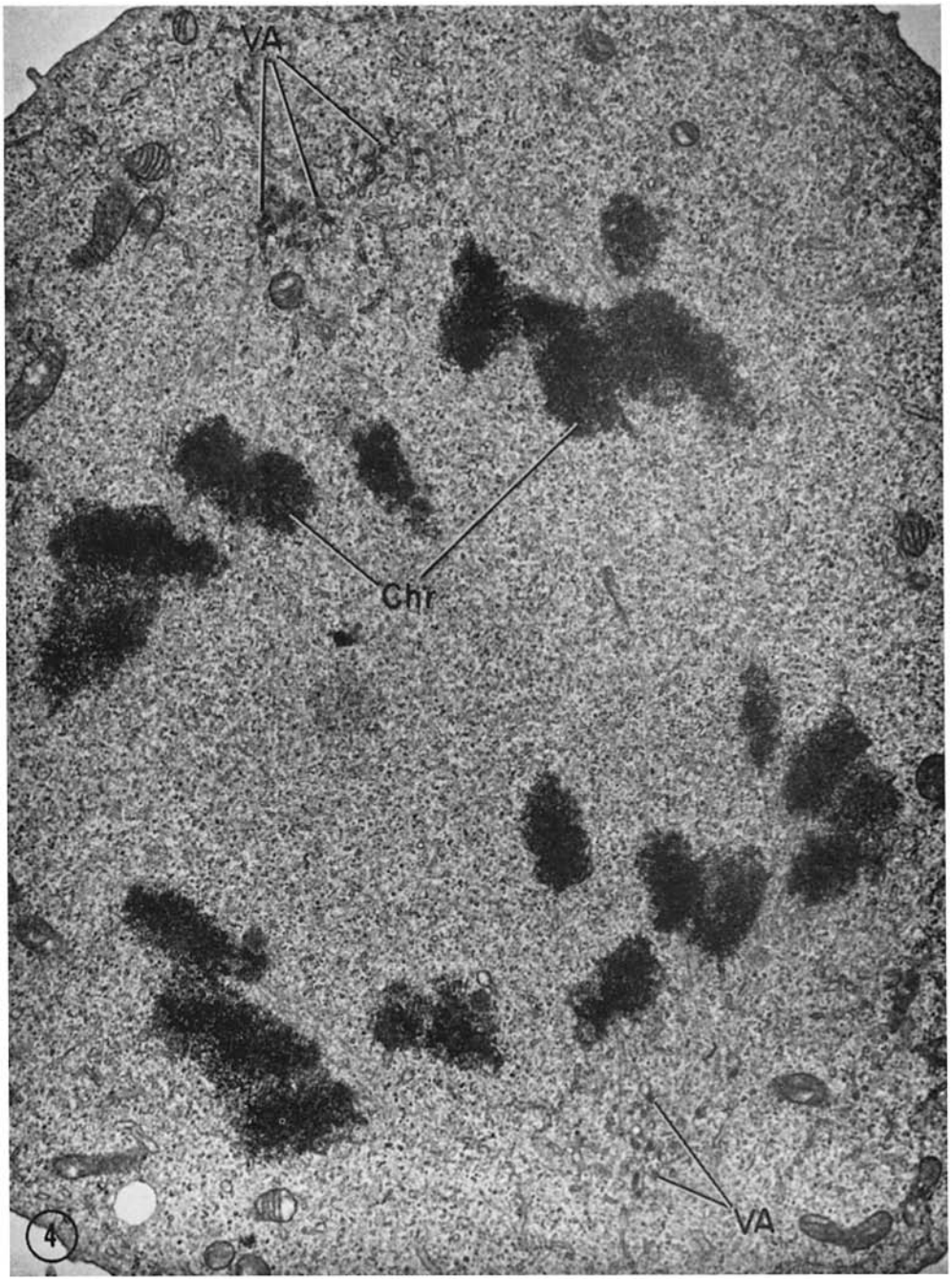


FIGURE 4 Early anaphase cell. Polar vesicular aggregates are apparent. × 10,000.

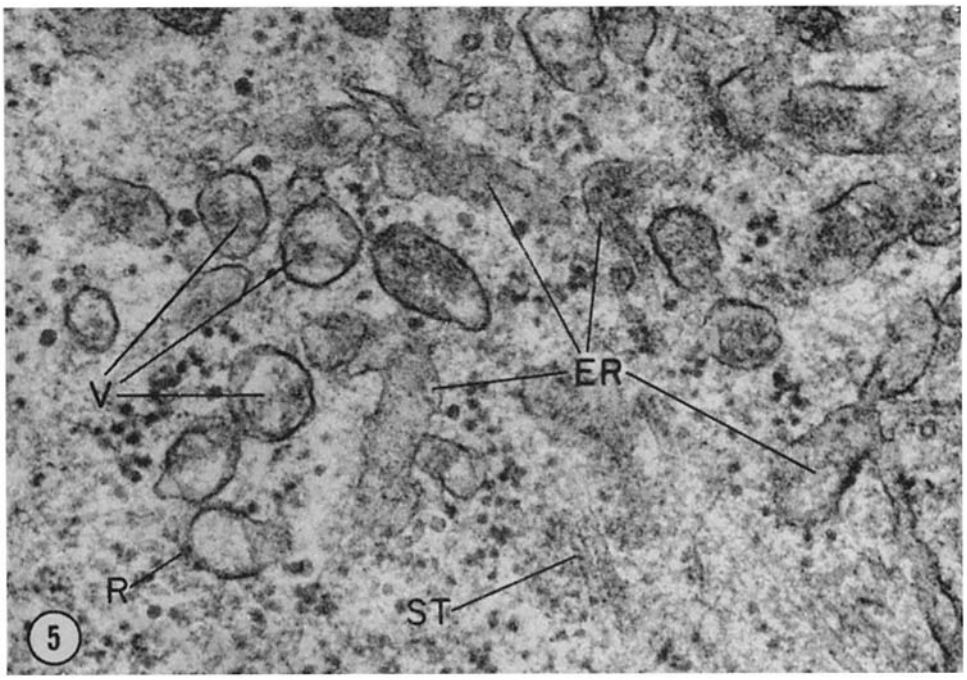


FIGURE 5 High magnification of polar vesicular aggregates showing intimate relationship of endoplasmic reticulum and a spheroid with attached ribosomes. × 63,000.

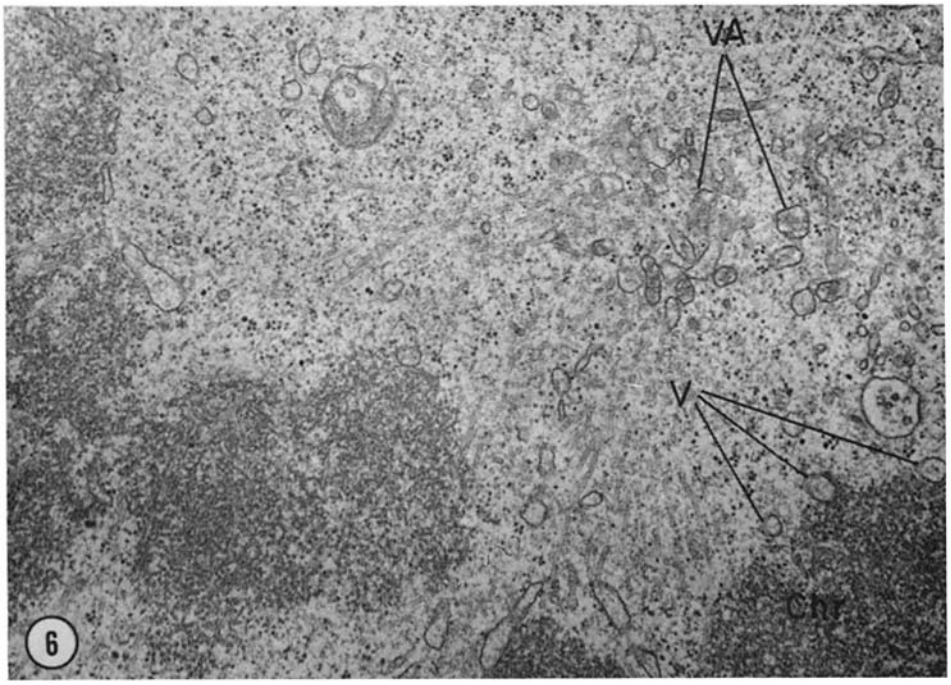


FIGURE 6 Lower magnification of polar aggregate in early anaphase cell showing several spheroids attached to the chromosomes at the stage of nuclear membrane reformation. × 20,000.

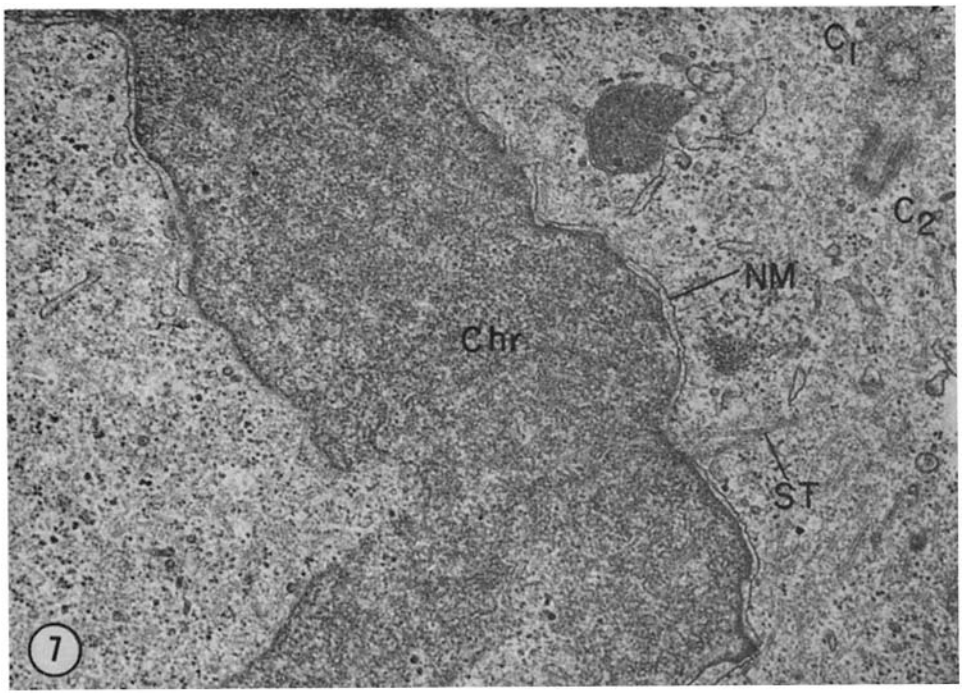


FIGURE 7 Mid-anaphase pericentriolar complex is seen to retain little of the activity found in the early stages. Spindle elements and vesicles are sparse. × 22,000.

can detect interzonal transitions that are similar to those seen in early anaphase around the centriole. itig. 8 shows an example of this at low magnifica-Fon. The mid-body is evident as increased electron opacity along the tracts of spindle tubules. *VA* leaders point to several aggregates of vesicles and short, sometimes electron opaque, rodlike segments. At higher magnification (Fig. 9), it is clear that the general pattern is similar to that already described for Fig. 5. There are elongated vesicles and lengths of rodlike segments bounded by a unit membrane. These segments have the dimensions of the spindle tubule. *1* and *2* indicate two structures of equivalent dimensions: one is a segment of spindle tubule while the other is electron-opaque and bounded by a unit membrane (see insert, Fig. 9). *3* points to an irregular, elongated element which is also membrane bounded and which appears to be in direct continuity with one or more spindle tubules. The coated vesicles (*CV*) of which there are four in this section and five in the previous figure are common in the Golgi region of interphase cells, and have been implicated as primary lysosomes (10).

Variations

The transitions in HeLa cells that we have illustrated above were intensively searched for in several other cell types including three diploid fibroblast strains cultured in this laboratory. The anaphase phenomena that we have observed in these cells is considerably less distinctive than those in HeLa although they may be related. Fig. 10, for example, is from a diploid fibroblast in early anaphase. Note the aggregates of vesicular elements within the spindle between the chromosomes and the centriole. While this localization is in contrast to the pericentriolar localization of these elements in HeLa, their function may be the same.

DISCUSSION

As chromosomes move from metaphase plate to poles, the chromosomal spindle tubules shorten. These tubules do not seem to contract since their diameter remains the same, and it therefore appears reasonable to suggest that they are somehow depolymerized at either their kinetochore or

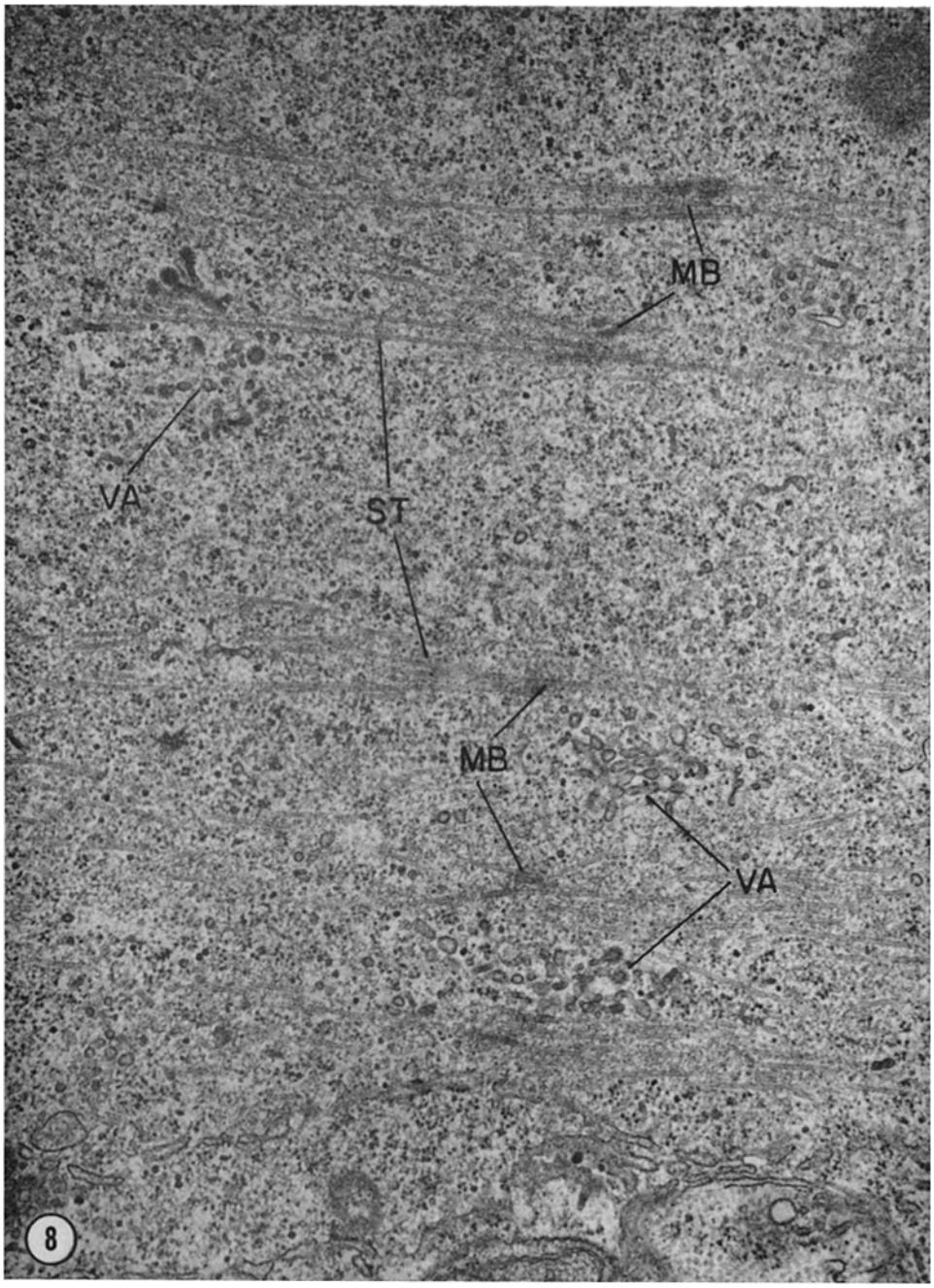


FIGURE 8 Mid-body region from same mid-anaphase cell illustrated in Fig. 8. Vesicular aggregates in close association with spindle tubules are conspicuous. Several coated vesicles are evident. × 22,000.

centriolar end. Harris has noted the absence of spindle in the centrosphere of the mitotic apparatus isolated from prometaphase cells (11), and Harris and Bajer (12) have shown a decreased peri-centriolar birefringence indicating that this region is one of decreased molecular organization. It, therefore, is conceivable that tubules in this area have an increased propensity to break down. This

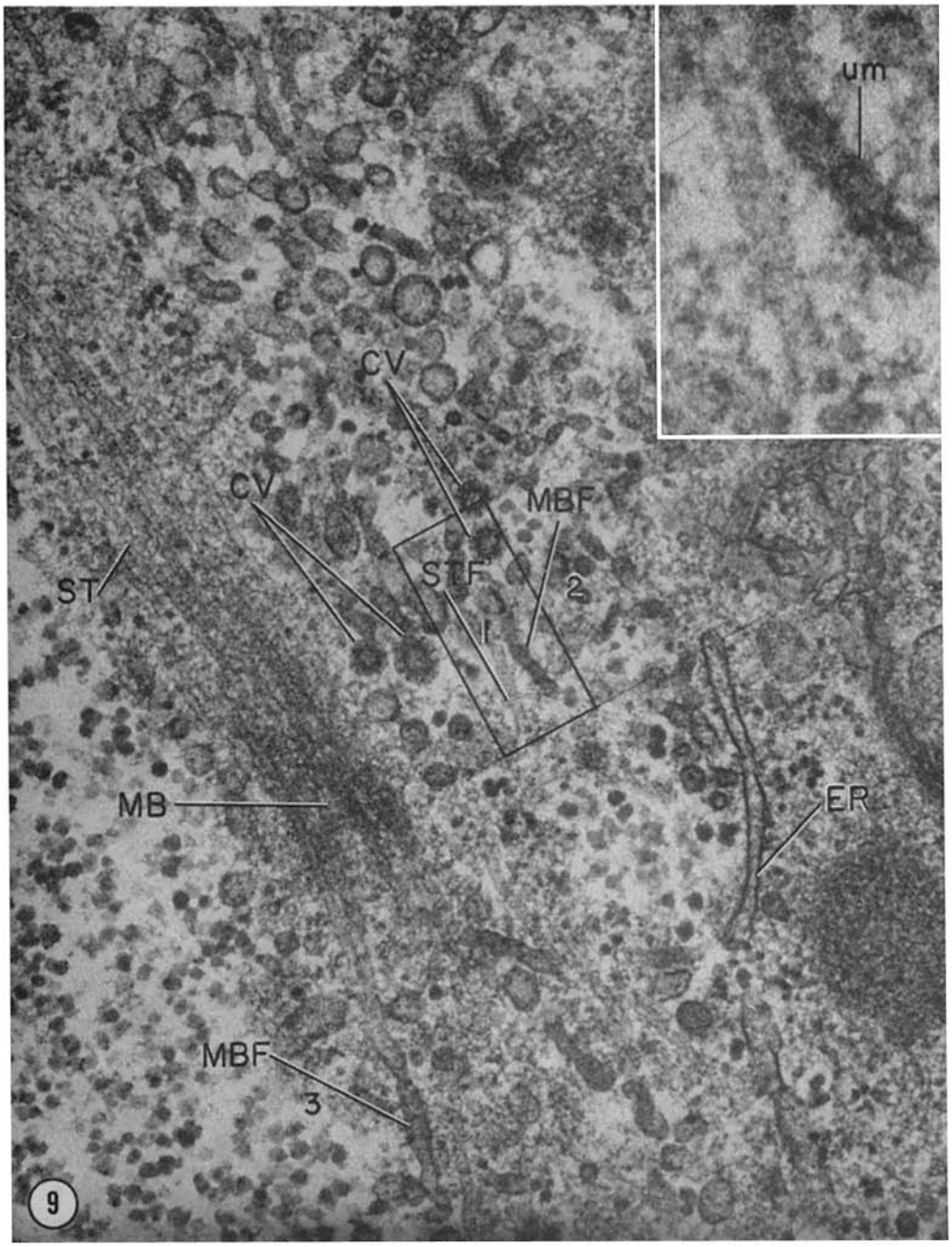


FIGURE 9 Higher magnification of interzonal region in mid-anaphase cell indicating spindle tubule fragmentation and encapsulation. Also note numerous coated vesicles. × 53,000. Insert: High power of area outlined by black square. Note unit membrane bounding one osmiophilic spindle fragment. × 185,000.

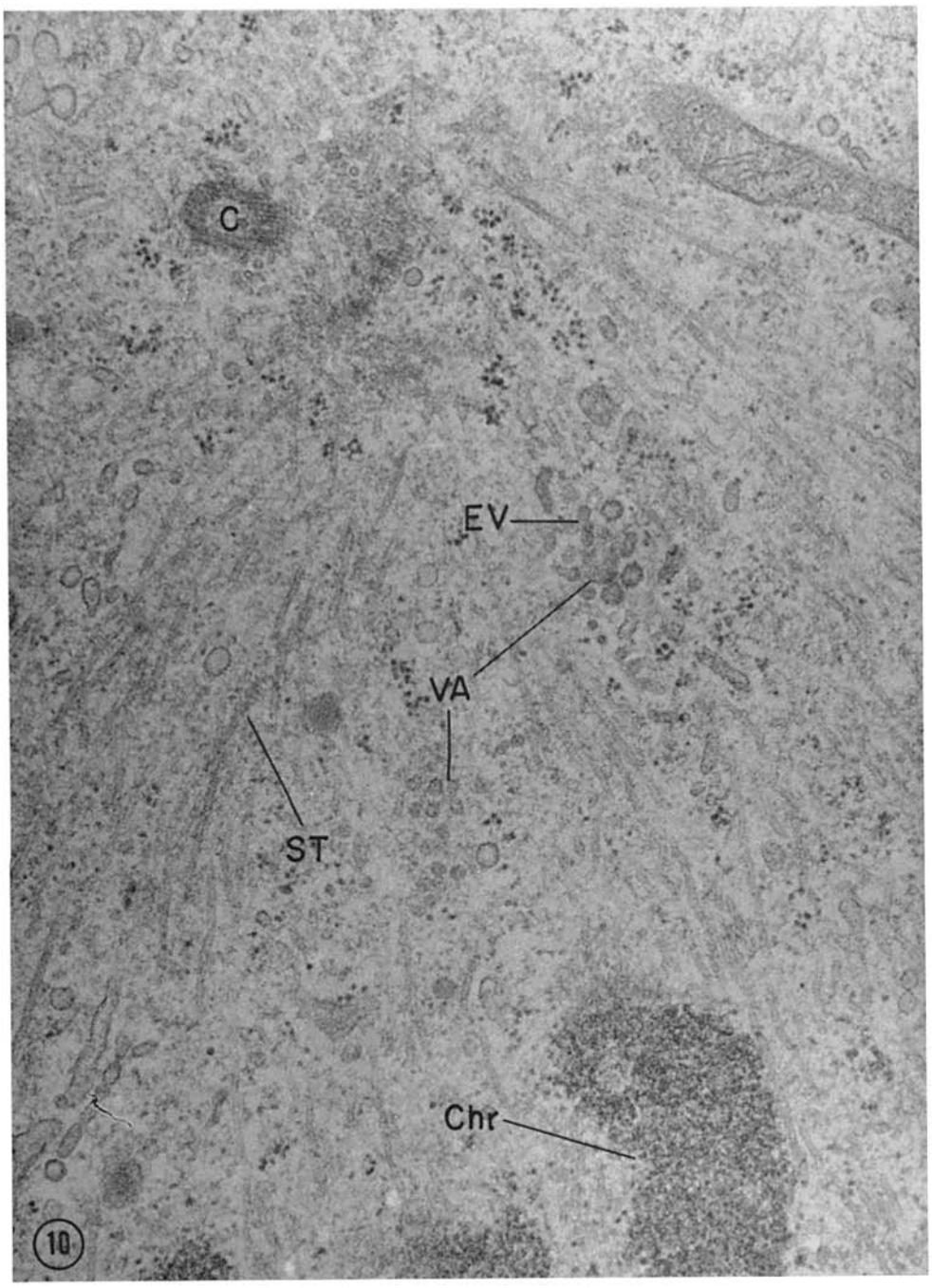


FIGURE 10 Intraspindle vesicular aggregates near, but not at, pole of diploid fibroblast in early anaphase. × 30,000.

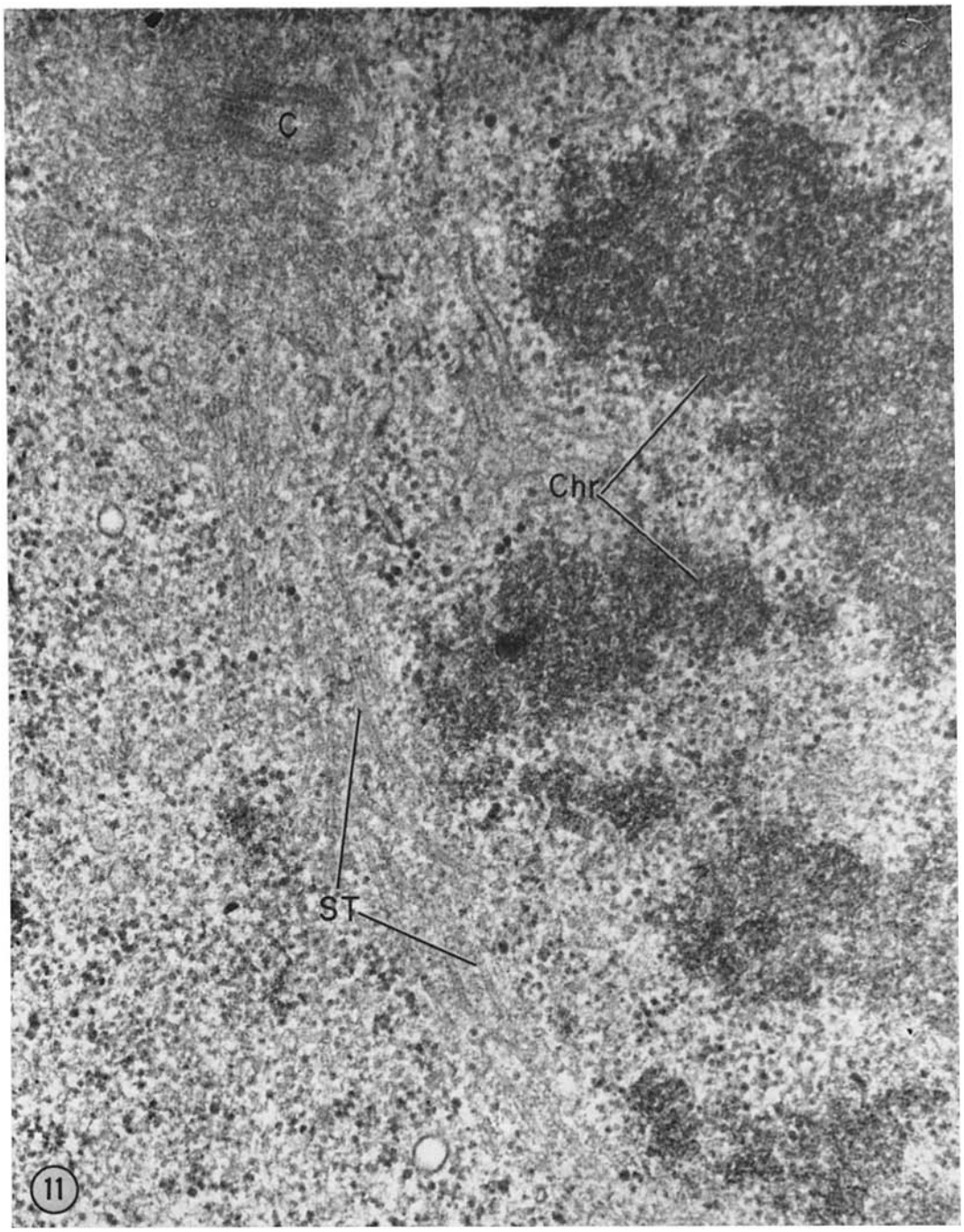


FIGURE 11 Cell in metaphase treated with colchicine 6 min. before fixation. Note that chromosomal tubules are intact except in pericentriolar region where the inhibitor seems to have preferentially depolymerized them. × 60,000.

is also supported by the effects of colchicine added to metaphase Hela cells. For example, in Fig. 11 we note that 6 min after exposure to the drug (1 μg/ml) pericentriolar tubules have been selectively dispersed while the remainder of the chromosomal tubules appear intact. The pictures described above may be one possible way that this breakdown is normally expedited in the mammalian HeLa cell, i.e. the spindle tubules are fragmented and encapsulated at their centriolar end. *1–5* in Fig. 3 provide an illustrative basis for this hypothesis as follows: *1* designates a typical spindle tubule fragment. Just prior to the onset of anaphase, encapsulation of these fragments occurs: the first step in the process consists of juxtaposing a unit membrane along the border of the open-ended tubule fragment (*2*). *3* indicates the next step; here the fragment is completely encapsulated by a unit membrane but still retains the dimensions of the spindle tubule. *4* and *5* show the expansion of the elongated, fragment-containing vesicle into a more spherical form. By anaphase, the process is further advanced in that most of the fragments and elongated vesicles are gone and polar vesicular aggregates are now prominent. Polar vesicles have previously been associated with nuclear membrane reformation (13, 14) and, while it is clear that polar aggregates in general do not necessarily originate at their polar locations (15), the closeness in time of the stages shown in Figs. 3 and 4 strongly suggests that these two figures represent a continuum. In other words, the anaphase polar spheroids of Fig. 4 are derivatives of the metaphase structures in Fig. 3.

The process that we call spindle tubule fragmentation and encapsulation is detectable before the visible onset of karyokinesis as shown in Fig. 1. Although we obviously cannot say with any conviction that this process plays a role in the initiation of chromosome movement, it is conceivable that centriole-to-kinetochore connections are important in maintaining the chromosomes on the metaphase plate, with spindle tubule depolymerization at the centriolar end allowing the migration of the chromosomes.

The spindle tubule transitions shown in the mid-body region during mid-anaphase (Figs. 8 and 9) may depict another view of the general way in which HeLa cells dispose of unnecessary spindle tubules. On the other hand, it is interesting to consider a second explanation which evolves from the findings of Allenspach and Roth (16). These authors have commented on spindle tubule elongation during anaphase and have suggested that "intususception" of spindle tubule elements may be responsible. The midbody vesicular complexes of Figs. 8 and 9 could provide a morphological basis for such spindle elongation. In addition, we perhaps should keep in mind that this stage of anaphase is the time at which the Golgi apparatus, largely dispersed at metaphase, reforms. The presence of coated vesicles in the mid-body may have significance for this process since these structures are so frequently observed in association with the Golgi complex.

The finding that spindle tubule protein has a much longer half-life than the visible spindle[1] raises the possibility that encapsulation of tubule fragments by membranes which ultimately show characteristics of either the Golgi complex or ER could furnish a convenient storage depot for at least some tubule subunits. This is no to say that all spindle tubule protein is stored in vesicles or even that encapsulated tubule units remain in vesicles for any prolonged period. It is possible that the anaphase vesicles serve merely as a means of transporting subunits to other points in the cytoplasm where they are then released for use as necessary.

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