

RAPID COMMUNICATIONS

CONTINUATION OF MITOSIS AFTER SELECTIVE LASER MICROBEAM DESTRUCTION OF THE CENTRIOLAR REGION

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ABSTRACT

The centriole regions of prophase PTK₂ cells were irradiated with a laser microbeam. Cells continued through mitosis normally. Ultrastructural analysis revealed either an absence of centrioles or severely damaged centrioles at the irradiated poles. Microtubules appeared to focus into pericentriolar cloud material.

KEY WORDS laser · microbeam · centrioles · mitosis

In a previous study (2), it was demonstrated that damage produced in the pericentriolar material of prophase cells of rat kangaroo (*Potorous tridactylis*) by argon ion laser microbeam irradiation was effective in blocking the anaphase movements of chromosomes. However, these cells did appear to contain microtubules emanating from kinetochores, and they did undergo cytokinesis. The centrioles in these cells appeared normal ultrastructurally. In another study (3), cells undergoing an unusual meiotic-like reduction division were examined, and centrioles were found to be lacking from otherwise normal spindle poles. In these cells, microtubules appeared to be associated with electron-dense pericentriolar cloud material even though no centrioles could be found by careful examination of serial thin sections.

In the present study, we have examined the question of centriole function by producing selective laser microbeam damage in the centriole proper while apparently leaving the pericentriolar material unaffected. The cells were irradiated in prophase and subsequently went through normal anaphase movements and cytokinesis.

MATERIALS AND METHODS

Cells of the PTK₂ (*P. tridactylis*) rat kangaroo were grown in Rose culture chambers after the procedures described previously (2, 3). Immediately before micro-

beam irradiation, the cells were exposed to the vital dye acridine orange (AO) in a concentration of 0.25 $\mu\text{g/ml}$ culture medium for 5 min and then placed in normal medium for irradiation. Cells in early prophase were selected for irradiation. Only cells in which the centriolar duplexes were clearly visible in the perinuclear clear zone (4) were irradiated. After irradiation, the cells were followed by time-lapse videotape and fixed for electron microscope analysis at varying time periods. All ultrastructural procedures were identical to those described in our earlier studies (2, 4).

The laser microbeam employed the second harmonic blue wavelength, 473 nm, of a neodymium-YAG laser. These were the first experiments in which this particular wavelength has been used. Laser pulse duration was 100 ns, and peak output was 2-3 kW. Approx. 3% of this power was contained within the 0.5- μm focal spot of the microbeam. The basic configuration of the laser microbeam was the same as described in earlier studies (1).

RESULTS

Laser microbeam irradiation of both centriolar duplex regions in early prophase did not affect the ability of the cell to undergo mitosis. Two spindle poles were established; chromosomes aligned on a metaphase plate and underwent subsequent anaphase movements. These results were observed in seven repeats of the experiment.

Light and electron micrographs of a typical cell are presented. The light micrographs show the cell in prophase at the time of irradiation and in subsequent anaphase (Figs. 1 and 2). The arrow

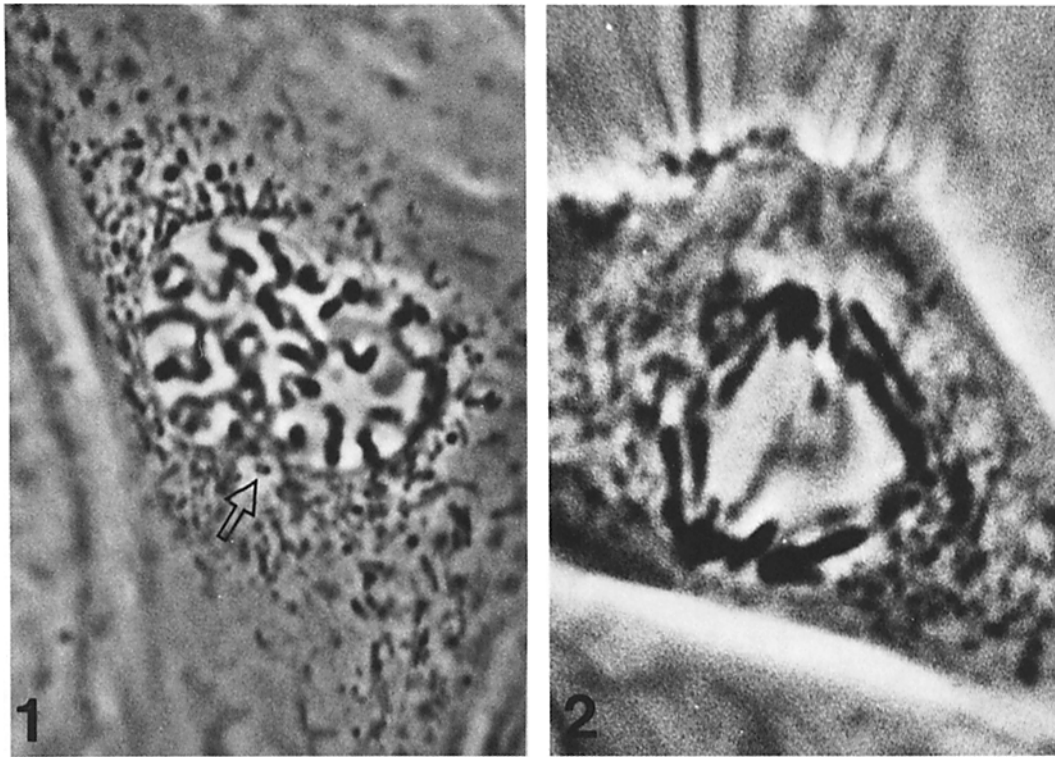


FIGURE 1 Phase-contrast light micrograph of prophase cell immediately after laser microbeam irradiation of region containing the two centriolar duplexes (arrow). $\times 1,000$.

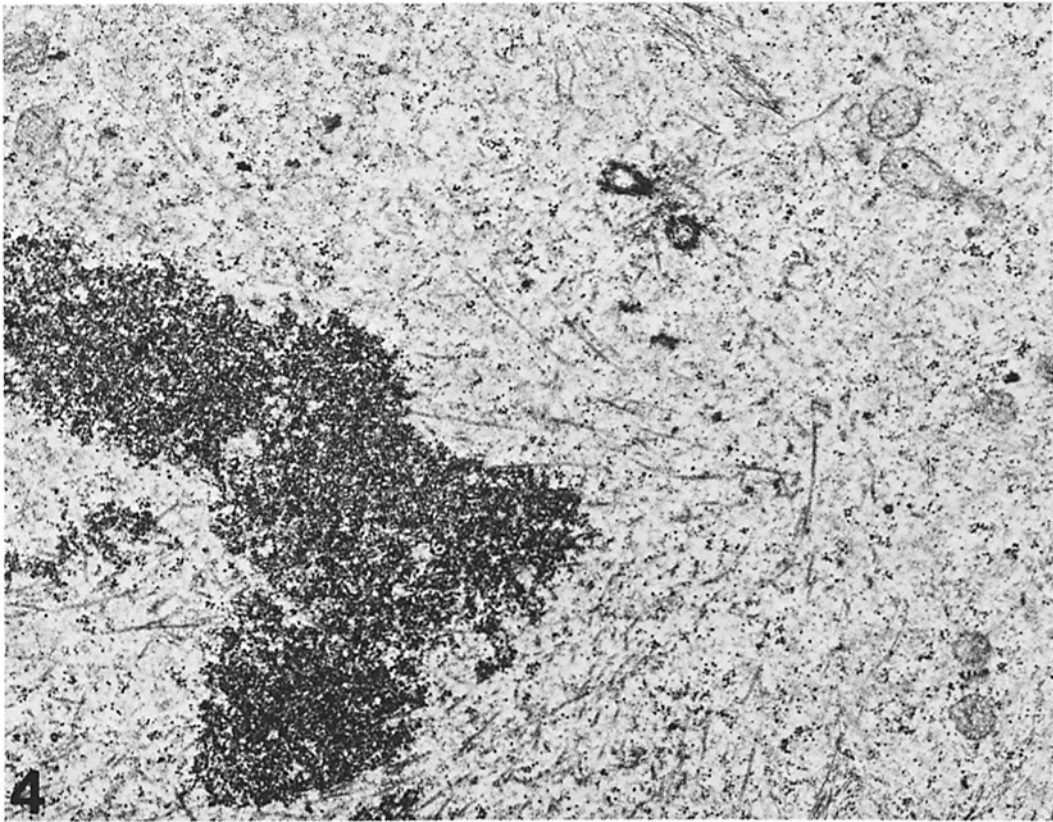
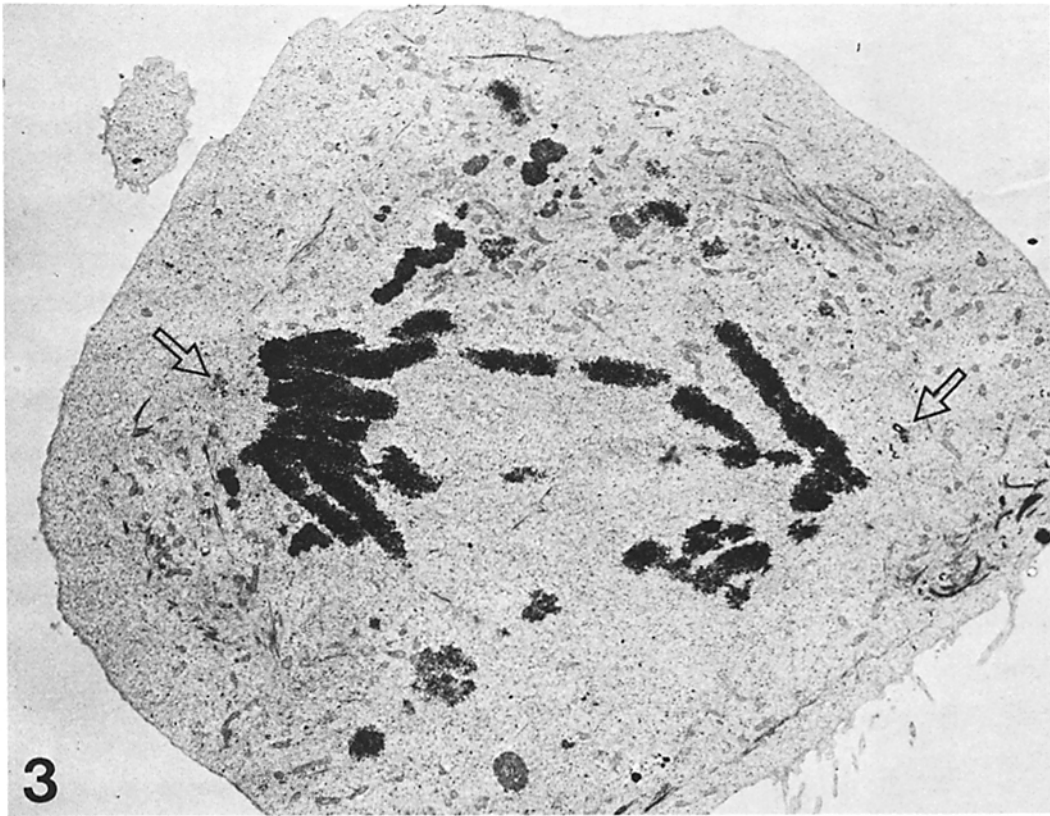
FIGURE 2 Phase-contrast light micrograph of same cell as in Fig. 1 in subsequent anaphase. The cell was fixed for electron microscopy immediately after this picture was taken. $\times 1,500$.

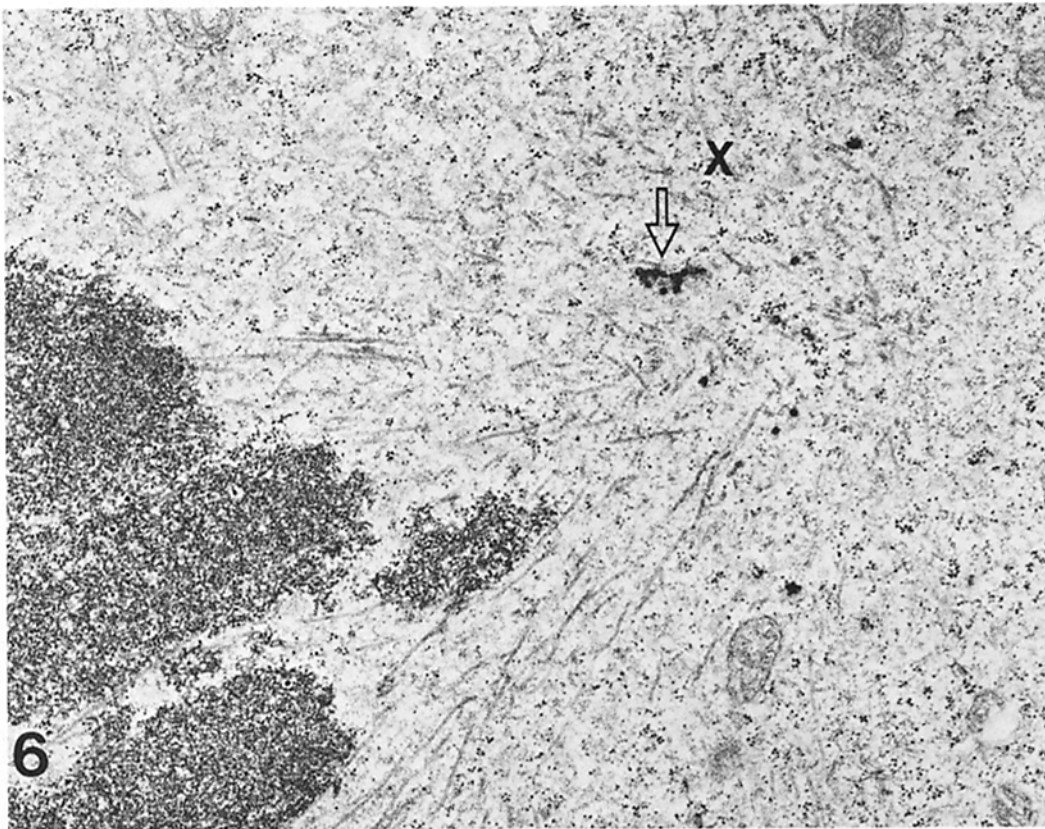
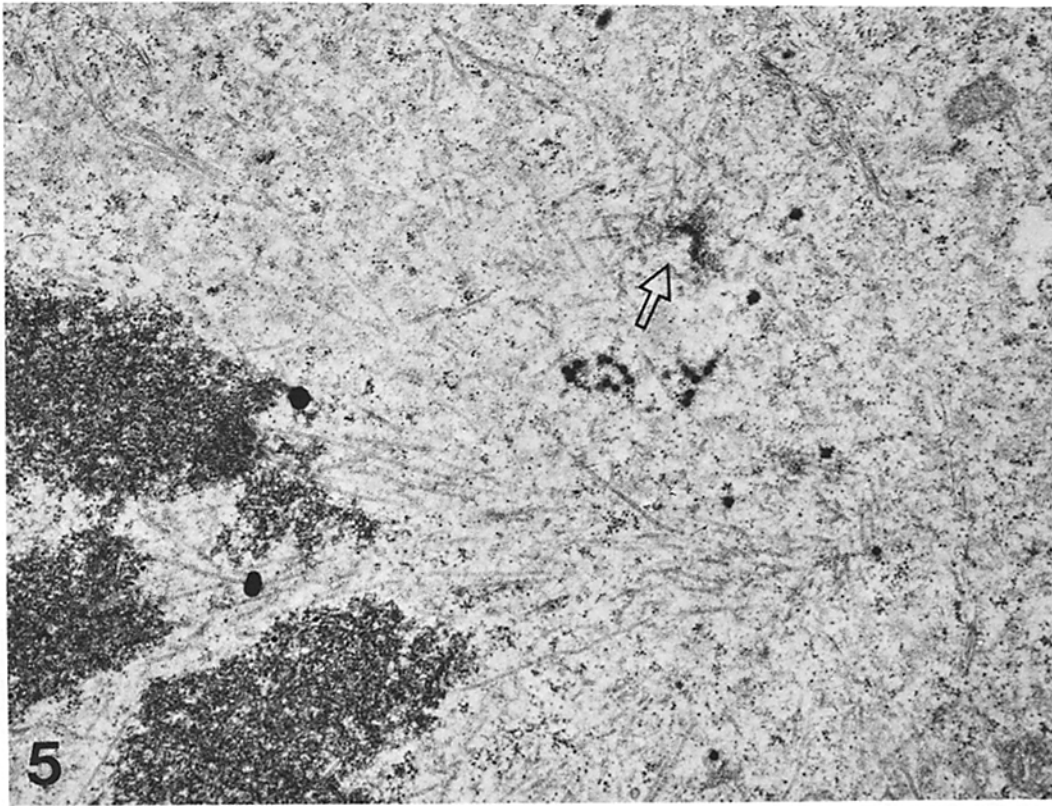
in Fig. 1 indicates the irradiated region consisting of two centriolar duplexes in a typical perinuclear clear zone. Careful electron microscope examination of serial sections of the anaphase cell revealed either the complete absence of centrioles from the spindle poles or the presence of damaged centrioles. This particular cell (see Fig. 3 for low-power micrograph) has a damaged duplex displaced off one pole and no duplex at the second pole. Higher-magnification serial sections of the pole containing the damaged duplex (Figs. 4, 5,

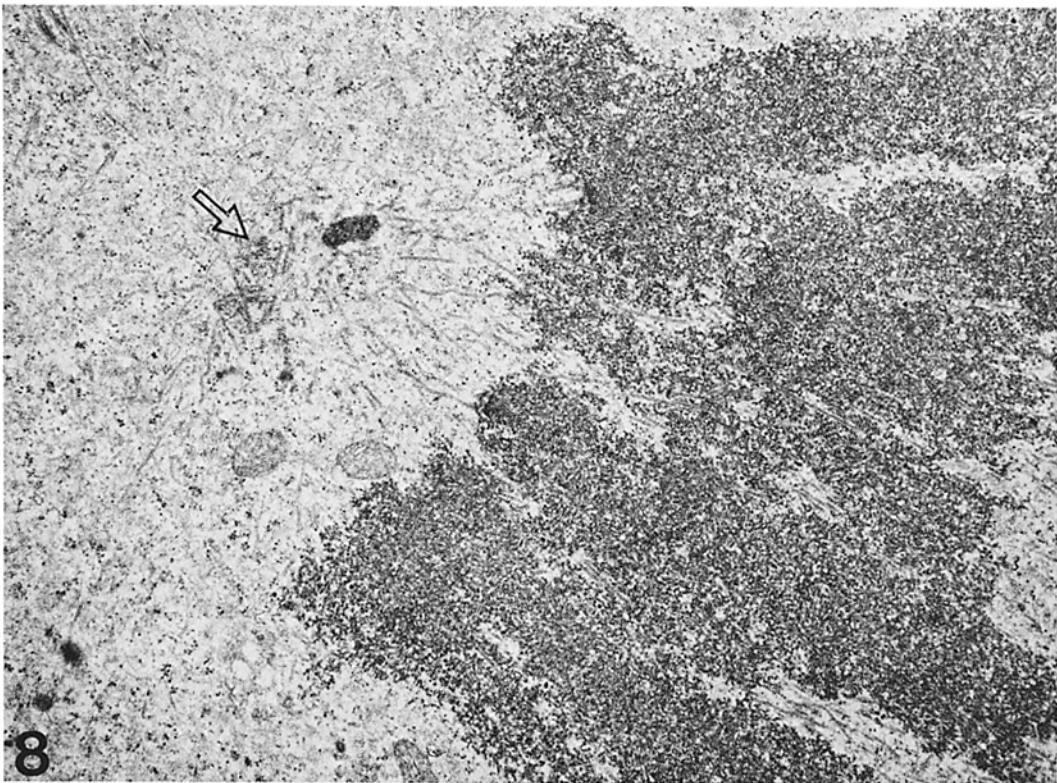
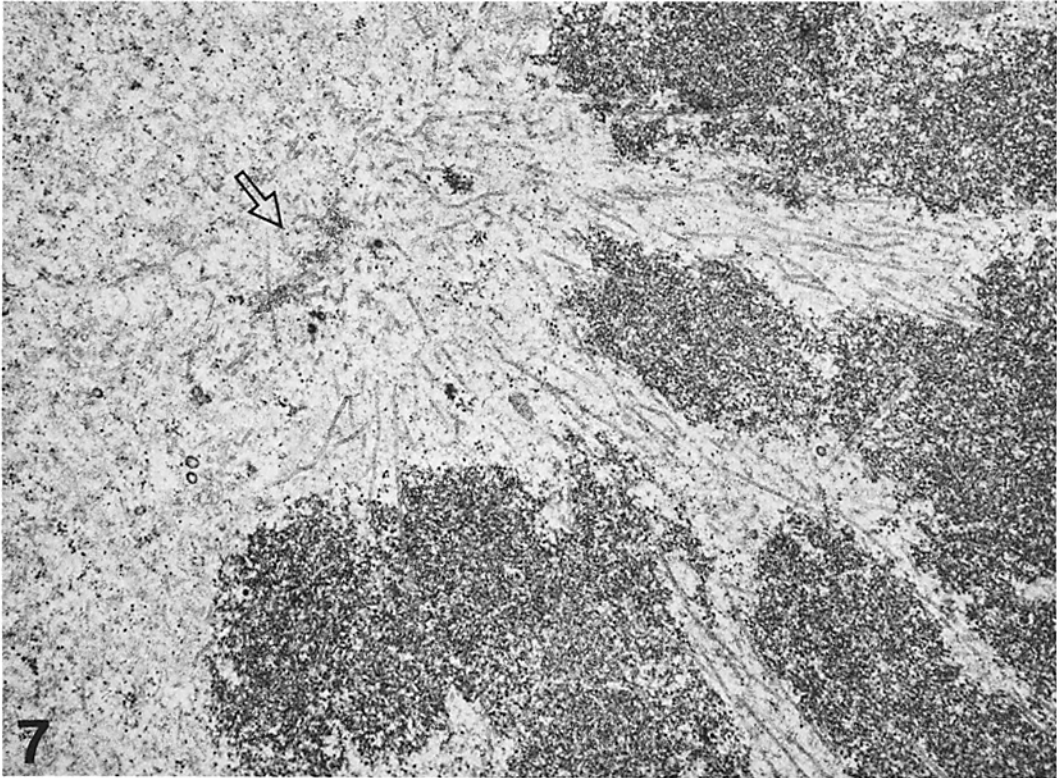
and 6) clearly show its abnormal ultrastructure and, furthermore, demonstrate that the bulk of the microtubules are focused into a region away from the centrioles. Fig. 6 is a serial section taken just out of the plane of the centriolar duplex. The X on this figure indicates the region of the cell lying in a plane just below the centrioles. High-magnification serial sections through the other pole of this cell reveal no centrioles or centriolar remnants but do reveal rather typical-looking electron-dense pericentriolar material (Figs. 7 and 8)

FIGURE 3 Low-power electron micrograph of the cell in Fig. 2. Right arrow indicates abnormal centriole duplex displaced slightly off the pole. Left arrow indicates pericentriolar cloud material at pole. See Figs. 3-8 for high-magnification serial sections of both poles. $\times 2,990$.

FIGURE 4 High-magnification electron micrograph of right spindle pole from Fig. 3. Note that there are some microtubule fragments associated with the abnormal duplex but that the majority of spindle microtubules are focused into a region below the duplex. Figs. 5 and 6 are serial sections of this region. $\times 14,520$.







into which are focused the microtubular elements.

Similarly, in another cell examined ultrastructurally, one spindle pole had no centrioles at all, but electron-dense material resembling pericentriolar material was observed with numerous microtubules. At the other spindle pole, only one severely abnormal centriole was detected.

DISCUSSION AND CONCLUSIONS

This laser microbeam study confirms the notion that centrioles may not play a critical active role in the actual process of cell division. Our earlier studies on the meiotic-like reduction division cells suggested this idea because no centrioles were formed in at least two of the four spindle poles associated with the second "meiotic" division (3). Furthermore, in the study immediately preceding that one (2), mitosis was disrupted by laser destruction of the pericentriolar cloud with apparently little or no damage produced in the centriole proper. In the present study, we have destroyed or severely damaged the centriole but apparently not the pericentriolar material. The cells have continued through mitosis. Chromosomes moved through anaphase normally, and cytokinesis occurred.

Just what the role of the centriole is in mitosis (if any) still remains unresolved. Our results strongly suggest that the centriole does not play an active role in the mitotic process itself; however, we cannot eliminate the possibility of a passive role. For example, it is possible that the centriole is involved in the production (or organi-

zation) of some essential chemical component in prophase or just before prophase. Once this material has been produced (perhaps the pericentriolar cloud or something associated with it), the centriole is no longer needed for mitosis to continue. Laser destruction of the centriole at that time would not be expected to affect cell division. However, destruction of the essential material, such as the pericentriolar cloud, would disrupt mitosis. This hypothesis is consistent with all of our recent laser microbeam studies (references 2, 3, and data reported here).

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FIGURE 5 Serial section of pole containing damaged duplex. Note the remnant of the centriole (arrow) with associated microtubule fragments. Also note that the majority of the microtubules are focused below the centriole. $\times 16,500$.

FIGURE 6 Serial section of pole depicted in Figs. 4 and 5. This section is in a plane just below the centriole. Note the "virus-like" particles that were visible in the other serial sections (arrow). Also note that the spindle microtubules are focused into the same general region as in Figs. 4 and 5. $\times 16,500$.

FIGURE 7 A serial section through the left spindle pole depicted in Fig. 3. Note that there is no centriole or centriole remnant but that there is electron-dense material resembling typical pericentriolar cloud material (arrow). The spindle microtubules are focused into this region. $\times 16,500$.

FIGURE 8 Another serial section through the same pole depicted in Fig. 7. $\times 16,500$.