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Centriole Behavior in Early Mitosis of Rat Kangaroo Cells (PTK_2)

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Abstract. Behavior of the centriolar duplexes during early mitosis was investigated. In general, both duplexes are capable of migrating about the cell as a unit with little change in their center to center spacing prior to separation to form the spindle poles. This duplex separation may occur at any point within the mid-prophase-prometaphase period. If it is delayed to prometaphase, transitory monopolar spindles were observed.

Introduction

Events during spindle formation have been extensively documented at both the light and electron microscope level (Robbins and Gonatas, 1964; Krishan and Buck, 1965; Murry *et al.*, 1965; Brinkley and Stubberfield, 1970; Nicklas, 1971). Recently, Roos (1973) presented a comprehensive study of mitotic events in rat kangaroo cells. This study suggested that centriolar separation may not always precede nuclear membrane breakdown but may be delayed to prometaphase. Further, the position of the centriolar duplexes may vary greatly with respect to the prophase nucleus.

In the present study, rat kangaroo cells (PTK_2), and particularly a clonal subline established in our laboratory, were utilized to further investigate early mitotic phenomena. The optical properties of this clone allowed visualization of the centrosomal regions throughout mitosis. Variation in patterns of centriolar movement and observations concerning their relationships to early organization of the mitotic spindle are presented.

Materials and Methods

Rat kangaroo cells (PTK_2) obtained from the American Type Culture Collection and a clonal subline (PTK_2W) derived from an apparent tetraploid cell that had in culture reverted back to near-diploid ($2N=14$) were grown as monolayer cultures in Eagle's Minimum Essential Medium with 15% fetal calf serum. Cells harvested from stock cultures were seeded in Rose chambers for analysis. PTK_2W cells were primarily used due to the clarity of the centrosomal region during mitosis. However, all patterns were documented in the established nonclonal PTK_2 line.

Still and cinephotographic records of centriolar behavior were recorded and timed using a Sage series 500 cinephotomicrographic apparatus operated at 30 frames per minute, utilizing Kodak Plus-X Reversal film, mounted on a Zeiss photomicroscope with a 100× oil immersion phase objective. Rose chambers were maintained at 37°C during photography using a Sage air curtain incubator.

For electron microscopy, 50 cells in which duplexes could be recognized were followed by phase microscopy to various points in mitosis. The cells were fixed by injecting 3% glutaraldehyde into the chambers and treated as described previously (Rattner and Berns, 1974). Serial sections prepared on an LKB Ultratome III with a diamond knife were examined in a Siemens Elmiskop IA microscope operated at 60 KV.

Results

Two events typify the behavior of the centriolar duplex in early mitosis (1) initial orientation to the nucleus, (2) duplex separation and the establishment of presumptive spindle poles. Considerable variation was seen in each of these phenomena (Fig. 1).

Duplex Orientation. During movements which orient the centriolar duplexes to the nucleus, the distance between the duplexes remains constant such that the two duplexes act as a unit within the cell (Fig. 1a—c). Such movements were either completed prior to prophase or occurred within the prophase-prometaphase period and required 15 minutes or less for completion.

In thin section, cells fixed while in the process of establishing this orientation revealed two duplexes with a center to center spacing of 0.6–1.3 μ. A radial array of microtubules was observed about the duplexes and frequently small bundles of microtubules were observed extending towards the plasma membrane (Fig. 2).

Duplex Separation. Centriolar duplexes frequently separated and moved to the poles during prophase (Fig. 1e—h). Generally both duplexes were motile. However in some cases, one duplex remained stationary while the other migrated (Fig. 1i—l). The duration of detectable movement was generally 7–10 minutes. Where the duplexes could be followed throughout migration, the region between duplexes generally appeared clear with occasional mitochondria oriented along the axis of separation. However, once the duplexes had reached the presumptive poles, organelles within the region of separation appeared either to maintain their prior orientation or to increase in number and orient randomly.

When seen in thin section, duplexes displaying prophase separation were initially surrounded by a radial array of microtubules. Subsequent to separation, microtubule profiles appeared between and around duplexes (Fig. 3). These tubules persisted and increased in length until each duplex reached the presumptive pole. Microtubules between duplexes did not appear to form a discrete band but appeared to radiate from the duplex in all directions. Single elements extending directly between duplexes were observed infrequently. In single sections or by serial reconstructions of some cells in which the duplexes had just reached their final position after separation, microtubules between duplexes were no longer apparent (Fig. 4). Rather, microtubules appeared to persist only in the duplex region until the onset of nuclear envelope breakdown when microtubules appeared about and through the nucleus interconnecting the duplexes. This transition occurred quite rapidly and was often coincidental with breakdown of the nuclear envelope. In general, this morphology was observed in cells in which the path of separation formed an arc about the nucleus and did not correspond with the axis of the presumptive spindle. When the path of separation remained linear, the microtubular elements between duplexes appeared to persist.

Frequently, duplexes maintained a constant center to center spacing until after breakdown of the nuclear membrane. Gradually with nuclear membrane dissolution, the chromosomes arranged themselves around a single bi-duplex pole. The chromosomes formed either a raidal or fan-shaped array. These monopolar configurations were observed for 5–15 minutes. In thin sections of cells forming a monopolar spindle at the prophase-prometaphase transition, the two duplexes

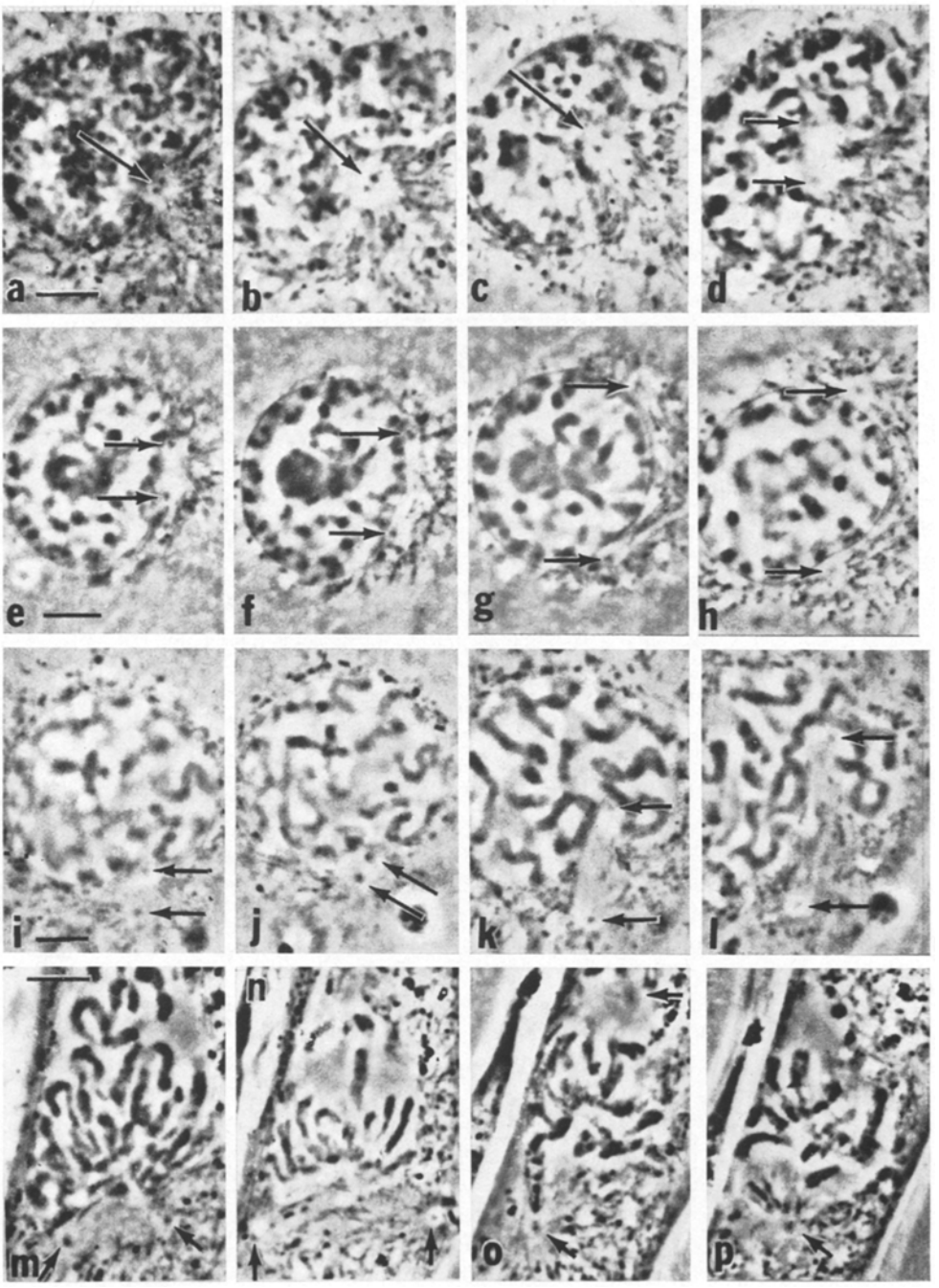


Fig. 1a—p. Prophase to prometaphase. (a)—(d) Early prophase cell where coordinated duplex migration results in a repositioning of the duplexes from the peripheral to the central portion of the nucleus. Subsequently, separation and migration are initiated across the nuclear surface (d). (e)—(h) Early prophase cell in which duplex separation and migration occur along the nuclear surface. Both duplexes are motile. (i)—(l) Mid-prophase cell in which migration occurs across the nuclear surface. Only one duplex appears motile. (m)—(p) Prometaphase monopolar cell in which duplex separation occurs parallel to the chromosomal mass. Subsequently, one duplex migrates about the chromosomal mass resulting in a reorientation on the spindle axis. (a)—(l) Bar = 3 μ, (m)—(p) bar = 1 μ

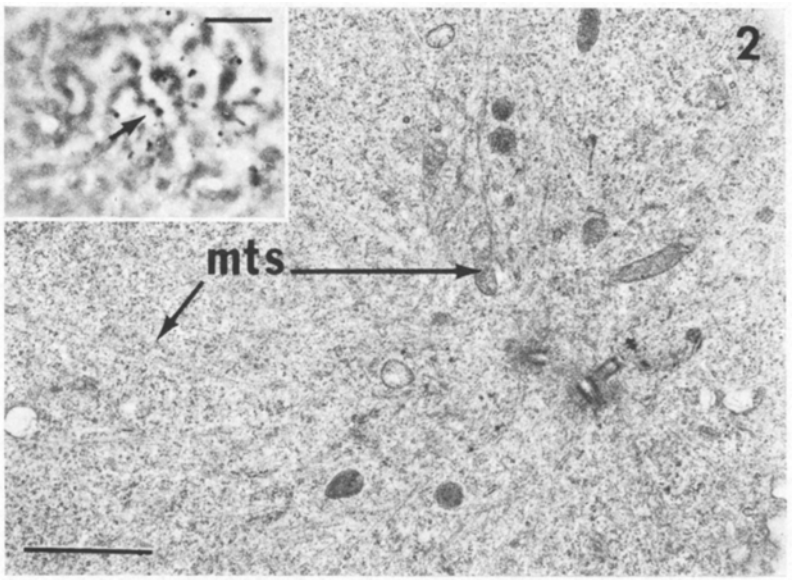


Fig. 2. Low power micrograph of bi-duplex movement across an early prophase nucleus. Duplexes maintain a close center to center spacing and microtubules (*mts*) are seen extending radially from the duplex region (Bar = 1 μ). Inset: phase micrograph (arrows-centrosomal region). (Bar = 5 μ)

maintained center to center spacing of 0.6–1.3 μ. Microtubules extended from the two duplexes around the outer margins of the nuclear envelope and into the nucleus and frequently passed along the chromosomes (Fig. 5). In general, only one sister kinetochore displayed microtubule association thus establishing the monopolar organization. This pattern was disrupted by the initiation of centriolar separation. In this situation, either one centriole remained stationary or both participated in the movement. If separation was in the same plane as the flattened mass of chromosomes, both duplexes were generally motile. In some cells, the two centrioles simply separated from each other, and their path of separation became the main axis for the mitotic spindle (Fig. 6). In other cells a second rotary movement involving one or both duplexes followed the separation of the duplexes such that the final spindle axis became displaced from the original line of centriolar separation by as much as 90° (Fig. 1 m—p). Separation could also occur with the axis of separation perpendicular to the plane of the chromosomal mass. In this pattern, it was common for one centriole to remain stationary. The motile duplex either separated from its partner on a straight ling transecting the chromosomal mass, or it separated along a curved path moving out and around the mass. Occasionally, the chromosomal mass remained associated with one duplex while the other moved away from the mass (Fig. 7). In each of these movements, microtubular profiles between duplexes increased in length during duplex separation. At the completion of separation, microtubules between duplexes appeared to persist and the chromosomal mass appeared to reorient so that the interduplex microtubules became part of the spindle. Once bipolarity was

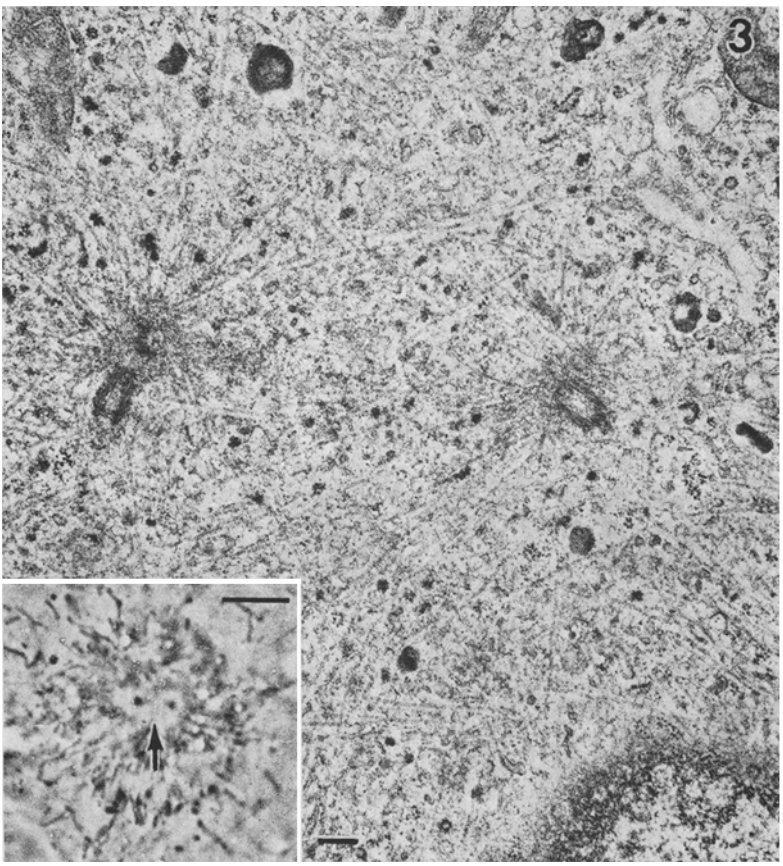
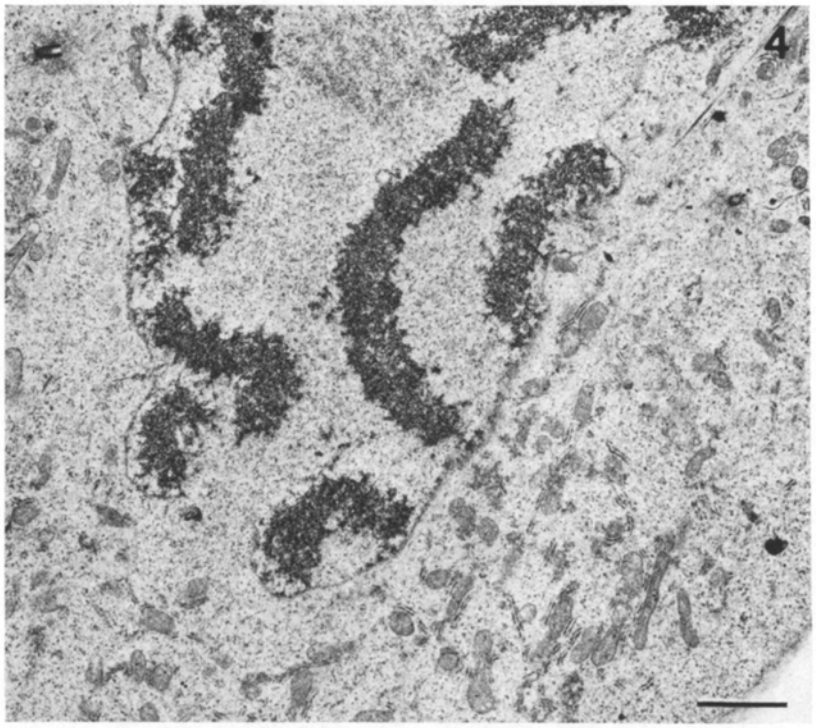


Fig. 3. Early prophase cell in which initial separation has begun across the nuclear surface (Bar= 1 μ). Inset: phase micrograph (arrows-centrosomal region). (Bar= 5 μ)

established, shortening and lengthening of the distance between the duplexes were common. Transition from a monopolar to a bipolar spindle was usually seen within 10–15 minutes.

Discussion

The behavior of the centriolar duplexes during early mitosis in rat kangaroo cells has been investigated. In general, the duplexes function as a unit prior to separation and are capable of joint movement about the cell. Similar observations have been reported during mitosis of the newt (Taylor, 1959). Such movement in PTK_2 cells is associated with an extensive array of microtubules which extend radially about the duplexes. These arrays, however, are less extensive than those associated with the duplexes in interphase (Rattner and Phillips, 1973). Therefore, the initiation of centriolar movement may correspond with a general reorganization of the cellular microtubular network. Final positioning of the duplexes prior to their separation can occur at any point within the prophase-prometaphase period.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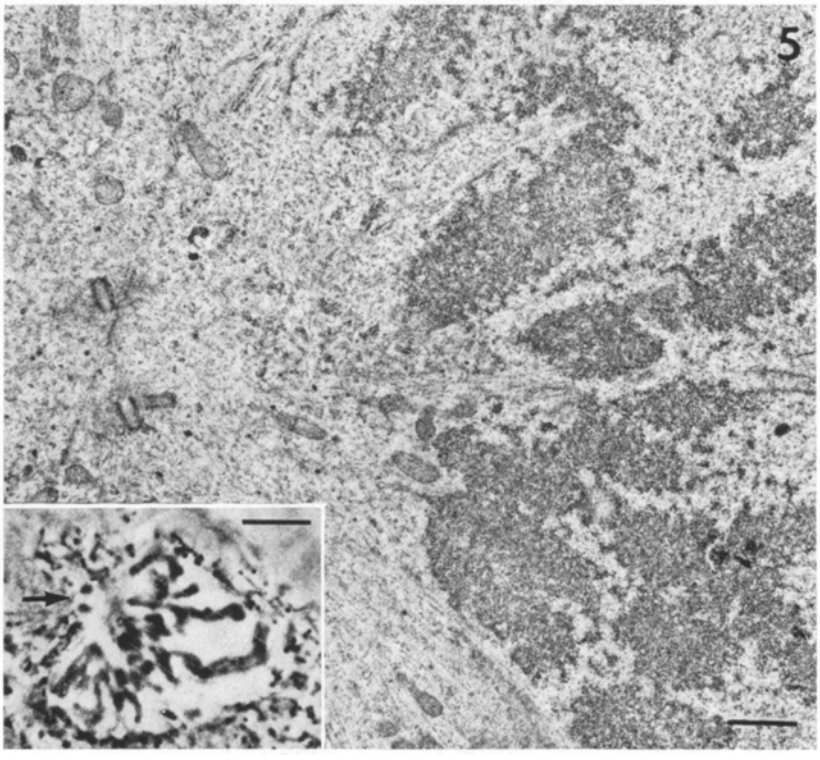
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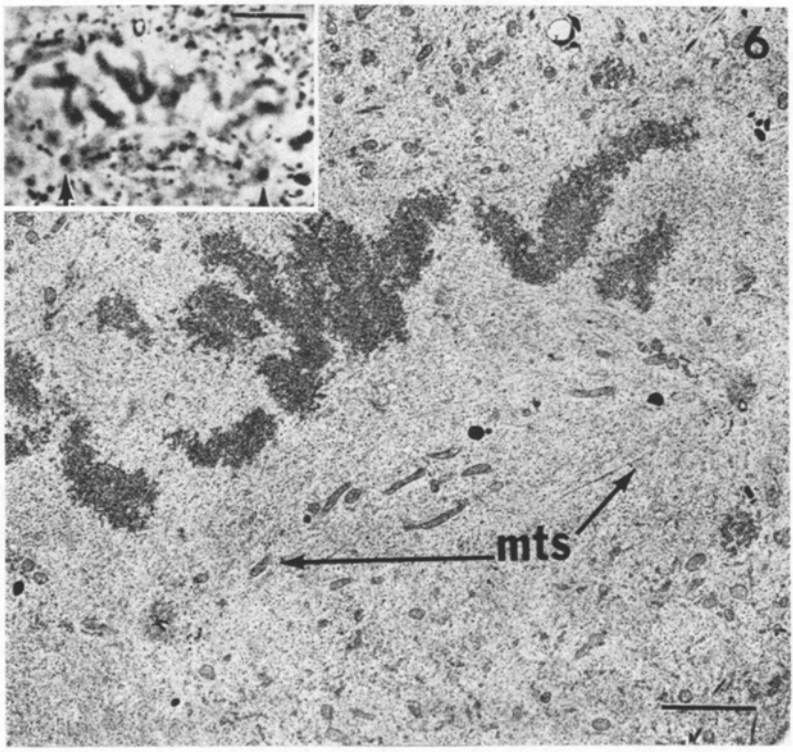

Fig. 4. Late prophase cell where duplexes have reached their final positions. Microtubules are present only in the duplex regions. Note numerous mitochondrial profiles which are now present in the region along the nuclear surface previously occupied by microtubular profiles. (Bar = 1 μ)

Fig. 5. Prometaphase cells containing monopolar spindle (Bar = 1 μ). Inset: phase micrograph (arrows-centrosomal region). (Bar = 5 μ)

Fig. 6. Prometaphase cell in which duplex separation has occurred after a monopolar configuration. Chromosomes are displaced along one side of the microtubular profiles (*mts*) placed between separating duplexes (Bar = 1 μ). Inset: phase micrograph (arrows-centrosomal region). (Bar = 5 μ)

Duplex separation and the establishment of bi-polarity also may occur at any point within the mid prophase-prometaphase period. In some cells in which centriolar separation occurs during prophase and the path of separation does not correspond to the final axis of the mitotic spindle, the microtubular elements between the duplexes do not appear to persist and contribute to the mitotic spindle. Instead, microtubules which appear at the time of nuclear envelope breakdown and which cross the disintegrating nucleus establish the mitotic spindle. In studies on newt mitosis, Taylor (1959) observed that when duplex separation could be detected in prophase, the birefringence present during separation persisted as the spindle was established. We note a similar occurrence when the path of separation remains linear and coincides with the axis of the presump-

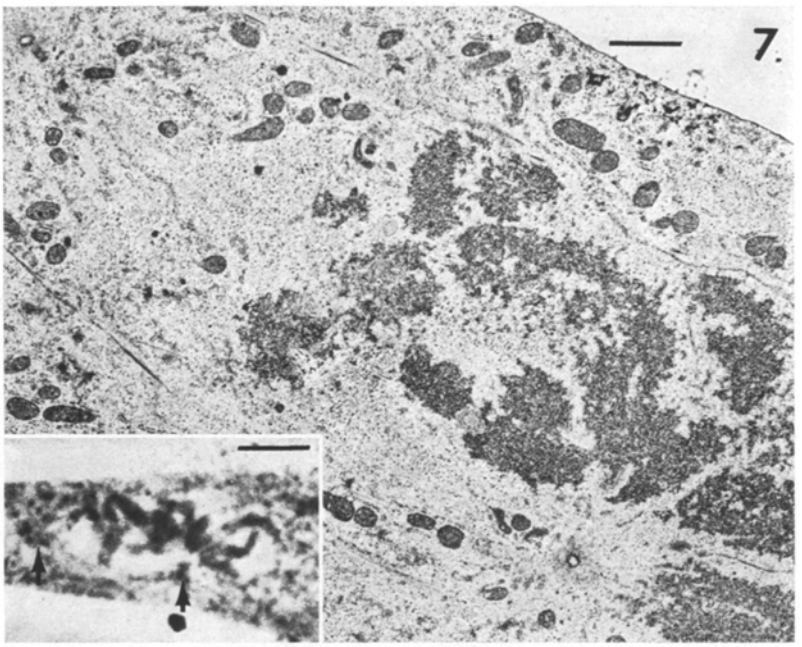

Fig. 7. Prometaphase cell: subsequent to monopolarity, one duplex has migrated while the other duplex has remained associated with the chromosomal mass (Bar=1 μ). Inset: phase micrograph (arrows-centrosomal region). (Bar=5 μ)

tive spindle or in cells which the duplexes migrate at prometaphase. These observations may suggest that perhaps the state of the nuclear envelope and the axis of separation play a role in determining the utilization of the microtubules which exist between duplexes in the initial formation of the mitotic spindle.

Delayed duplex separation results in the brief formation of a monopolar spindle. This configuration is similar to that induced by antimicrotubule agents, such as, colcemid (Brinkley *et al.*, 1967). Centriolar migration subsequent to a monopolar configuration indicates that the initial axis of duplex separation may reorient within the cell such that the final axis is up to 90° from that initially indicated.

Whereas both duplexes may migrate together prior to separation, once separation has occurred, one or both of the duplexes may be motile. In general, movement of a single duplex is most frequently observed in cases where either the nucleus proper or the chromosomal mass must be transected. It is unknown whether the reported variation in duplex behavior is specific for PTK_2 cells and perhaps is a result of their naturally flattened condition in mitosis. Little information is available in other cell types. However, some cases of prometaphase separation in L cells have been observed (Rattner, unpublished). This study suggests that duplex separation and movement are highly variable and are not dependent on prophase events though these generally tend to occur at the same time.

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