THE ROLE OF THREE CYTOPLASMIC FIBERS IN BHK-21 CELL MOTILITY

I. Microtubules and the Effects of Colchicine

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ABSTRACT

Microtubule breakdown in the presence of 5 or 40 μg/ml of colchicine is observed in BHK-21/C13 fibroblast-like cells. Several morphological and physiological effects are noted in the absence of microtubules: (*a*) the cells transform from fibroblast-like to epithelial-like cells; (*b*) the normal pattern of intracellular birefringence changes and a juxtanuclear cap of birefringent filaments is formed; (*c*) time-lapse cinematography demonstrates that cell locomotion is inhibited in colchicine-treated cells, even though membrane ruffling persists. The results are discussed in terms of the specific roles of microtubules in cultured cell motility and possible functional relationships of the three types of cytoplasmic fibers seen in BHK-21 cells.

INTRODUCTION

BHK-21/C13 (1) cell motility has been functionally associated with the presence of three cytoplasmic fibers, which have been described as microtubules (approximately 250 A in diameter), filaments (approximately 100–120 A in diameter), and microfilaments (40–60 A in diameter) (2, 3).

Cytoplasmic microtubules have been implicated in the normal motile processes of a variety of cells, from the movement of heliozoan axopods to pigment granule migration in Fundulus melanophores (4, 5, 6). The drug colchicine, which reversibly breaks down microtubules, has been used to define the motile functions of microtubules in many cell systems (16, 22, 27). This drug presumably acts by binding to protein subunits of the microtubule walls (7).

We have utilized colchicine to determine the possible functions of microtubules in normal BHK-21 cell motility. This study has also helped to define possible functional relationships between the three types of cytoplasmic fibers found in BHK-21 cells.

MATERIALS AND METHODS

Cell Cultures

BHK-21/C13 cells were grown in BHK-21 medium supplied by Grand Island Biological Co., Grand Island, N. Y. The medium was supplemented with 10% tryptose phosphate broth, 10% calf serum, and 100 units/ml each of penicillin and streptomycin. Stocks of growing cells were maintained at 37°C in Falcon plastic tissue culture dishes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) kept in a humidified atmosphere of 95% air-5% CO_2. Every 2 wk, frozen stocks of cells were thawed and cultured, and older growing stocks were discarded. Cells were removed from the plastic dishes by treatment with trypsin-EDTA (ethylenediaminetetraacetate) solution, supplied by Grand Island Biological Co., and were either subcultured into new Petri dishes or onto cover slips housed within Petri dishes.

Light Microscopy

Cover slips containing cells were mounted on slides and sealed with paraffin. To ensure that the cells were

not crushed or compressed, broken pieces of no. 1 glass cover slips were placed at the four corners of the cover slip between the cover slip and the slide before sealing. These preparations were not used for more than 1 hr, during which time no obvious morphological changes were seen.

Cells were observed with a Zeiss Photomicroscope equipped with phase contrast, polarized light, and Nomarski differential interference optics. Light micrographs were taken utilizing either a 60W tungsten or high pressure mercury arc light source with green interference and heat filters. Cells were maintained at 37°C during observations with a Sage Air Curtain incubator (Sage Instruments, Inc., White Plains, N.Y.).

Sykes-Moore chambers were utilized for long term time lapse cinematography. In order to see cells growing in these chambers, a long working distance phase condenser was required. Movies were taken with a Sage Time Lapse system (Sage Instruments Inc.).

Electron Microscopy

Cells were grown at 37°C and then fixed on Falcon tissue culture dishes, at room temperature for 1 hr. The fixative consisted of 1% glutaraldehyde in phosphate buffer (pH 7.2–7.4), containing 0.1 M sucrose, 0.003 M $MgCl_2$, and 0.003 M $CaCl_2$ (2). After rinsing in the phosphate buffer, the cells were postfixed for 1 hr in 1% OsO_4 dissolved in the same buffer.

Some cell preparations were flat embedded on the plastic dish in Epon (8), while others were scraped off during dehydration and embedded as a pellet in BEEM capsules. To remove the flat-embedded cells from the Falcon culture dishes, they were immersed in liquid nitrogen for a few seconds and then placed at room temperature. The plastic dish could be pulled off in pieces before equilibration of the dish at room temperature. Since the cells appeared to be identical with regard to their over-all shape and ultrastructure after both of these preparative procedures, the pellet technique was adopted as the method of choice. It was found that cells were much easier to locate in the pellet, and a much larger population of cells could be observed in the electron microscope over a relatively short period of time.

Thin sections were made on an LKB Ultrotome, mounted on collodion-carbon coated grids, and stained with uranyl acetate (9) and lead citrate (10).

RESULTS

The Morphology of Normal BHK-21 Cells

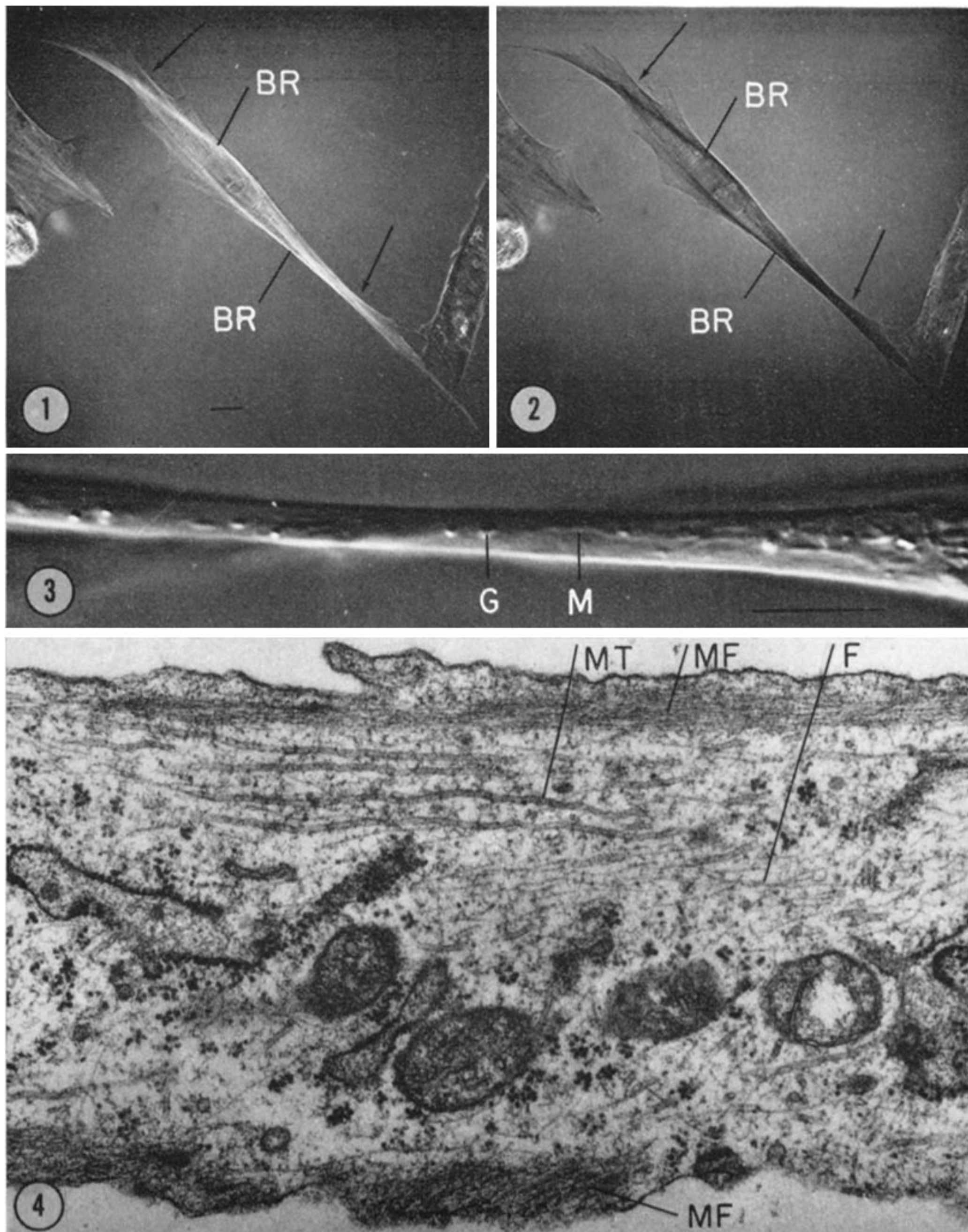
Living BHK-21 cells which have been allowed to attach and spread on a glass or plastic substrate possess birefringent streaks running longitudinally along the long axis of their major cell processes (2). A living BHK-21 cell observed with polarized light optics and possessing two major cell processes is seen in Figs. 1 and 2. Spread cells observed with Nomarski differential interference optics contain mitochondria and granules oriented longitudinally along their major cell processes (Fig. 3). When cells are observed with the electron microscope microtubules, filaments, and microfilaments are seen to be longitudinally oriented along the major process (Fig. 4). All three types of fiber may contribute to the birefringence seen within living cells (2, 3).

The Morphology of Colchicine-Treated BHK-21 Cells

Low density cell cultures were plated on cover slips in normal BHK-21 medium and allowed to attach and spread for 12–18 hr. Most of the nondividing cells appeared fibroblastic in shape and possessed one or more major cell processes (Figs. 1 and 2). The normal medium was removed and colchicine, at a concentration of either 5 μg or 40 μg/ml in BHK-21 medium, was added to these cultures. Within 1 hr, the fibroblastic appearance of cells began to disappear. 2–3 hr after adding colchicine, the majority of cells appeared more epithelial-like than fibroblastic (Fig. 5) and, in most instances, no major cell processes could be found (Fig. 6).

When colchicine was removed after 3–4 hr and the cells were washed several times in normal medium and then maintained in normal medium, the cells returned to their normal fibroblastic morphology. The time course of recovery was dependent on the concentration of colchicine used. After removing 5 μg/ml colchicine, the recovery was rapid and the majority of cells appeared fibroblastic within 1–4 hr. The 40 μg/ml colchicine treatment resulted in a much slower recovery of normal morphology, and often required up to 10–12 hr. Recovery from colchicine treatment was also slower when cells were exposed for longer time intervals at either of the concentrations used.

Cells placed from a suspension into medium containing 5 or 40 μg/ml of colchicine attached and began to spread on glass cover slips within 30 min. After 2–3 hr, many epithelial-like cells were observed. These cells appeared identical to cells which had been treated with colchicine after



FIGURES 1 and 2 A fully spread BHK-21 fibroblast-like cell observed at opposite compensator settings with polarized light optics. Note the presence of two major cell processes (arrows) and birefringent fibers or streaks (*BR*). × 40 lens. Scale lines represent 10 μ.

FIGURE 3 A Nomarski differential interference micrograph of a major cell process viewed with an oil immersion lens (× 100). Filamentous mitochondria (*M*) and granules (*G*) move bidirectionally in oriented paths long the length of these cell extensions. Scale line represents 10 μ.

FIGURE 4 An electron micrograph of a section cut longitudinally through a major cell process similar to the ones designated by arrows in Figs. 1 and 2. Most of the 250 A microtubules (*MT*), 40–60A microfilaments (*MF*), and 100–120A filaments (*F*) are oriented along the long axis of the major cell process. × 38,700.

attachment and spreading in normal medium (Figs. 5 and 6).

In addition to changing their shape, colchicine-treated cells contain a sharply delineated juxtanuclear cap which is easily visualized with the phase contrast microscope (Fig. 6). The cap, in some instances, is located at the periphery of one part of the nucleus, and, in other instances, is spread out around it. Filamentous mitochondria and phase-dense granules are seen to be unoriented and randomly organized in a perinuclear array, away from the periphery of the cell (Fig. 6). Membrane ruffling is seen at many points along the cell surface (Fig. 6).

Cells treated with colchicine for longer time intervals (24–72 hr) consist of two distinct morphological types: epithelial-like and spherical. The epithelial-like cells get larger with time and single cells are seen to fill the microscope field seen with a 25 × objective. Normally, many cells are seen in the field at this magnification. Most of these "giant" cells contain many small nuclei (Fig. 7) which appear to arise by nuclear budding (Fig. 8). These multinucleated cells do not reverse their morphology even after 24 hr in normal medium. Similar multinucleated cells in the presence of colchicine have been reported by other workers (33).

Most of the spherical cells observed in colchicine-treated cultures contain a random array of metaphase chromosomes (Fig. 9). Cells normally round up before mitosis (3, 11), but in the presence of colchicine they are arrested in metaphase and cannot respread on the glass substrate.

Colchicine-Treated Cells Observed with Polarized Light

Colchicine-treated cells with an epithelial-like morphology (Fig. 10) were observed with polarized light optics, in order to determine whether there were changes in the pattern of birefringence seen in normal spread cells. A distinct cap of dark and light banded birefringent material was seen adjacent to the nucleus (Fig. 11). This banded pattern of birefringence indicates that the material is oriented in several directions with respect to the crossed polars. The position of these birefringent caps coincides with the position of the caps seen with phase contrast microscopy (Fig. 6).

In addition to the birefringent caps, fine birefringent fibers are seen in living, epithelial-like, colchicine-treated cells and are more easily seen after 18–24 hr of treatment. These birefringent fibers form a network which traverses the cytoplasm in several directions (Fig. 12). They frequently seem to end at or near a ruffling margin at the cell surface (Fig. 12). They also cross over the nuclear region in many cells (Fig. 13). These fibers can also be seen with Nomarski differential interference optics (Fig. 14). By focusing through the cells carefully with a 100 × oil immersion objective, the Nomarski system reveals that these fibers are almost exclusively located on the side of the cell which is attached to the substrate.

Electron Microscopy of Colchicine-Induced Epithelial-like Cells

At different time intervals after the addition of colchicine, cells were monitored with the phase contrast microscope and then fixed for electron microscopy. There were no microtubules within 2–3 hr after addition of 5 μg or 40 μg/ml colchicine. This was the case regardless of whether cells were allowed to attach and spread in the presence of colchicine or whether colchicine was added to cells which had been allowed to attach and spread before treatment. The loss of microtubules occurs during the first few hours of colchicine treatment and is coincident with the conversion from the fibroblast morphology to the epithelial-like morphology.

The region of the birefringent cap, when observed at low magnification, appears as a fibrous region excluding most large cytoplasmic organelles (Fig. 15). At higher magnification, the region is seen to consist almost exclusively of 100–120A filaments, which are oriented in large bundles and appear to come in and out of the plane of the thin section (Fig. 16). The juxtanuclear organization of the filaments fits well with the pattern of birefringence seen in living cells.

Bundles of microfilaments are localized just under the cell membrane and appear unchanged in colchicine-treated cells. They are oriented in a manner which suggests that they represent the fine birefringent fibers described in living colchicine-treated cells (Fig. 17).

Cells which were returned to normal medium after several hours' to overnight treatment in colchicine were also followed at timed intervals and fixed for electron microscopy. Microtubules begin to reappear in these cells within 30 min after return to normal medium. The reestablish-

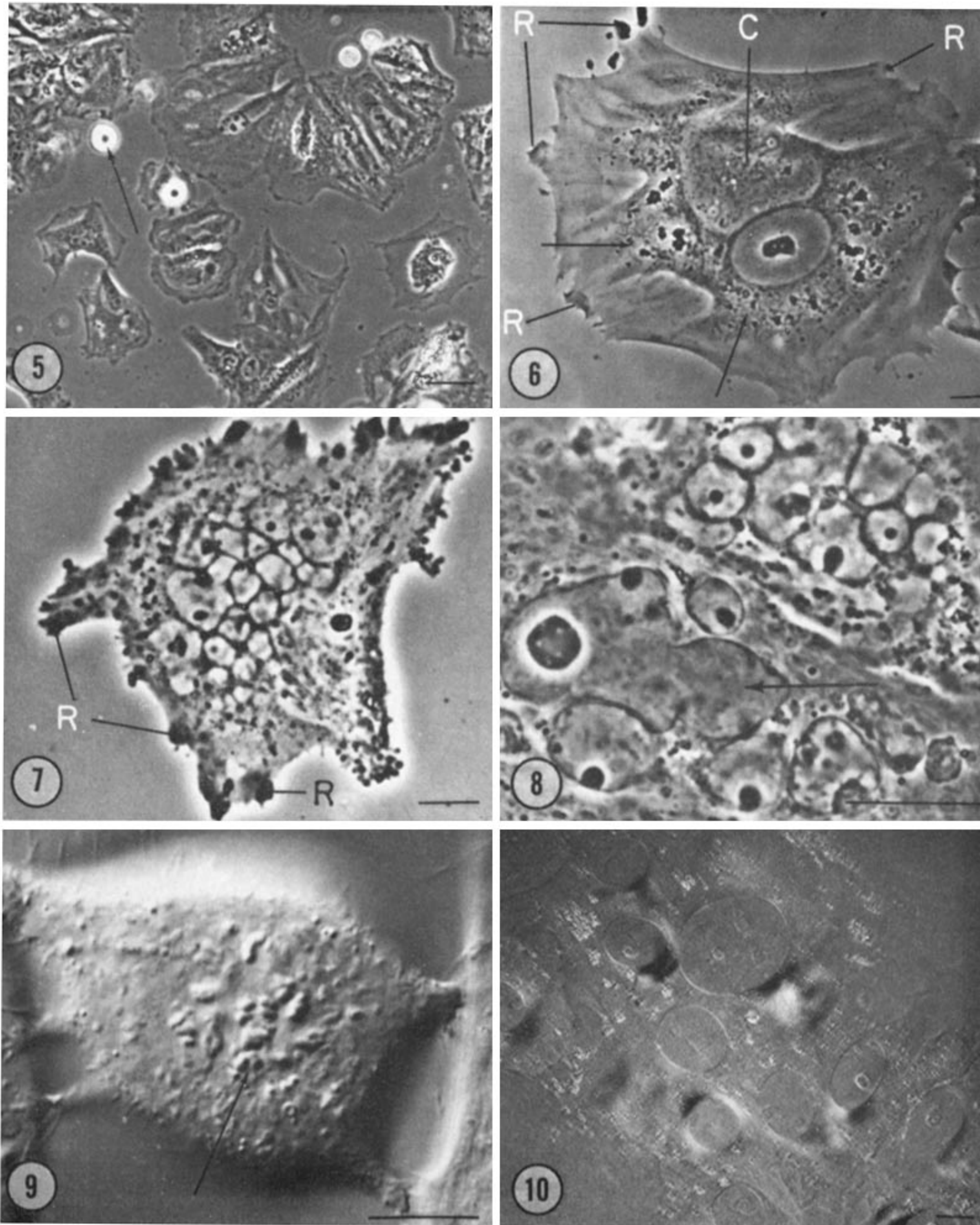


FIGURE 5 Low magnification survey view of cells treated with 5 μg/ml of colchicine for 3 hr. These cells had been grown in normal medium for 18 hr before being transferred to medium containing colchicine. Most of the cells are spread as a relatively uniform sheet around the nucleus, and lack the asymmetric cell processes seen in normal cells. There are also several spherical cells (arrow), which are arrested metaphase cells. Phase contrast, × 25 lens. Scale line represents 50 μ.

FIGURE 6 Higher magnification micrograph of a colchicine-treated epithelial-like cell. A nuclear cap is present (*C*), as well as several ruffled membrane regions (*R*). Note the perinuclear array of randomly oriented mitochondria and granules (arrows). Phase contrast, × 40 lens. Scale line represents 10 μ.

FIGURE 7 Cell treated with 5 μg/ml of colchicine for 30 hr. Many small nuclei are apparent. Note the presence of several ruffling regions at the edge of the cell (*R*). Phase contrast, × 40 lens. Scale line represents 10 μ.

FIGURE 8 The nuclear blebbing phenomenon seen in a cell treated with 5 μg/ml of colchicine for 30 hr (arrow). Phase contrast, × 100 oil immersion lens. Scale line represents 10 μ.

FIGURE 9 Cell arrested in metaphase in medium containing 5 μg/ml of colchicine. Chromosomes are randomly oriented in the cytoplasm (arrow). Nomarski differential interference, × 100 oil immersion lens. Scale line represents 10 μ.

FIGURE 10 Cells treated in colchicine (5 μg/ml) for 6 hr. These cells had been grown in normal medium for 18 hr before the addition of colchicine. Birefringent caps are evident in most cells. Note the dark and light pattern of birefringence. Polarized light, × 10 lens. Scale line represents 10 μ.

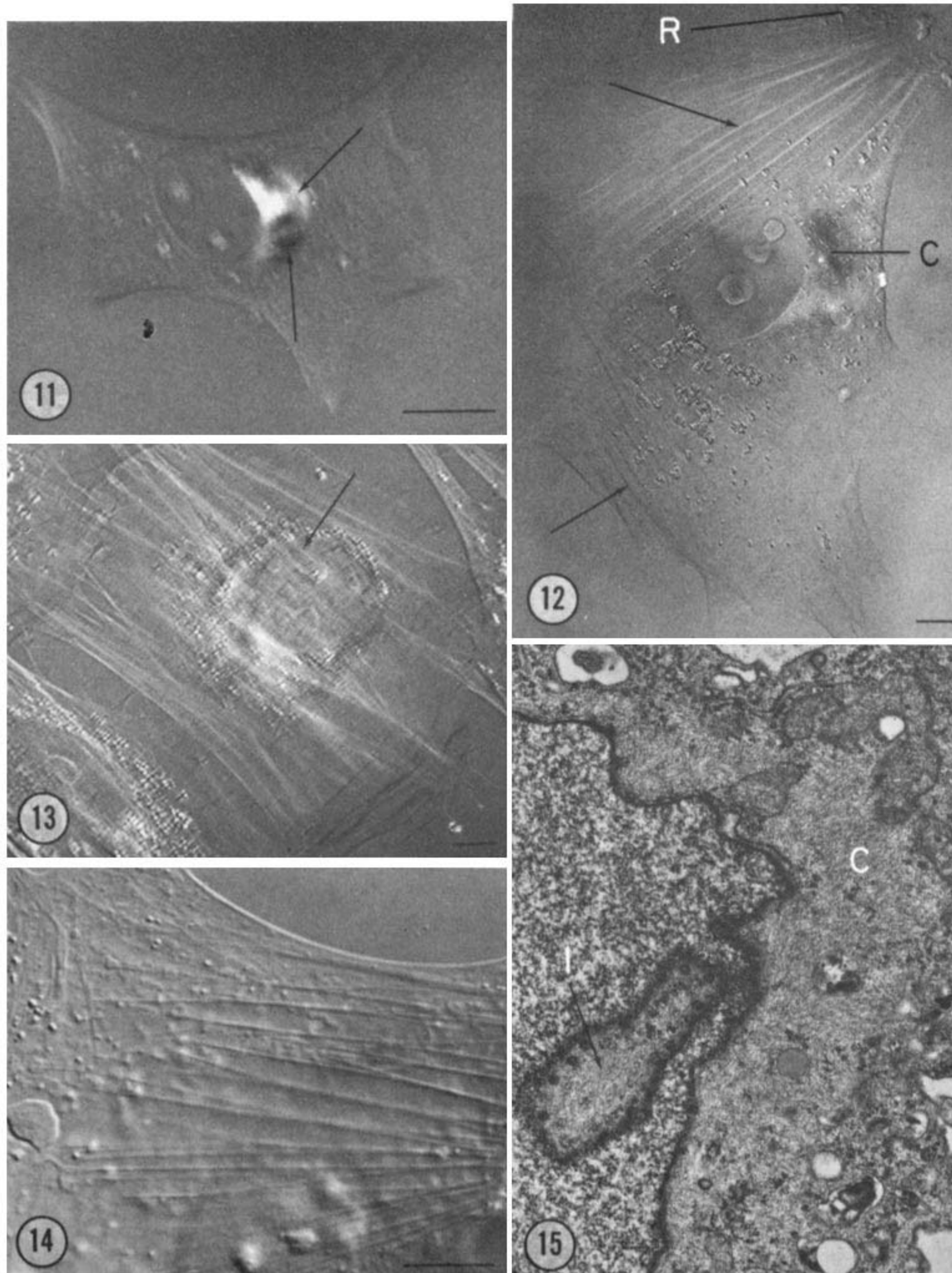


FIGURE 11 Higher magnification view of the birefringent cap of a colchicine-treated cell. Note dark and light regions (arrows). Polarized light, × 40. Scale line represents 10 μ.

FIGURES 12 and 13 Epithelial-like cells exposed to 5 μg/ml of colchicine for 24 hr. Fine birefringent fibers oriented in several directions are present (arrows). Note the birefringent cap (*C*) in Fig. 12 and fibers passing over the nucleus in Fig. 13. *R*, ruffled membrane region. Polarized light × 40. Scale lines represent 10 μ.

FIGURE 14 A cell viewed with Nomarski optics demonstrating the network of fibers just under the attachment side of the cell. The position of the fibers was determined by gradually focusing up and down with the fine focus control. × 100 oil immersion lens. Scale line represents 10 μ.

FIGURE 15 Low magnification electron micrograph of a colchicine-treated cell containing a juxtanuclear cap of filaments (*C*). Note the inpocketing of the nuclear membrane, which also contains filaments (*I*). × 14,200.

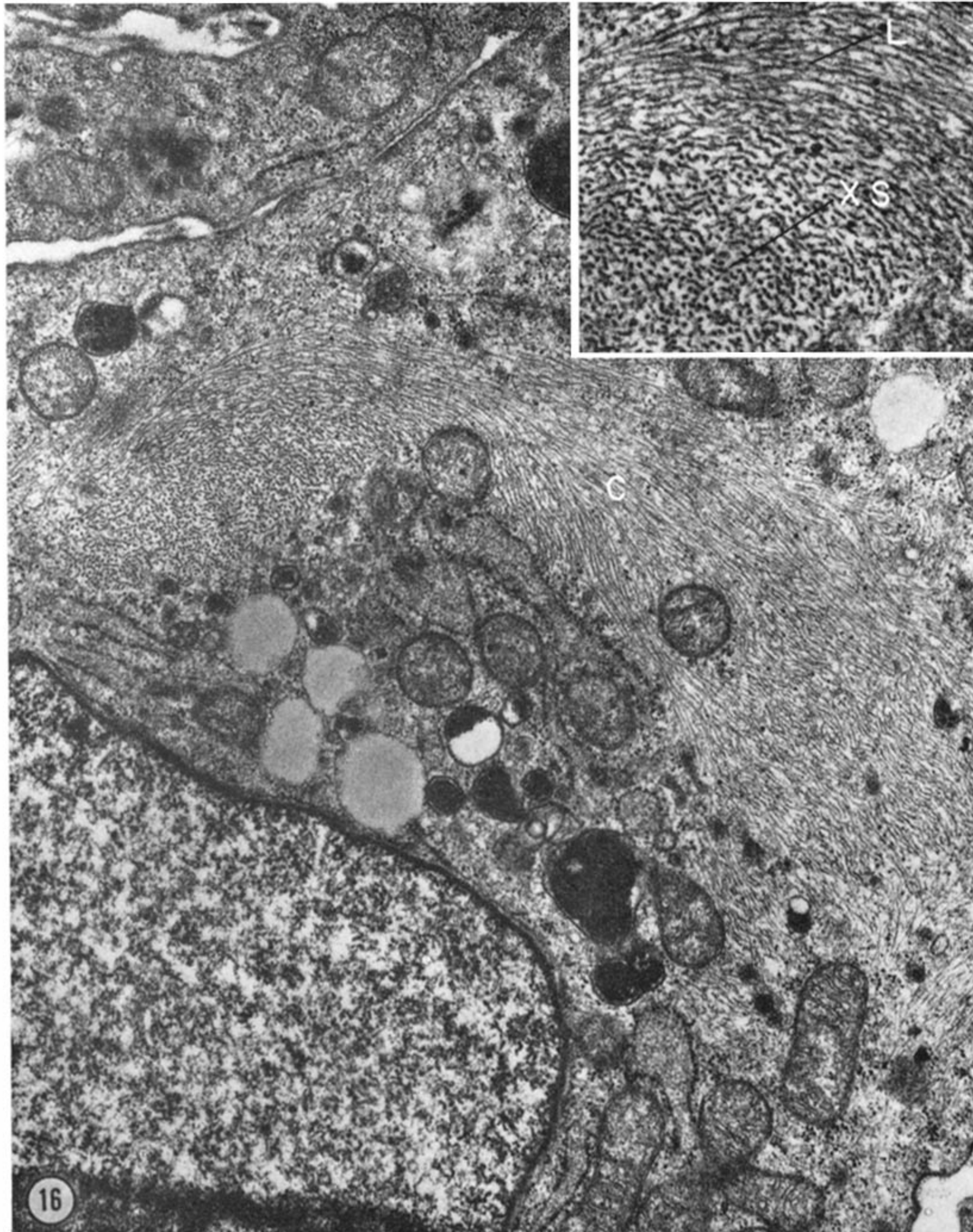


FIGURE 16 Higher magnification electron microscope view of a juxtanuclear cap (*C*). These caps contain oriented bundles of 100–120A filaments which come in and out of the plane of section. The inset shows longitudinal (*L*) and cross sections (*XS*) of filaments. Fig. 16, × 22,200. Inset, × 47,200.

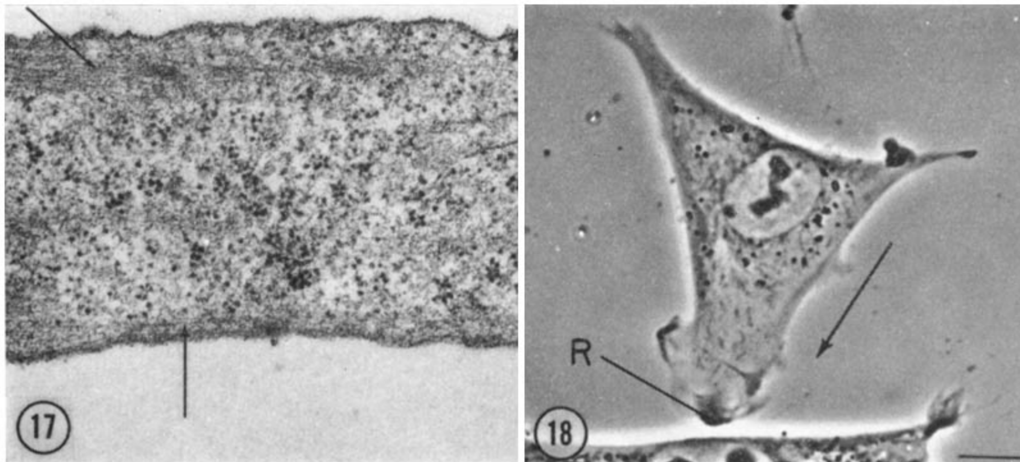


FIGURE 17 Electron micrograph of normal bundles of microfilaments located just under the plasma membrane of colchicine-treated cells (arrows). × 34,500.

FIGURE 18 A moving BHK-21 cell. The cell possesses a leading edge which is ruffling (*R*). The cell is moving in the direction of the arrow. Phase contrast, × 40 lens. Scale line represents 10 μ.

ment of fibroblastic cell shape and the formation of major cell processes is coincident with the formation of increasing numbers of oriented microtubules.

Time Lapse Cinematography of Normal and Colchicine-Treated Cells

Time lapse movies taken of normal BHK-21 cells for periods up to 8 hr demonstrated that cells do translocate across a glass substrate, with a ruffled membrane at their leading edge (Fig. 18). This phenomenon has been described for many types of cultured cells, and the ruffling edge has been implicated as an integral part of the locomotion system (12, 13).

Cells treated with colchicine and observed after the epithelial-like configuration had been attained were also filmed for periods up to 8 hr. During this time interval no cell showed obvious movement across the glass substrate, even though membrane ruffling was evident throughout. Ruffling occurred at several places around the cell periphery (Figs. 6 and 7), but no one ruffle seemed to be able to become a leading edge.

DISCUSSION

Microtubules: Cell Shape and Cell Spreading

The results of colchicine treatment on BHK-21 cells indicate that microtubules are involved in the formation and maintenance of fibroblastic shape in normal cells. Microtubules are oriented longitudinally along the major cell processes of BHK-21 cells, which may reach lengths of up to several hundred microns (2). Microtubules rapidly disappear after exposure to colchicine, resulting in an epithelial-like cell morphology. Microtubules have been implicated in similar structural maintenance roles in a variety of other cell types (4, 5). By allowing cells to attach and spread in colchicine, it was demonstrated that microtubules are not needed by the cell for attachment and spreading on a glass substrate.

The Relationship Between Filaments and Microtubules

Both microtubules and filaments are found longitudinally oriented along the major cell processes of spread BHK-21 cells (2). Invariably, microtubules in major cell processes are seen surrounded by several parallel filaments (2). In colchicine-treated cells, the 100–120A filaments are arrested in a juxtanuclear position, indicating an inability to move away from the nucleus in the absence of microtubules. A similar juxtanuclear region of 100–120A filaments is seen just after normal cells are allowed to attach to a glass substrate (3). This sphere is birefringent and during the spreading process gives rise to some of the birefringent fibers observed in spread cells

(3). Electron microscope studies demonstrate that as the birefringent sphere "reels out" fibers, filaments are simultaneously dispersed into longitudinally oriented arrays in the major cell processes being formed during cell spreading (3). This dispersal of oriented filaments is coincident with the formation of increasing numbers of visible microtubules in the major cell processes formed as a cell spreads from a spherical to a fibroblastic configuration (2, 3). This evidence suggests that filaments are dependent on microtubules for their normal distribution in spread cells.

It has been suggested that colchicine treatment of nerve microtubules (neurotubules) results in a reversible conversion of microtubules into filaments (neurofilaments) (14). This is based on an increase in the number of 100A filaments observed in the perikarya of nerve cells treated with colchicine (14). A similar interconversion of microtubules and 80–100A filaments, on the basis of increased numbers of filaments, has also been proposed in virus-induced syncytia of BHK-21 cells treated with colchicine (15), and in macrophages exposed to colchicine (16). These results in nerve and other cells are similar to those described in this paper for colchicine-treated BHK-21 cells. We feel, however, that in BHK-21 cells there is an apparent increase in numbers of filaments in the absence of microtubules, due to their juxtanuclear accumulation. Sections through this region contain a high concentration of filaments and would give the impression of an increase in numbers. Thin sections through other regions of colchicine-treated cells show that filaments are no longer distributed throughout the cell as they are in control cell populations.

The tubular nature of 100–120A filaments has been reported in BHK-21 cells (2) and in nerve cells (17). Wuerker and Palay (17) compared the wall thickness of microtubules and filaments of the anterior horn cells in rats and demonstrated that the thickness of the microtubule walls is at least twice that of the filament walls. We have found a similar discrepancy in the wall thicknesses of microtubules and filaments in ultrathin sections of BHK-21 cells.[1] On the basis of these measurements of wall thickness and the proposed protofilament substructure theory of microtubules (18), it is difficult to accept the idea that microtubules and filaments consist of the same monomer subunits, unless the packing of subunits differs in the two types of cytoplasmic fibers. The recent finding that microtubule protein and filament protein isolated from nerve axoplasm differ in molecular weight, size, electrophoretic mobility, immunological specificity, and amino acid composition supports the idea that microtubules and filaments do not consist of the same protein subunits (19, 20).

The Microtubule-Filament Complex and Intracellular Organelle Orientation and Movements

It was proposed previously that the microtubule-filament complex found in the major cell processes of BHK-21 cells provided a possible two-component system for oriented intracellular organelle and particle movements (2). Mitochondria and particles are seen to move in a discontinuous, bidirectional, oriented fashion within the major cell processes of spread cells (Fig. 3). Colchicine treatment and the consequent disruption of the microtubule-filament complex results in a disoriented perinuclear array of mitochondria and particles (Fig. 6). Microtubules have also been purported to play an important role in intracellular (saltatory) particle movements in several other cell types (21, 22). Thus the available evidence supports the idea that the microtubule-filament complex is involved in some manner in orienting organelles, as well as in intracellular particle movements. A detailed analysis of organelle movements, both in the presence and in the absence of microtubules in cultured nerve and BHK-21 cells is now in progress.[2]

Membrane Ruffling and Cell Locomotion

Ruffling, or membrane undulation, is thought to be directly involved in the locomotion of cultured fibroblasts by forming intermittent contacts with the substrate which, in turn, result in the net translocation of whole cells (13, 30, 31). A moving cell always possesses a ruffled membrane at its leading edge. There is very little, if any, ruffling at other points along the cell surface (Fig. 18) (12, 13). Abercrombie (12) has described the phenomenon of the initiation of locomotion as being due to one of several ruffling regions be-

[1]Goldman, R. D. Unpublished observations.

[2]Chang, C. M., and R. D. Goldman. Unpublished observations.

coming the prominent leading edge, while other edges lose their ruffling capacity. In colchicine-treated BHK-21 cells, membrane ruffling is observed at several points along the cell periphery; however, no one ruffling edge seems to be capable of taking over as the leading edge, and the cells do not translocate during the time intervals of observation. However, normal cells were seen to translocate during the same time period. Similar results have been obtained by Gail and Beene (32) using Colcemid on 3T3 cells, but these cells continue moving at a much slower pace in Colcemid. These authors did not use electron microscopy, however, so it is possible that some microtubules remained in the cells. The lack of a normal, oriented microtubule-filament complex, in colchicine-treated BHK-21 cells, indicates that these cytoplasmic fibers may be involved in determing which ruffling edge becomes the leading edge.

Bundles of microfilaments retain their normal distribution in colchicine-treated cells (Fig. 17), and in living cells they appear as fine birefringent fibers (Fig. 12) which frequently end at a ruffling edge of a cell. On the basis of the submembranous localization of microfilaments (Fig. 4) (2, 11) and the fact that similar microfilaments in other cell systems bear a definite physiological and chemical resemblance to muscle actin (23, 24, 25), it seems likely that they are involved in membrane ruffling. This is further substantiated by studies with cytochalasin B which inhibits membrane ruffling and disorganizes submembranous bundles of microfilaments (26, 27).

Cell Attachment and Spreading in the Absence of the Normal Microtubule-Filament Complex

Cell attachment and spreading proceeds in the absence of visible microtubules, and therefore microtubules do not seem to be directly related to these phenomena. Filaments remain in a juxtanuclear position during cell attachment and spreading and therefore do not seem to play an important role in cell spreading. Microfilament bundles, however, form oriented arrays in spread colchicine-treated cells, and are therefore a likely candidate for direct involvement in this aspect of cultured cell motility. The observation that they seem to be preferentially located just under the attached surface of colchicine-treated cells (Fig. 14), suggests that they may also be involved in some manner with attachment to the substrate. Jones (28) and Jones (29) have put forth the hypothesis that a contractile layer just under the cell membrane might be involved in cell adhesion.

The results with colchicine-induced breakdown of microtubules suggest that they play important roles in the determination of fibroblastic cell-shape, the formation of major cell processes, and cell locomotion. The fact that filament distribution is also affected by the absence of microtubules suggests a functional relationship between the two types of cytoplasmic fibers. Microfilaments, on the other hand, seem to be independent of the microtubule-filament complex and probably play important roles in cell spreading and membrane ruffling.

REFERENCES

The author is most grateful for the assistance of Miss Anne Bushnell throughout the period of this investigation.

This work has been supported by grants from the American Cancer Society (E-639), the National Science Foundation (GB-23185), and the Damon Runyon Memorial Fund for Cancer Research, Inc. (DRG-1083).

Received for publication 20 May 1971, and in revised form 30 June 1971.

1. Stoker, M., and I. Macpherson. 1964. *Nature (London)* **203**:1355.
2. Goldman, R. D., and E. A. C. Follett. 1969. *Exp. Cell Res.* **57**:263.
3. Goldman, R. D., and E. A. C. Follett. 1970. *Science (Washington).* **169**:286.
4. Tilney, L. G., and K. R. Porter. 1965. *Protoplasma.* **60**:317.
5. Tilney, L. G., Y. Hiramoto, and D. Marsland. 1966. *J. Cell Biol.* **29**:77.
6. Bickle, D., L. G. Tilney, and K. R. Porter. 1966. *Protoplasma.* **61**:322.
7. Borisy, G. G., and E. W. Taylor. 1967. *J. Cell Biol.* **34**:525.
8. Luft, J. J. 1961. *J. Biophys. Biochem. Cytol.* **9**:231.
9. Stempak, J., and R. Ward. 1964. *J. Cell Biol.* **22**:697.
10. Reynolds, R. 1963. *J. Cell Biol.* **17**:208.
11. Follett, E. A. C., and R. D. Goldman. 1970. *Exp. Cell Res.* **59**:124.
12. Abercrombie, M. 1961. *Exp. Cell Res. Suppl.* **8**:188.
13. Ambrose, E. J. 1961. *Exp. Cell Res. Suppl.* **8**:54.
14. Wisniewski, H., M. L. Shelanski, and R. D. Terry. 1968. *J. Cell Biol.* **38**:224.
15. Holmes, K. V., and P. W. Choppin. 1968. *J. Cell Biol.* **39**:526.

16. Bhisey, A. N., and J. J. Freed. 1971. *Exp. Cell Res.* **64**:419.
17. Wuerker, R. B., and S. L. Palay. 1969. *Tissue and Cell.* **3**:387.
18. Gall, J. G. 1966. *J. Cell Biol.* **31**:639.
19. Huneeus, F. C., and P. F. Davison. 1970. *J. Mol. Biol.* **52**:415.
20. Davison, P. F., and F. C. Huneeus. 1970. *J, Mol. Biol.* **52**:429.
21. Rebhun, L. I. 1971. *Int. Rev. Cytol.* In Press.
22. Freed, J. J., and M. M. Lebowitz. 1970. *J. Cell Biol.* **45**:333.
23. Ishikawa, H., R. Bischoff, and H. Holtzer. 1969. *J. Cell Biol.* **43**:312.
24. Pollard, T. D., E. Shelton, R. Weihing, and E. Korn. 1970. *J. Mol. Biol.* **50**:91.
25. Nachmias, V. T., H. Huxley, and D. Kessler. 1970. *J. Mol. Biol.* **50**:83.
26. Carter, S. B. 1967. *Nature (London).* **213**:261.
27. Wessells, N. K., B. Spooner, J. Ash, M. Bradley, M. Luduena, E. Taylor, J. Wrenn, K. Yamada. 1971. *Science (Washington).* **171**:135.
28. Jones, B. M. 1966. *Nature (London).* **212**:362.
29. Jones, P. C. T. 1966. *Nature (London).* **212**:365.
30. Ingram, V. M. 1969. *Nature (London).* **222**:641.
31. Abercrombie, M., J. Heaysman, and S. Pegrum. 1970. *Exp. Cell Res.* **59**:393.
32. Gail, M. H., and C. Beene. 1971. *Exp. Cell Res.* **65**:221.
33. Kihlman, B. A. 1966. Actions of Chemicals on Dividing Cells. Prentice Hall, New York. 260 pp.