Laser Irradiation of Centrosomes in Newt Eosinophils: Evidence of Centriole Role in Motility

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ABSTRACT Newt eosinophils are motile granulated leukocytes that uniquely display a highly visible centrosomal area. Electron microscope and tubulin antibody fluorescence confirms the presence of centrioles, pericentriolar material, and radiating microtubules within this visible area. Actin antibodies intensely stain the advancing cell edges and tail but only weakly stain pseudopods being withdrawn into the cell. Randomly activated eosinophils follow a roughly consistant direction with an average rate of 22.5 μm/min. The position of the centrosome is always located between the trailing cell nucleus and advancing cell edge. If the cell extends more than one pseudopod, the one closest to or containing the centrosome is always the one in which motility continues.

Laser irradiation of the visible centrosomal area resulted in rapid cell rounding. After several minutes following irradiation, most cells flattened and movement continued. However, postirradiation motility was uncoordinated and directionless, and the rate decreased to an average of 14.5 μm/min. Electron microscopy and tubulin immunofluorescence indicated that an initial disorganization of microtubules resulted from the laser microirradiations. After several minutes, organized microtubules reappeared, but the centrioles appeared increasingly damaged. The irregularities in motility due to irradiation are probably related to the damaged centrioles. The results presented in this paper suggest that the centrosome is an important structure in controlling the rate and direction of newt eosinophil motility.

Cell movement is a fundamental process of particular relevance to problems in developmental biology, cell biology, oncology, and immunology. Recent advances in immunofluorescence and electron microscopy have resulted in considerable research on the mechanisms of cell motility. As a result of many studies, it is fairly well accepted that the cytoskeleton is a major element in the cellular motile apparatus (1–5). Cytoskeletal proteins such as actin and myosin are thought to generate forces necessary for movement (5–8). In addition to their role in cell division, microtubules (tubulin and associated proteins) have been studied as support structures involved in maintaining cell shape and polarity (5, 9, 10), intracellular partical transport (11), and as structures involved in directed movement of leukocytes (1, 12, 13). Recently, the microtubule-organizing centers have been suggested as the structures involved in direction determination (14–17).

Eosinophilic granular leukocytes of the newt *Taricha granulosa* are especially favorable for studies of cell motility and, in particular, the role of the centrosome and cytoskeletal elements in the process of cell movement. They are teardrop-shaped cells that move as rapidly as 35 μm/min. The nucleus is always contained in the trailing portions of the cell, and the leading cytoplasmic regions are rich in lysosomal enzyme-containing granules. The unique feature of these cells is a small, highly visible area devoid of granules between the nucleus and advancing cytoplasmic edge. This area contains a centriolar duplex, granular pericentriolar material, and a radiating array of microtubules. Under phase-contrast or differential interference contrast microscopy, it is possible to visualize the position of the microtubule-organizing center as the cell moves, and as it responds to chemotaxic signals. In this paper, the cell system is described and an ultraviolet laser microbeam is used to damage these organizing centers and study their role in cell migration.

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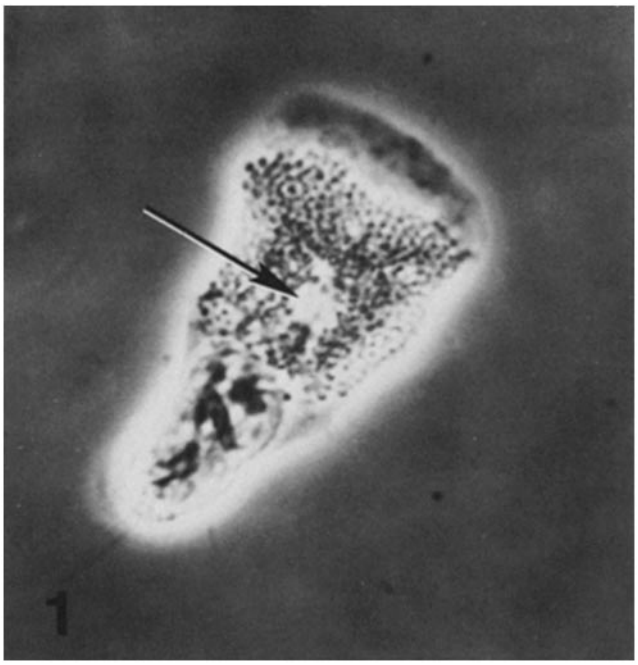


FIGURE 1 Phase-contrast photograph of a moving newt eosinophil, demonstrating the clear leading edge, granules, centrosome (arrow), and trailing nucleus. Cell is moving diagonally to the upper right-hand corner. × 950.

MATERIALS AND METHODS

Cell Preparation: *Taricha granulosa*, purchased from Carolina Biological Supply Co. (Burlington, NC), were kept in water at ambient temperature and fed a weekly diet of raw beef liver.

To obtain eosinophils, a newt was wrapped in a cotton sponge to prevent urine contamination of the blood. A small portion of the tail was cut off and blood was dropped onto 30-mm round quartz coverslips (Esco Products, Inc., Oak Ridge, NJ) containing several drops of amphibian medium (Gibco Laboratories Inc., Grand Island, NY) supplemented with 10 U/ml heparin (Sigma Chemical Co., St. Louis, MO). The coverslip was then placed in a moist chamber at room temperature to prevent desiccation and the cells were allowed to settle and attach for 20 min. In the interim, a thin ring of silicon grease was applied to a glass slide, forming a well which was filled with appropriate medium. The blood-containing coverslip was tilted and gently rinsed with fresh medium, removing the erythrocytes. It was inverted over the prepared slide and compressed to form a seal. This resulted in a relatively clean preparation of leukocytes and allowed for easy removal of the coverslip for fixation. "Chambers" such as these were used within 4 h of preparation. Although motility and adhesion decreased over a longer period of time, cells remained viable for at least 24 h following a setup. All experiments were performed at room temperature (22°C). Various types of media were prepared and used to obtain good adhesion, activation, motility, and viability of the eosinophils and other leukocytes (see Results). The medium found to give the best results was amphibian medium supplemented with 10% newt serum. This medium was used in all of the laser experiments.

Light Microscopy: The preparations were observed on either a Zeiss Photomicroscope III or a Zeiss Axiomat microscope equipped with phase-contrast optics. The Axiomat was interfaced with a De Anza image processing computer (18). A cell-tracking program developed in this laboratory (19) allowed the computer to recognize a selected cell and automatically keep that cell in the center of view during the course of an experiment. The image processing system digitized the cell's video image which was then processed to allow computer recognition of the cell boundaries. From this, the cell center could be calculated. By comparing cell center locations over time, the computer could detect any cell movement. Movement was then corrected for by sending electrical impulses to the motorized stage and thus returning the cell to the center of view. Error due to random shape change was minimal and did not affect the tracking process. After the tracking period, the computer printed out the path cells had moved as well as total and net distance.

Cells were photographed with Kodak Panatomic-X and developed in Kodak Microdol-X. A Sony TVO-9000 video cassette recorder was used to obtain time-lapse sequences of cell movement.

Identification of the cells as eosinophils was confirmed by a descriptive analysis as well as by staining with Wright's blood stain (J. T. Baker Chemical Co., Phillipsburg, NJ) (20).

Laser Irradiations: Cells were irradiated using the fourth harmonic 266-nm ultraviolet wavelength from either a pulsed Quantel model YG 481A Nd YAG laser or a pulsed Quantronix model 116R Nd YAG laser (21). Laser energy was controlled with neutral density filters (Oriel Corp. of America, Stamford, CT) placed in the light path. Energies per focused pulse were measured with a Scientech No. 362 energy power meter and found to be 65 nJ ± 3% with pulse durations of 10 ns (Quantel) or 80 ns (Quantronix). The lasers were diverted by a series of mirrors and a dichroic filter into a Zeiss Axiomat (Quantel) or Photoscope III (Quantronix) microscope modified with quartz optics, and focused to a 3-μm spot by a Zeiss 32X Ultrafluar objective. Single pulses were obtained by an electronic shutter synchronized with the laser. The computer and video equipment used to image the cells and target the laser have already been described (18).

Electron Microscopy: Cells were fixed in 3% glutaraldehyde in amphibian media at room temperature for 1 h, then overnight at 4°C. Osmification, embedding, sectioning, and staining procedures were followed as described in reference 22. Serial silver sections were observed on slotted carbon/Formvar-coated grids in a Philips 300 transmission electron microscope operated at 80 kV.

Indirect Immunofluorescence: Cells were fixed for 30 min at room temperature with 3% formaldehyde in PBS. Except for treatment after permeabilization with 1% BSA and 10% fetal calf serum in PBS for 30 min, the cells were processed as described in reference 23. This pretreatment significantly reduced the random antibody binding.

Cells were viewed on a Zeiss RA microscope equipped with epifluorescence illumination and Zeiss fluorescein filter set No. 487709. Photographs were taken using Kodak Tri-X film and developed in Kodak Microdol-X.

Tubulin and actin antibodies were generously provided by Dr. Scott Peterson and Ms. Ann Siemens (University of California at Irvine). Actin antibodies were prepared against chicken gizzard actin by the method of Lazarides (24) and tubulin antibodies were prepared from bovine brain tubulin according to the method of Asnes and Wilson (25).

RESULTS

Newt eosinophils are granulated cells roughly 60 μm long and 40 μm wide (Fig. 1). They are usually teardrop shaped but display many morphologies while moving. A multilobed nucleus is always located to the rear of a moving cell and, except for the advancing cell edge and the centrosome, the cytoplasm contains many small 1–2-μm diam phase-dense granules. A striking feature of these cells is an area ~4 μm diam between the nucleus and advancing cell edge which is devoid of granules. This clear zone contains the Golgi complex and centrosome (Fig. 2).

In order to track by computer and effectively observe the cells without interruption, the erythrocytes had to be removed. Using amphibian media supplemented with heparin, we isolated the leukocytes by allowing them to attach to a coverslip and then gently rinsing away the erythrocytes. However, this procedure resulted in most leukocytes halting their movement and rounding up. Various combinations of media and leukocyte activators were used to obtain good adhesion and motility. Amphibian medium alone (280 vs. 290 mOsmol blood) provided good initial attachment but cells rounded within 1 h. Similarly, PBS, and PBS with 20 μg/ml phytohemagglutinin, provided good initial attachment but with subsequent cell rounding and loss of movement. Bovine serum, whether heat inactivated or not, was ineffective as an activator. Cells did not even attach to the coverslip. Initial experiments with the peptide chemotatic factor *N*-formyl-methionyl-leucyl-phenyl-alanine (Sigma Chemical Co.) as well as horse serum did stimulate attachment and motility but for the eosinophils only. Other types of leukocytes remained rounded. In contrast, newt serum alone or diluted to 10% in amphibian medium induced eosinophils as well as other

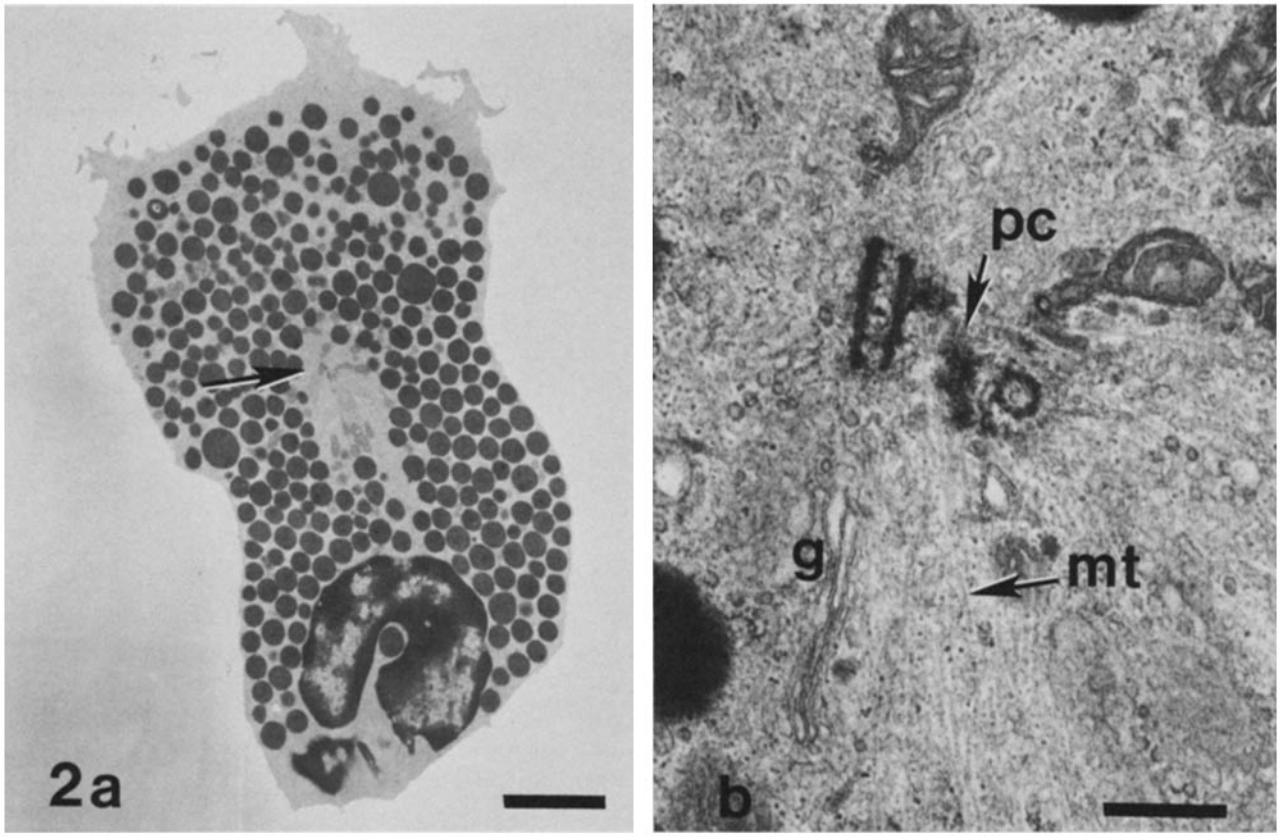


FIGURE 2 (*a*) Low-power electron micrograph of eosinophil. Centriole duplex (arrow) is visible within the clear area. Bar, 5 μm. × 2,700. (*b*) Higher magnification of centrosome in *a* showing the centrioles, pericentriolar material (*pc*), Golgi complex (*g*), and microtubules (*mt*). Bar, 0.5 μm. × 31,000.

leukocytes to attach, spread, and move. No difference in motility could be detected between cells in newt serum alone or in serum diluted to 10% in amphibian medium. Coating of the coverslips with poly-L-lysine, 0.2% BSA, or fetal calf serum did not increase attachment or motility. Likewise, no observable differences were detected between quartz and glass substrates.

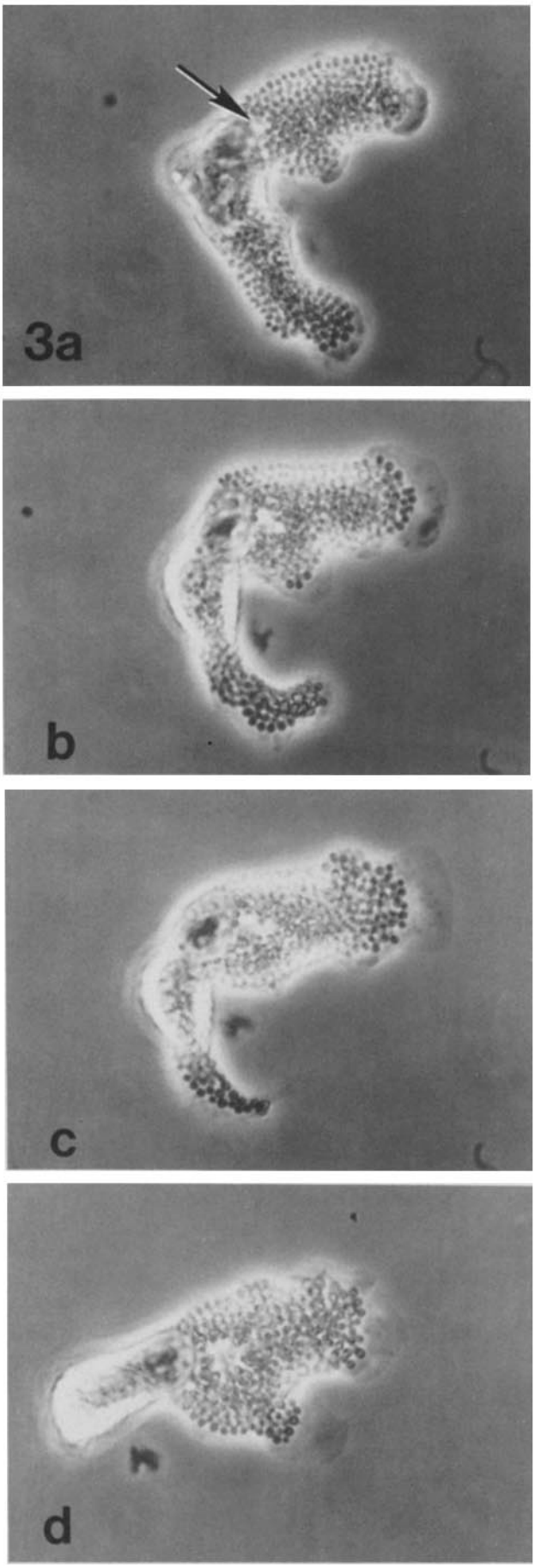
These cells displayed a rapid ameboid type of movement. A clear, nongranular portion of the cell flowed out from an advancing pseudopod, after which the cell seemed to push cytoplasm (with granules) into it. Contraction waves (6) were seen but no ruffling of the leading cell membrane edge was observed. Granules also were observed moving rapidly within the cytoplasm. They seemed to flow in bulk as the cell advanced as well as in bulk back towards the centrosome if a pseudopod was withdrawn. The eosinophils often paused briefly and extended (pushed out?) more than one pseudopod (branching) (Fig. 3). This branching process was observed in roughly one third of all cells examined and usually resulted in a direction change. When branching occurred, the pseudopod closest to or containing the centrosome dictated the direction of continued cell movement (Fig. 3). The centrosome, in every case observed, was always located between the nucleus and advancing cell edge. As the cell moved, its position was sometimes close or sometimes distant from the nucleus.

The migration patterns of over 60 different cells were observed by computer tracking. The migration rate averaged 22.5 μm/min. The fastest cells were recorded at 35 μm/min. Most cells were tracked for a period of 10 min and a few cells for as long as 1 h. No difference was detected in their behavior after 1 h of tracking. Fig. 4 represents several examples of cell movement. The randomly migrating cells roughly followed a consistent direction but with many small variations along the way.

Fluorescence analysis of newt eosinophils with tubulin antibodies revealed a brightly staining centrosome with many microtubules radiating outwards (Fig. 5). In most cases, bundles of microtubules seemed to be orientated toward or away from the nucleus. Actin antibodies intensely stained the clear leading edges and tails of the cells (Fig. 6). It also appeared that only those pseudopods actively moving forward were brightly stained with the actin antibody. Pseudopods being reabsorbed or retracted into the cell were only weakly fluorescent.

Electron microscope (EM)[1] analysis of 15 control (nonirradiated) centrosomes revealed a single duplex of centrioles (all with a 90° orientation), granular electron-dense pericentriolar material, and numerous radiating microtubules (Fig. 2). Most microtubules appeared to emanate from the pericentriolar material (Fig. 7) and radiate outwards between the cytoplasmic granules. Ultrastructural analysis revealed that most granules were membrane bound and contained an electron-dense uniform core with occasional membrane-like structures inside (Fig. 8). Rarely, granules were observed with internal crystalline arrays or an internal ghost-like structure.

[1] Abbreviation used in this paper: EM, electron microscope.



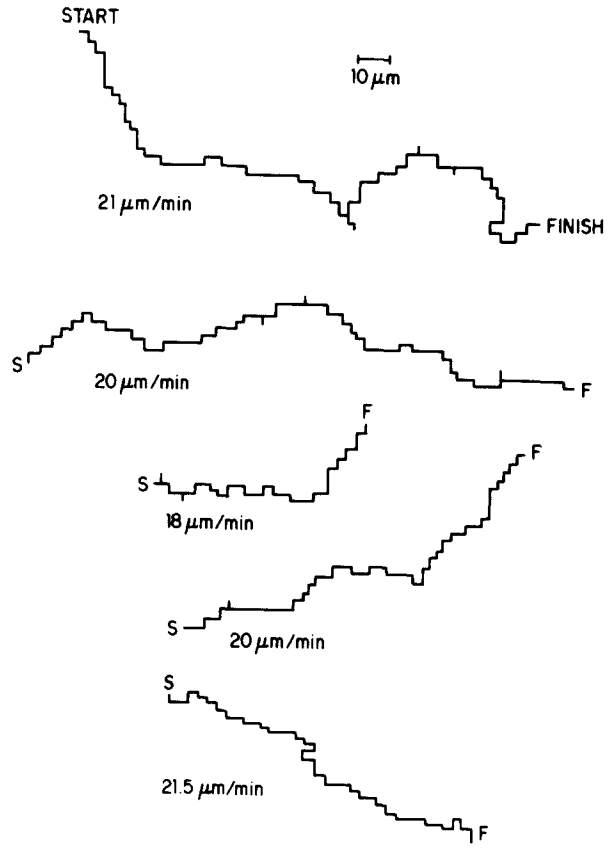


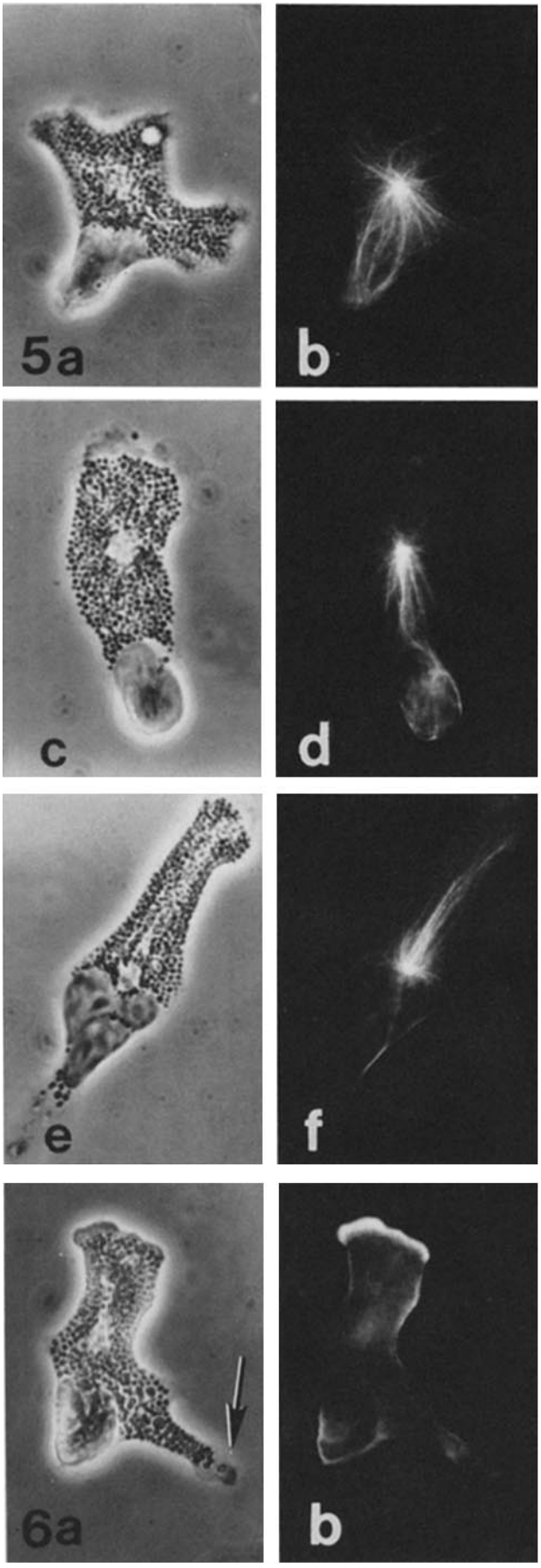
FIGURE 4 Computer-derived tracks of randomly moving eosinophils. Cells did not always move from right to left; tracks were positioned to conserve space. In general, cells followed a consistent direction but with many small deviations.

Microfilaments were found predominantly in the subcortical regions of the cell and intermediate filaments were found throughout the cell interior. The clear areas of the advancing membrane edges contained very dense arrays of microfilaments just inside the cell membrane. The more central regions of the clear areas contained a granular type of cytoplasm rich in ribosomes and short lengths of microfilaments (Fig. 9).

Precise diamond knife alignment before serially thin-sectioning cells made it possible to examine the centriole orientation with respect to cell direction in the 15 control cells. In only two cells (13%) was the same orientation found (one centriole perpendicular to the substrate, one parallel to the substrate pointing to the direction of movement) as that suggested for migrating 3T3 fibroblasts (14). In only 40% of the cells was there a centriole perpendicular to the substrate. However, in 73% of the cells, there was a centriole pointing to within 30° of the direction a cell was moving.

Before laser irradiation, randomly activated eosinophils were computer tracked for 5 min to determine their rate and direction of movement. Most cells moved continuously and followed a consistent direction. More than 60 newt eosino-

FIGURE 3 Sequence showing cell movement. (*a*) Cell has two pseudopods extended; arrow denotes centrosome. (*b–d*) Lower pseudopod is reabsorbed and centrosome containing pseudopod is extending as cell moves to the right. Time course of sequence is 1.5 min. × 710.



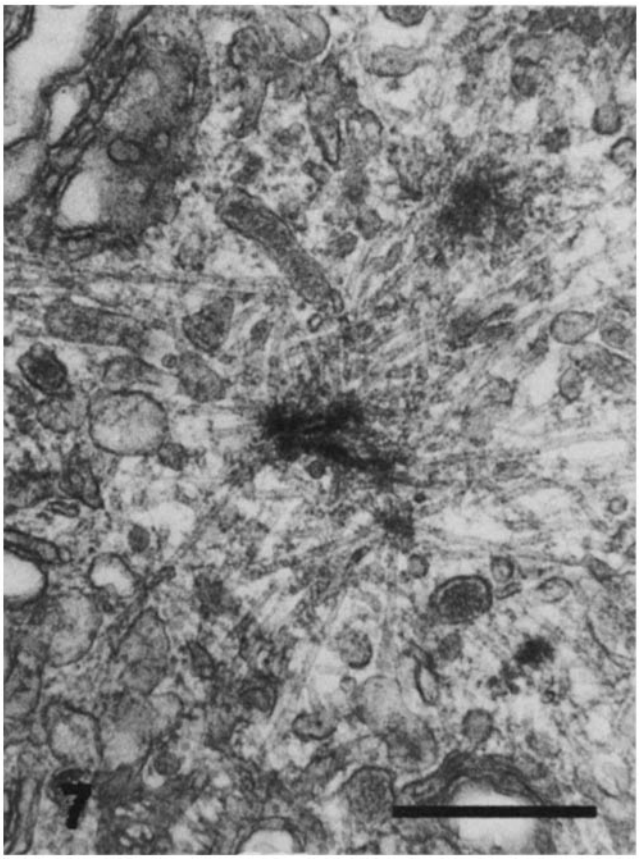


FIGURE 7 Electron micrograph of pericentriolar material. Most microtubules in the centrosome appeared to originate from this structure. Bar 0.5 μm. × 54,000.

phils were irradiated by directing one to five pulses of UV laser light into the centrosome region. The most immediate visual effect was typically a phase darkening and a shrinkage or disappearance of the clear centrosomal area. The clear zone usually reappeared within a few minutes. Another, although infrequent, response was a rapid, short movement of the clear zone towards either the leading cell edge or the nucleus. Within a few mintues after irradiation, the cell typically stopped moving and rounded up (Fig. 10). Following irradiation and during rounding, granule movement continued within the cell.

Shortly after rounding, most cells (92%) again flattened and continued movement. However, their motility was not as fast or as consistent as before irradiation. Pseudopods were extended and cells would slowly move a short distance and round up again. This process was repeated many different times and in many different directions. Fig. 11 represents

FIGURES 5 and 6 Fig. 5: Phase-contrast (*left*) and corresponding tubulin fluorescence (*right*) photographs of three different cells. Most microtubule bundles are toward or away from the nucleus. × 810. Fig. 6: Phase-contrast (*a*) and corresponding actin fluorescence (*b*). The cell appears to have been moving toward the top of the photograph and the pseudopod (arrow) extended away from the primary (centriole containing) pseudopod is weakly stained. × 810.

computer tracks of typical cells before and after irradiation. Irradiated cell motility decreased to an average of 14.5 μm/min. Postirradiated track paths were much more irregular and inconsistent than nonirradiated cells and net distances covered were greatly reduced. The cells appeared unable to undergo the consistent, directed movements that were observed in nonirradiated cells. In a very few cases, postirradiated cells did sustain a directed motility, although in all cases the rate of movement was significantly lower. Cells were routinely observed for 10–15 min after irradiation although some were followed for as long as 1 h. In contrast to centrosome irradiations, cytoplasmic control irradiations (five cells) did not affect motility. Visible lesions were produced in the cell granules but in all cases, rate and direction of cell movement remained unaffected. Preirradiated control cells averaged 22.4 μm/min and postirradiated controls averaged 22.8 μm/min.

EM analysis of 13 cells fixed within 5 min of irradiation revealed that most microtubules associated with the centrosome appeared disrupted (Fig. 12 and Table I). Most cells had very few visible microtubules in the clear region. These centriolar duplexes had lost their 90° orientation and two duplexes had visible structural damage (Fig. 13). Damage was manifested as a loss of distinct centriolar structure, fragmentation, or a loss of the typical 90° duplex orientation. 12 cells were fixed after at least 10 min following irradiation. Microtubules appeared reorganized in the majority of these cells, although damage to the centrioles appeared to be much greater. Structural centrosomal damage appeared in 42% of the cells (vs. 17% in cells fixed immediately after irradiation) and 90° orientation was lost in 66.7% of the cells. Other laser-associated damage was occasionally found in the granules and the Golgi complex (Fig. 14).

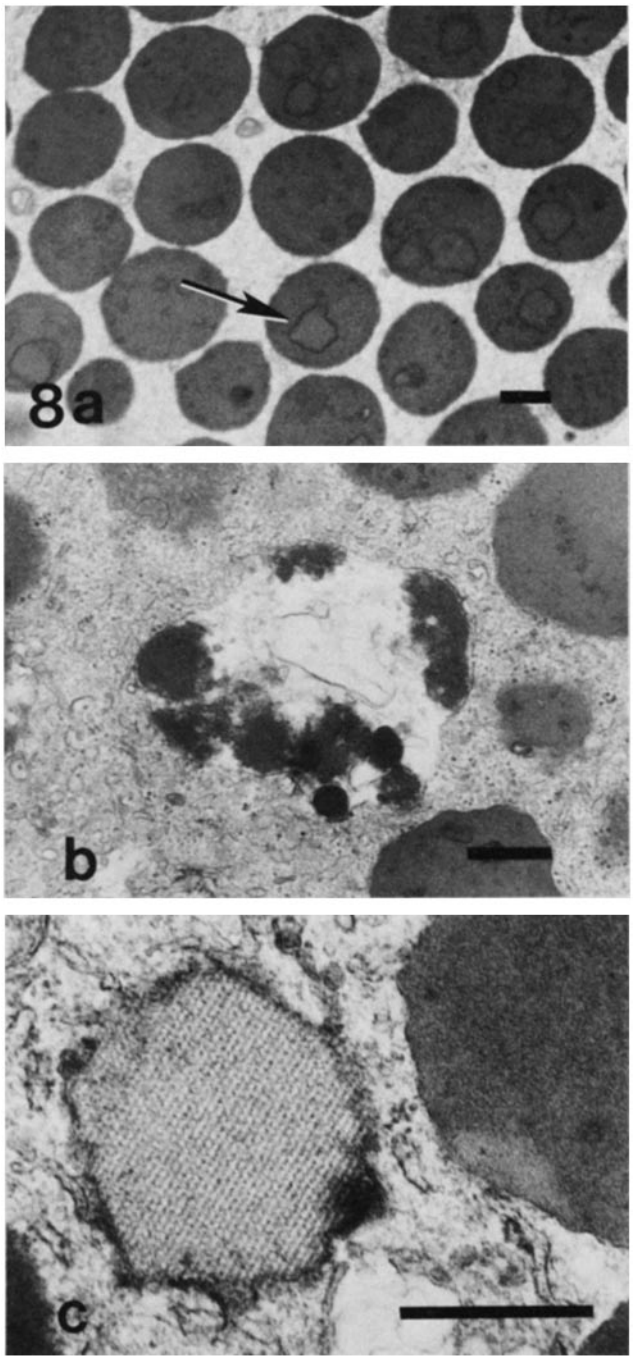


FIGURE 8 Cytoplasmic granules. (*a*) Characteristic uniform electron-dense granules with occasional membrane-like structures inside (arrow). (*b*) Granule ghost. Internal material seems to have been released. (*c*) Granule with a crystalline appearance. Bars, 0.5 μm. (*a*) × 13,000; (*b*) × 23,000; (*c*) × 53,000.

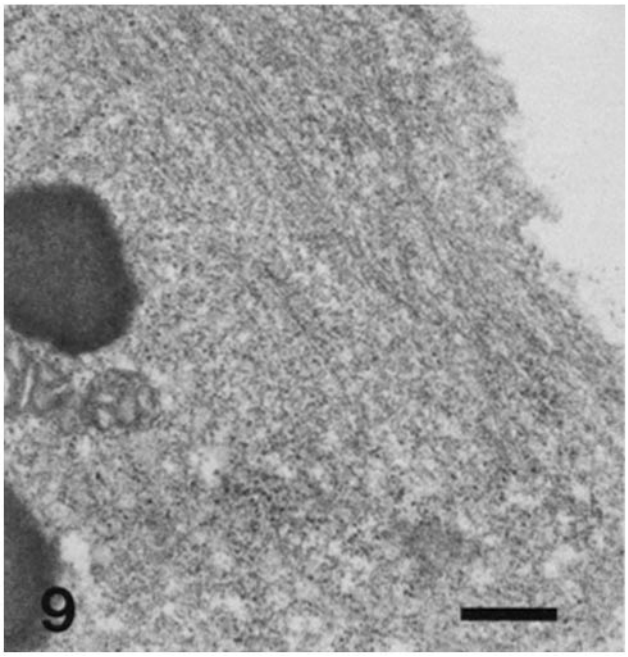
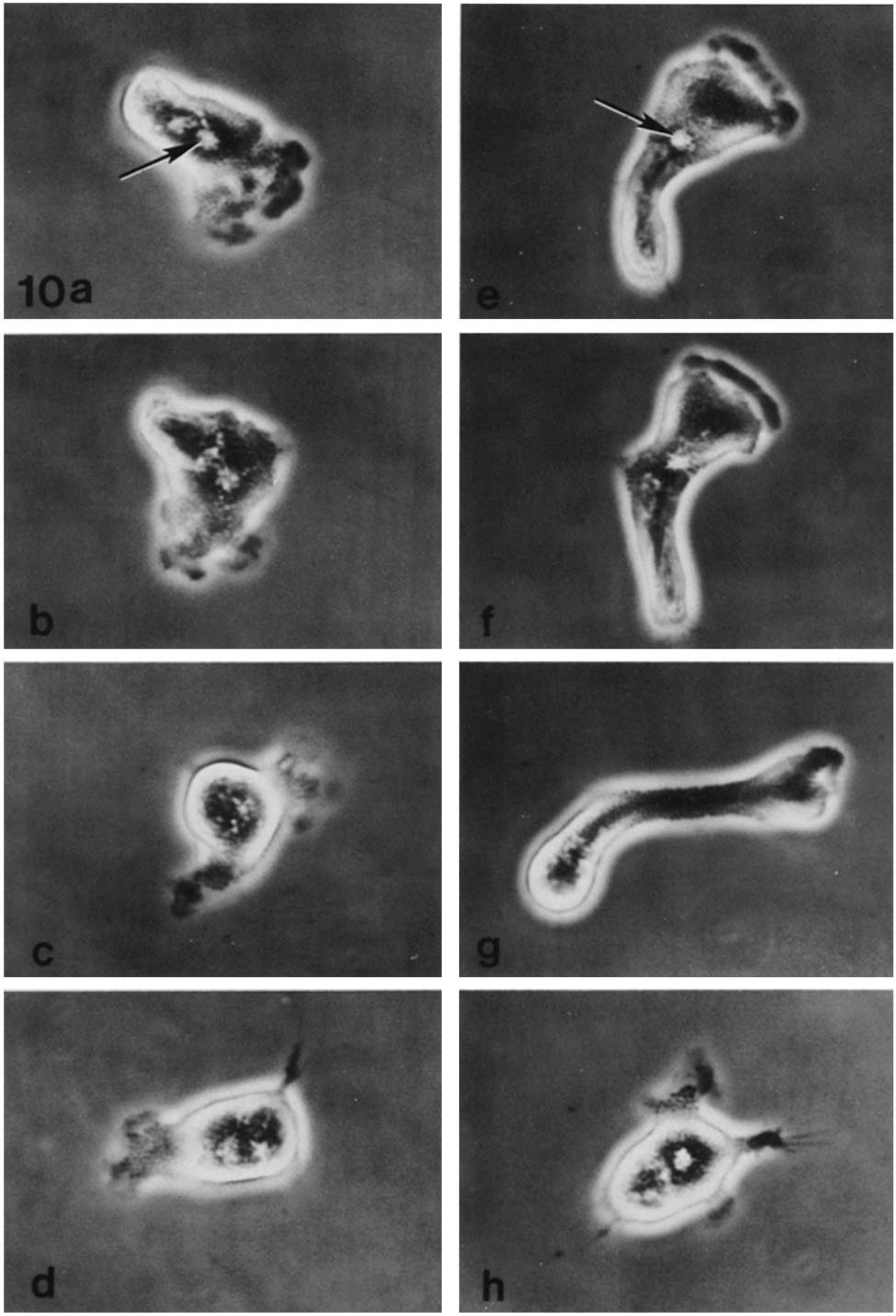


FIGURE 9 Clear portion of leading cell edges, containing only ribosomes and microfilaments. Bar 0.5 μm. × 26,000.

FIGURE 10 Laser irradiation of newt centrosomes. (*a* and *e*) Two different cells, pre-irradiation, with arrow marking the centrosome. (*b* and *f*) Immediately after irradiation. (*c*) 5.0 min post-laser; cell has rounded and has two small pseudopods. (*d*) 10.0 min post-laser; cell is still partially rounded but moving. This cell was not tracked. (*g*) 1 min post-laser. (*h*) 6 min post-laser. This cell's (*e–h*) motility decreased from 24.0 to 7.1 μm/min as a result of irradiation. × 700.


10a
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c
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Two cells with cytoplasmic irradiations distant from the centrosome were fixed for EM within 2 min. Their centrioles and microtubule arrays appeared identical to control cells.

Four cells were irradiated at their centrosomes, fixed within 2 min, and processed for indirect immunofluorescence with antibodies specific for tubulin. No distinct cytoplasmic microtubules were visible in these cells (Fig. 15).

The eosinophils also displayed a necrotaxic response. By killing nearby cells with the laser microbeam, eosinophils rapidly changed their direction. They moved toward the dead cell and engulfed or crawled over it (Fig. 16). In addition, rounded (presumably inactive) eosinophils could be activated by destroying a nearby cell. Within 5 min, a round cell would flatten and begin migrating towards the killed cell. It was found that the dead cells had to be within a relatively short distance (several cell lengths) or there was no effect upon migrating eosinophils.

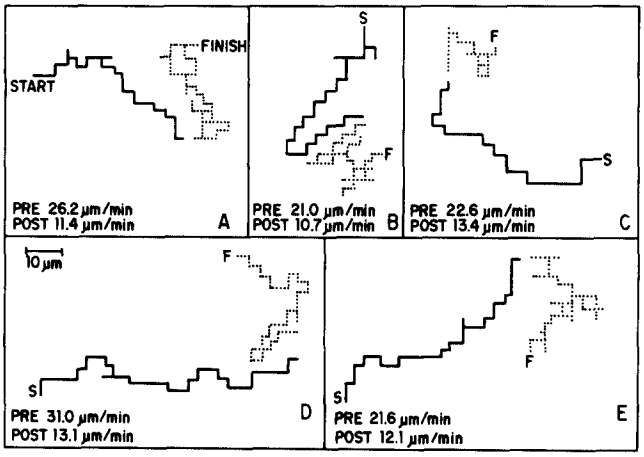


FIGURE 11 Computer tracks of selected cells before and after laser irradiation. Note how much more inconsistent the direction is after irradiation. Pre-irradiation (——), postirradiation (. . . . .).

DISCUSSION

Randomly activated newt eosinophils moved consistently and in a roughly straight direction at an average rate of 22.5 μm/min. These cells are stimulated by factors in the medium to attach and move and can probably be orientated by chemotaxis. The unique feature of these cells that makes them especially favorable for cell motility studies is their highly visible centrosomal region. Unlike most other motile (or nonmotile) cells, the centrosome position can be easily and directly visualized in the living cell. Recent interest in the position of the centrosome and its possible role as a directional or stabilizing device (14–17) exemplifies the importance of direct visualization in living cells. It is not known whether this feature is strictly unique to this cell type or if it is also seen in other cells. Presumably the "clear zone" results from granular exclusion by dense arrays of microtubules, much in the same manner as a mitotic spindle excludes organelles.

These eosinophils maintain a striking nucleus-centrosome-leading edge polarity during their rapid movement. A similar polarity has been described in many cell types, though to a lesser degree than described here (1, 10, 16, 17, 26). This

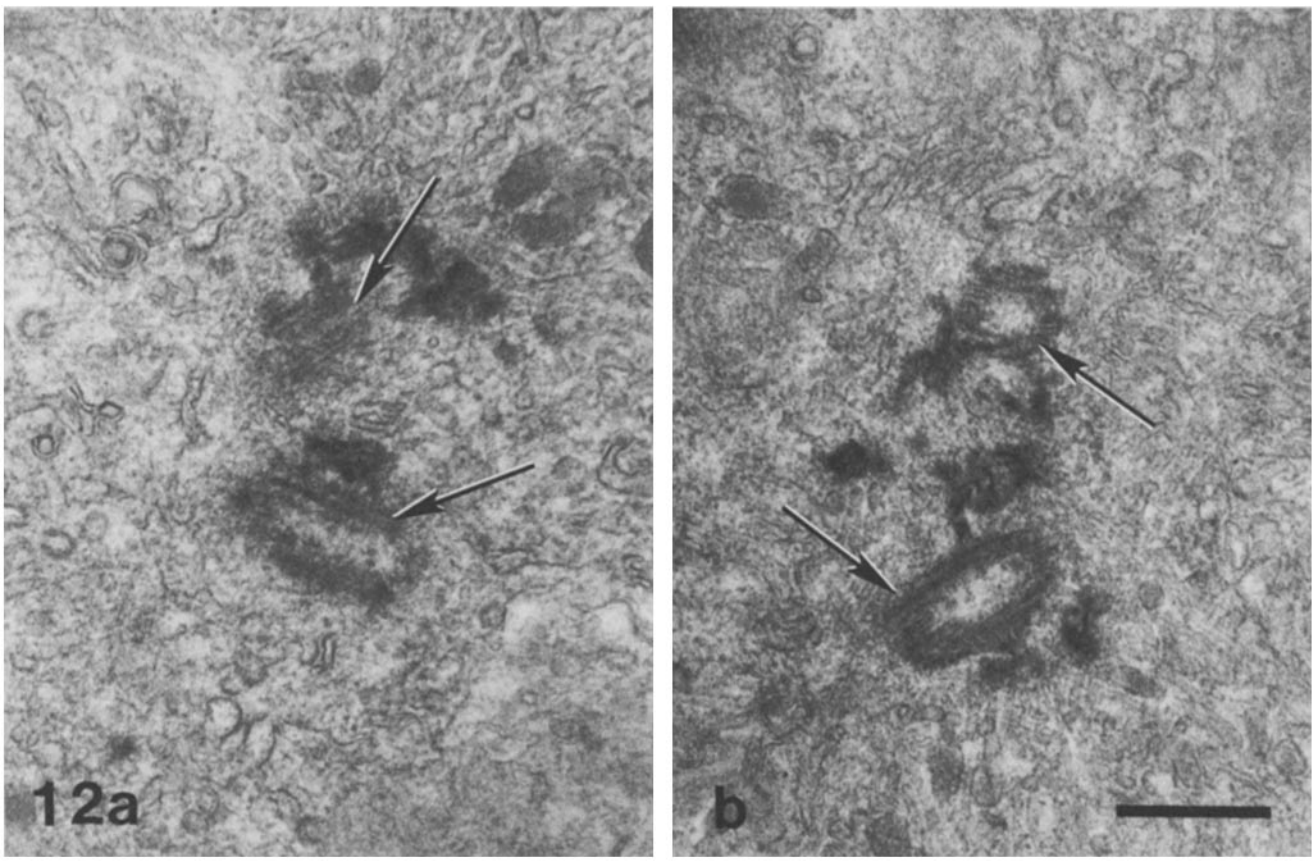
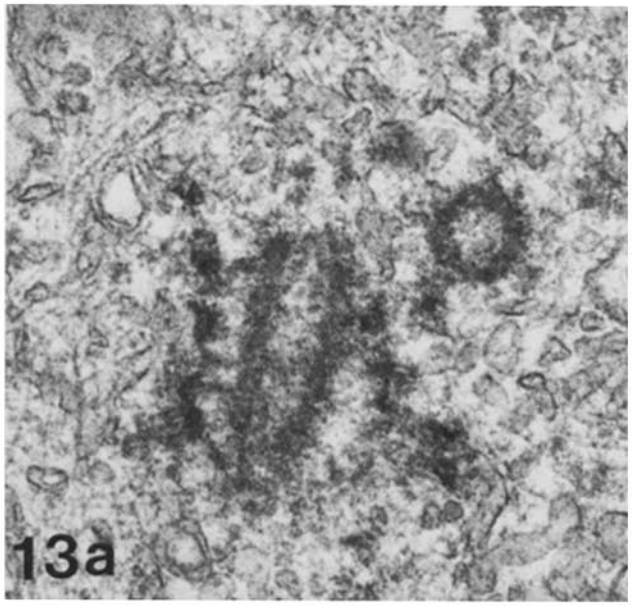
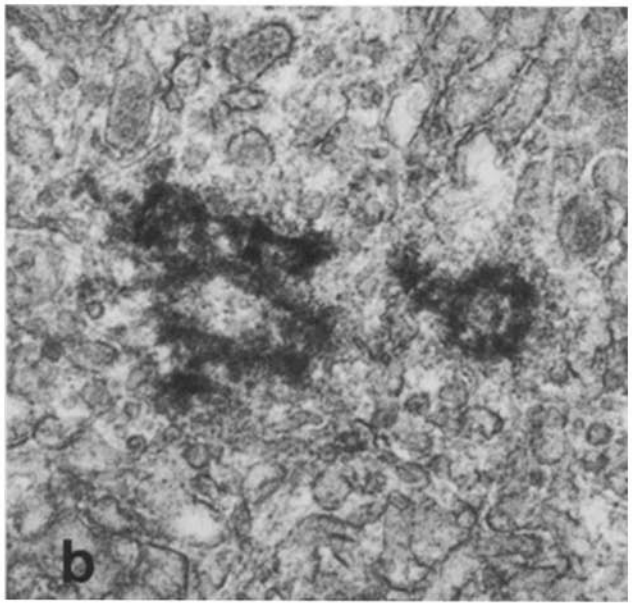


FIGURE 12 Laser-irradiated centrosomes. Two different pairs of centrioles (arrows) both fixed within 1 min of irradiation. Only very few microtubules were visible in these two cells. Bar 0.5 μm. × 40,000.





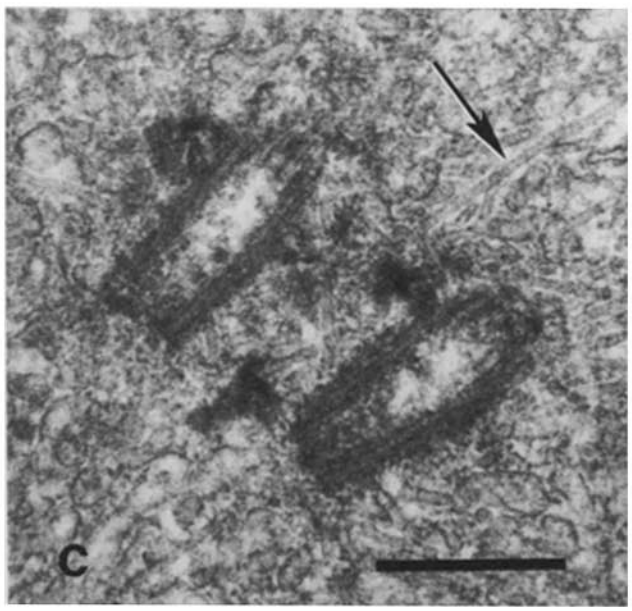
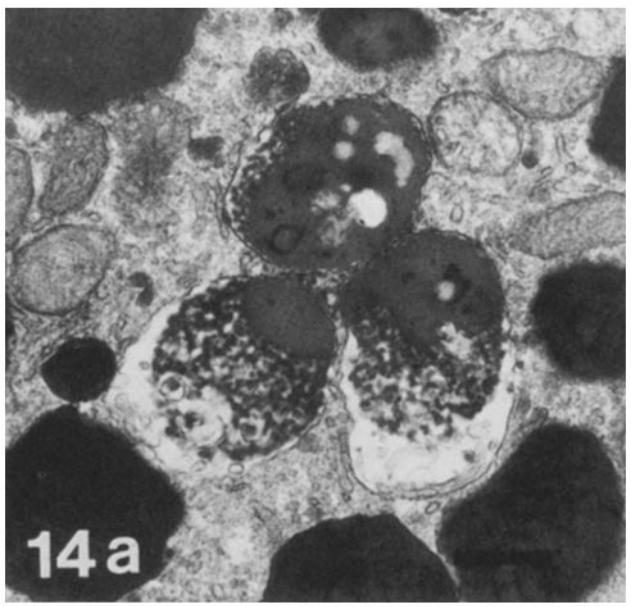


FIGURE 13 Laser-damaged centrioles. Serial sections were examined to confirm damage. Note that the centrioles (*a* and *b*) appear indistinct and somewhat distorted. c shows a loss of 90° orientation but microtubules (arrow) are present. (*a*) Cell fixed 12 min post-laser; motility decreased from 26.2 to 11.4 μm/min. This is the same cell as displayed in Fig. 11*A*. (*b*) Fixed 30 min post-laser; motility decreased from 24.2 to 13.9 μm/min. (*c*) Fixed 9 min, 30 s post-laser; cell was not tracked.



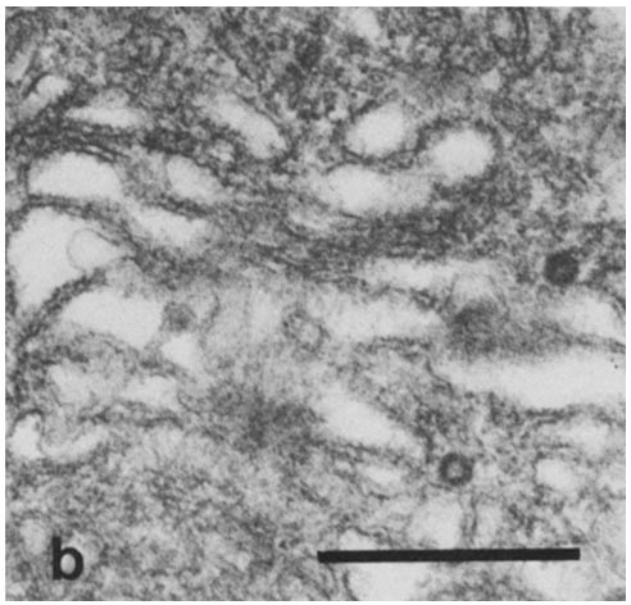


FIGURE 14 (*a*) Laser-damaged granules. Centrosome is located just off the upper right-hand corner of photograph. (*b*) Laser-damaged Golgi complex. Bar 0.5 μm. × 70,000.

consistent orientation provides at least circumstantial evidence that the centrosome is involved in cell guidance or intracellular organization. Conversely, it could also be argued that the rest of the cell incidently positions the centrosome. A previous study on this cell type (27), using colchicine as well as microtubule inhibitors on a variety of cell types (1, 10, 17, 26), showed that microtubule disruption tended to

TABLE I

EM Analysis of Cells Fixed Postirradiation

Number of Cells	Postirradiation	Number of microtubules		90° Centriole orientation	
				Yes	No
15	Controls, no irradiation	Many	15*	15	0
		Moderate	0	0	0
		Few	0	0	0
13	Fixed within 5 min of irradiation	Many	2	2	0
		Moderate	3	3	0
		Few	8	5	3 (2 dam)
12	Fixed ≥10 min after irradiation	Many	6	1	5 (1 dam)
		Moderate	3	2 (2 dam)	1
		Few	3	1 (1 dam)	2 (1 dam)

dam, damaged.

* The values in this column represent the number of cells with that quantity of microtubules.

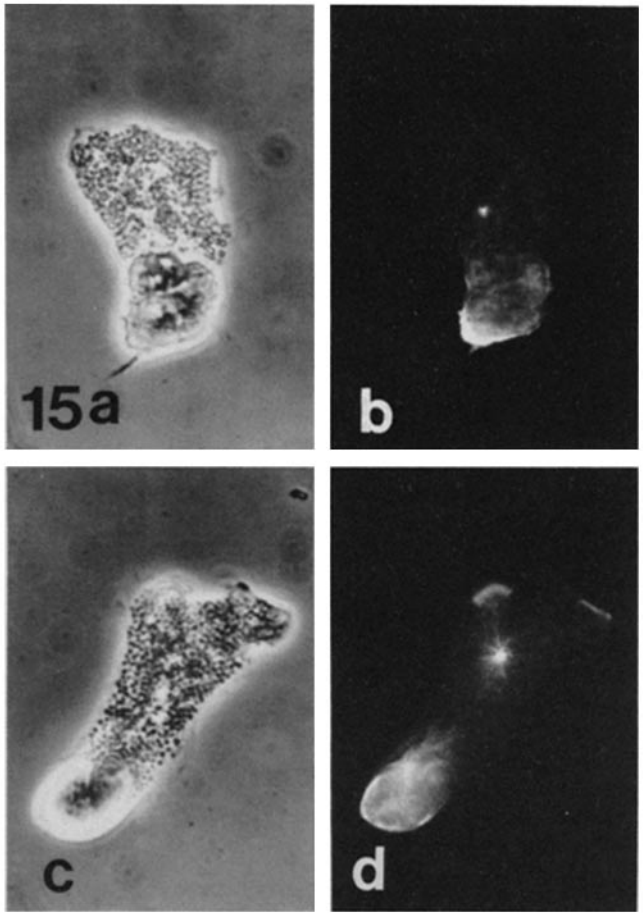


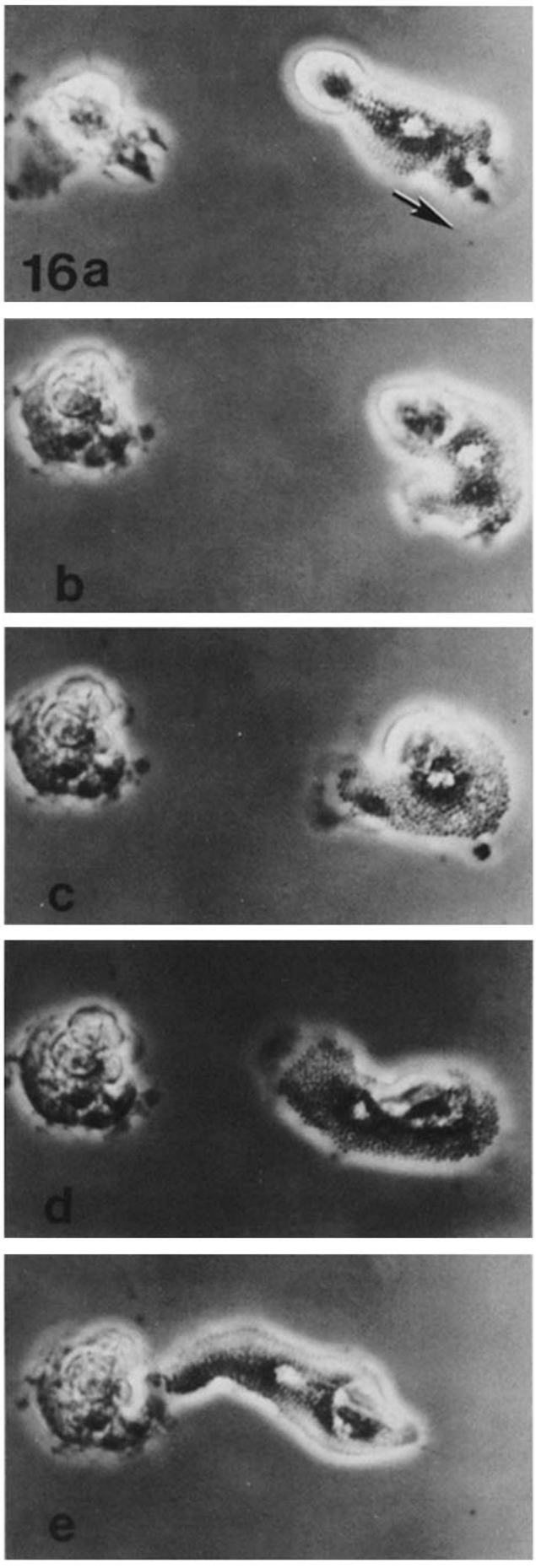
FIGURE 15 Phase-contrast (*left*) and corresponding tubulin fluorescence (*right*) photographs of two different cells. Centrosome is brightly stained but little or no microtubules can be seen. × 810.

disrupt this cell polarity (the nucleus lost its rearward orientation). These studies indicate that the centrosome probably is not casually positioned in the cell and perhaps does function in maintaining a certain degree of intracellular organization.

In this study, an ultraviolet laser microbeam was used to damage this centrosomal region. Though most cells were still motile after irradiation, movement was inconsistent, direction was irregular, and rate was much more reduced than in control cells. Cytoplasmic irradiations away from the centrosome produced no visible motility effects as detected by either light or electron microscopy. Therefore, the overall effect is not due to general ultraviolet irradiation on nonspecific cell components. The initial loss of cell movement correlated on an ultrastructural level with an initial disorganization in centrosomal microtubules and some damage to the centrioles. As time following irradiation increased, microtubules in most cells were seen to regenerate, corresponding to the cells regained ability to move. The loss of directed (straight line) movement correlates with a disruption of the centrioles. Damage to the centrioles appears greater the longer the period between irradiation and fixation. Perhaps centrioles are initially damaged but owing to their tenacity, several minutes are necessary for that damage to become visual. These results indicate that though the centrosomal region acts as a microtubule-organizing center and perhaps has some role in cell polarity, the centrioles within the centrosome play an important role in organizing cell movement. Furthermore, destruction of the centriole per se does not prevent subsequent microtubule organization by the undamaged centrosomal material (the pericentriolar material). This observation is supported by earlier laser microbeam studies on mitotic cells (28). In those studies, destruction of pericentriolar cloud material disrupted microtubule organization, but destruction of centrioles did not affect the organization of microtubules. It would be interesting to examine microtubule reorganization with tubulin antibodies at various times following irradiation and to correlate that with the cell motility behavior.

It is noteworthy that irradiated centrioles frequently lost their 90° orientation to each other. The cause of this phenomenon is presently not understood. Centrioles are known to separate when a cell divides and it has been shown that certain drugs can cause centriole separation when human neutrophils are activated to move (29).

Microtubule disruption by conventional drug inhibitors in motile leukocytes has had results that vary from no effect at all to significantly inhibiting motility (1, 12, 13, 30), even in the same cell type. Allison (10) presents movement tracings of mouse macrophages, both normal and colchicine treated, that are remarkably similar to the before and after tracks of irradiated eosinophils. Likewise, Allan and Wilkinson (31) report that the absence of microtubules impairs accurate turning in human neutrophils. In addition, most studies (but not all) indicate that the major effect of microtubule disruption on leukocytes seems to prevent the cells from orientating in chemotaxic gradients (1, 12, 13). They suggest that while something other than microtubules propels these cells (most



likely an actin-based microfilament system [1, 6]), the microtubules are involved as signal sensors or directional effectors. In the present study, however, microtubules appear normal several minutes after irradiation yet centrosomes and motility are disrupted. This suggests that an intact centrosome/microtubule complex is perhaps necessary for maintaining directional motility. Furthermore, the ability of the centrosomal region to reorganize microtubules even though the centrioles have been damaged suggests that microtubule organization is not a function of the centriole.

A previous study (14) has indicated that centrioles are specifically orientated and are the guidance structures in motile 3T3 fibroblasts. Orientation of the centriole duplex in newt eosinophils was not found to be consistent with that described for the 3T3 cells (14). However, there was a preference for at least one centriole to orientate itself and point generally toward the leading cell edge (73% of the cells). It must be remembered that these cells move much more rapidly than 3T3 cells and perhaps their centrioles are subject to much greater cytoplasmic stresses that could disrupt the specific orientation seen in other cells.

Actin antibodies intensely stain these eosinophils and EM observation reveals dense meshworks of microfilaments in the subcortical cytoplasmic regions. This pattern has been seen in similar cell types (32). Weak staining of retracting pseudopods suggest a rapid F-actin to G-actin transition occurs when pseudopods stop their outward movement. This phenomenon should be studied further.

Granule ultrastructure is consistent with that reported for eosinophils from other species (33, 34). Granule ghosts observed in the cytoplasm probably reflect lysed granules. Crystalline granular arrays have been reported in other granulocytes (35) and probably contain different components than in the more common granules.

Necrotaxis toward dead cells has been shown previously in in vitro studies (36) and is a basic property of leukocytes. Future studies on this cell type should include correlations between centrosome position, effects of irradiation, and the ability of the eosinophils to orientate and move toward a specific source.

In summary, these eosinophils provide a convenient yet very informative system in which to study many current aspects of cell motility. Laser irradiation of centrosomes has demonstrated an important role of the centriole in organizing cell movement.

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FIGURE 16 Necrotaxis toward laser-killed cell. (a) pre-irradiation; arrow denotes initial eosinophil direction. (b) 45 s after irradiation; cell on left has been killed and eosinophil is turning towards it. (c) 1 min, 45 s postirradiation. (d) 2 min, 45 s postirradiation. (e) 5 min postirradiation. × 590.

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