# THE EFFECTS OF CYTOCHALASIN B ON THE MICROFILAMENTS OF BABY HAMSTER KIDNEY (BHK-21) CELLS

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ABSTRACT

Attempts were made to test the motile functions of bundles of microfilaments found in baby hamster kidney (BHK-21) cells, by using cytochalasin B (CB). It was found that individual cells respond differently to the drug. These differential effects are quite obvious in both light and electron microscope preparations. Some cells contain normal bundles of microfilaments even after 24 hr in CB, and other cells form muscle-like configurations which also contain arrays of microfilaments. These varied effects suggest the existence of several types of microfilaments in BHK-21 cells, and make the interpretation of the motile role of microfilaments difficult to evaluate at the present time.

## INTRODUCTION

Cytochalasin B (CB)[1] (1) causes the disruption of microfilaments in the cleavage furrow of marine eggs and HeLa cells (2–4), and the reversible disorganization of microfilaments in several types of embryonic cultures (5–8). It has been suggested that these microfilaments are involved in primitive contractile processes and that they are similar to actin (8–10). We have attempted to use CB to test the specific function of the submembranous bundles of microfilaments found in baby hamster kidney (BHK-21) cells (9); however, preliminary evidence demonstrates that there is not a simple change in microfilament configuration in these cells. On the basis of the findings reported in this paper, we are not yet able to use CB to test specifically the function of microfilaments in BHK-21 cells.

[1] *Abbreviations:* CB, cytochalasin B; DMSO, dimethylsulfoxide.

## MATERIALS AND METHODS

### *Cell Cultures*

BHK-21/C13 cells (11) were grown in BHK-21 medium supplied by Grand Island Biological Company (Grand Island, N. Y.). The medium was supplemented with 10% tryptose phosphate broth, 10% calf serum, and 100 units/ml of penicillin and streptomycin before use. Stocks of growing cells were maintained at 37°C in Falcon plastic tissue culture dishes (Falcon Plastics, Div. of Bioquest, Oxnard, Calif.), kept in a humidified atmosphere of 95% air–5% $CO_2$. Every 2 wk, frozen stocks of cells were thawed and fresh cultures were started. Cells more than 2 wk old were discarded. Cells growing in plastic Petri dishes were removed by treatment with trypsin-ethylenediaminetetraacetate solution supplied by Grand Island Biological Co.

### *Light Microscopy*

Cells were grown on glass cover slips for observation with phase contrast and Nomarski differential

interference optics. 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 between the cover slip and the slide before sealing.

Light micrographs were taken with a Zeiss Photomicroscope (Carl Zeiss, Inc., New York) containing a green-filtered 60 w tungsten light source. Cells were maintained at 37°C during light microscope observations with a Sage Air Curtain incubator (Sage Instruments Inc., White Plains, N. Y.).

### *Electron Microscopy*

Cells were grown at 37°C and were fixed at room temperature on Falcon plastic tissue culture dishes. The cells were fixed for 1 hr in 1% glutaraldehyde in 0.05 M $KH_2PO_4$-NaOH buffer (pH 7.0–7.2) containing 0.1 M sucrose, 0.003 M $MgCl_2$, and 0.003 M $CaCl_2$ (9). After brief rinsing in 0.05 M $KH_2PO_4$-NaOH buffer (pH 7.0), the cells were postfixed for 1 hr in 1% $OsO_4$ dissolved in the same buffer. During dehydration in ethanol, the cells were scraped off the plastic Petri dish with a rubber policeman, and were centrifuged at ~600 rpm in a clinical centrifuge. The pellet of cells was embedded in B.E.E.M. capsules (Better Equipment for Electron Microscopy, Bronx, N. Y.) containing Epon (12). Cells treated in this manner appeared identical in morphology to cells which had been flat embedded on the Petri dish or had been flat embedded on Millipore filters (Millipore Corporation, Bedford, Mass.) (9).

Thin sections were made on an LKB Ultratome (LKB Instruments, Inc., Rockville, Md.) and were mounted on collodion-carbon-coated grids. The sections were stained with hot uranyl acetate (13) and lead citrate (14), and observed with an RCA EMU4 electron microscope.

### *CB Treatment*

Stock solutions of 100 μg/ml of CB were made up in 0.1% dimethylsulfoxide (DMSO) according to the technique of Carter (1). Freshly trypsinized (9) BHK-21 cells were placed into normal culture medium and allowed to attach and spread on the substrate for at least 6 hr. After initial attachment and spreading, fresh culture medium containing either a dilution of the CB stock solution, or an identical dilution of 0.1% DMSO, was added. The latter served as a control. The final concentrations used were 5 μg/ml or 10 μg/ml CB and approximately 0.01% DMSO.

## RESULTS

Cells grown in normal medium or in DMSO-containing medium were identical in appearance when observed with the phase contrast microscope. Fig. 1 shows a field of typical fibroblast-like BHK-21 cells after 24 hr of culture in medium containing 0.01% DMSO.

Populations of CB-treated cells observed after 12–24 hr of treatment with 5 or 10 μg/ml CB consisted of variable numbers of several morphological types. Some cells were rounded up, although still attached to the substrate, and others were still somewhat spread, containing long cell processes (Figs. 2 and 3). The latter type of cell differed from normal BHK-21 cells by having a "spiky" morphology (Fig. 3). Some cells contained one or two regions which appeared to be bubbling (Fig. 4). Still other cell types appeared to be well spread on the cover slips, but due to the inhibition of cytokinesis in the presence of CB they had two nuclei (Fig. 5). Cells possessing this type of morphology also exhibited a distinct cessation of membrane ruffling, which reappeared within minutes after CB was washed out of a culture after 12–24 hr of drug treatment (Fig. 6). Nuclear extrusion in the presence of CB was also frequently observed (Figs. 7 and 8). It is not possible to give percentages of each cell type, due to the variability from one preparation to the next. Most of these types of morphological changes in the presence of CB have been reported in other cultured cell types (1, 15).

When cells which had been treated with CB for 12–24 hr were returned to normal medium, ruffled membranes appeared within minutes (Fig. 6) and a morphologically normal cell culture was observed within a few hours. The only obvious difference was the unusually large number of binucleate cells in the population. This was due to the inhibition of cytokinesis by CB, in the presence of normal karyokinesis.

Electron microscopy of 24-hr cultures revealed that control cells maintained in medium containing the appropriate dilution of 0.1% DMSO possessed the normal distribution of microtubules, microfilaments, and filaments described in previous publications (9, 10, 16, 17). Bundles of microfilaments are seen just under the cell membrane in control cells (Figs. 9 and 10).

Many of the cell types observed in CB-treated cultures with the phase contrast microscope were also observed with the electron microscope. The "rounded" type of CB-treated cell (see *R* in Fig. 2) revealed the presence, in some but not in all preparations, of bundles of fibers which possessed a muscle-like configuration (Figs. 11–13). Fig. 11

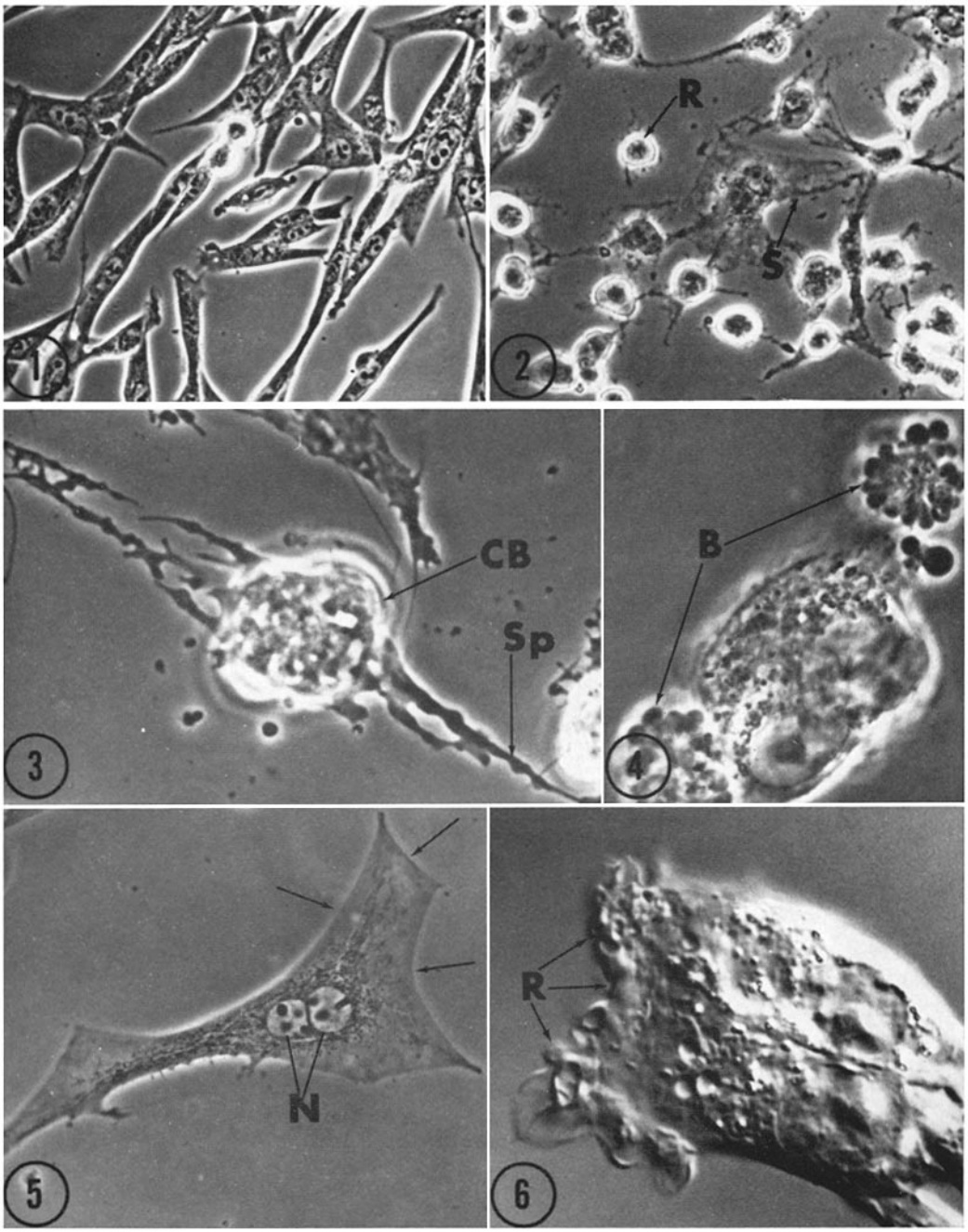



FIGURE 1 BHK-21 fibroblast-like cells after 24 hr in DMSO. Phase contrast. × 1120.

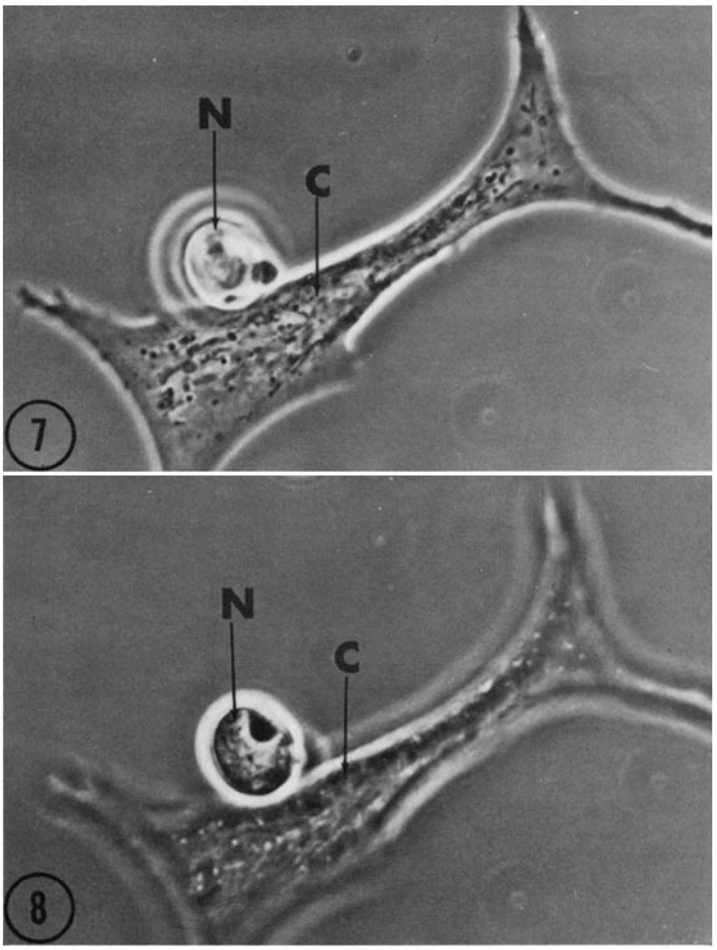
FIGURE 2 BHK-21 cells observed 24 hr after placing in medium containing 10 μg/ml of CB. *R*, round cell; *S*, a spread cell. Phase contrast. × 1250.

FIGURE 3 A spiky cell observed at higher magnification, after 24 hr in medium containing 10 μg/ml of CB. Note the rounded cell body (*CB*) and the spiky processes (*Sp*). Phase contrast. × 4480.

FIGURE 4 Cell showing the bubbling effect (*B*). These bubbling regions may be seen by focusing up at the surface of the cell. 10 μg/ml CB for 24 hr. Phase contrast. × 5000.

FIGURE 5 A well-spread cell maintained in 10 μg/ml CB for 24 hr. This cell has two nuclei (*N*) and demonstrates the lack of ruffled membranes in the presence of CB (arrows). Phase contrast. × 1800.

FIGURE 6 A cell observed within minutes after washing out CB, after a 24-hr, 10 μg/ml CB treatment. Note the appearance of ruffling (*R*). Nomarski differential interference. × 5120.




FIGURES 7 and 8 A CB-treated cell extruding its nucleus. Fig. 7 is focused on the cytoplasmic portion of the cell (*C*), and Fig. 8 is focused on the nucleus being extruded (*N*). Phase contrast. × 4480.

shows a region of one of these cells which contains many bundles of fibers. Striated bodies with electron-opaque periodicities spaced 100 mμ apart are also seen in cells containing these bundles (Fig. 11 and insert). At higher magnification (Fig. 13), longitudinal sections of these bundles showed alternating, thick, dense filaments which are 150–180 A in diameter, and microfilaments which are 50–80 A in diameter. Suggestions of cross-links between thick and thin filaments were frequently observed in longitudinal sections (Fig. 13). These bundles of alternating thick and thin filaments also bear a remarkable resemblance to muscle cells when observed in cross section (Fig. 12). Frequently, the thick filaments appeared to have a less dense core in cross-sectional views. No microfilaments were seen just under the cell membrane in these rounded cells. There was also a conspicuous absence of microvilli at the surface of these cells. Normal cells which are rounded up for cell division, or cells which are induced to round up following trypsinization, are usually covered with microvilli which contain bundles of microfilaments (10).

Cells possessing long cell processes (Figs. 2 and 3) were also observed with the electron microscope and were seen to contain the normal complement of longitudinally-oriented 40–60 A microfilaments, 200–250 A microtubules, and 100 A filaments found in normal cells (9) (Figs. 14 and 15).

## DISCUSSION

The results of these experiments with CB indicate that a simple change in the configuration of micro-

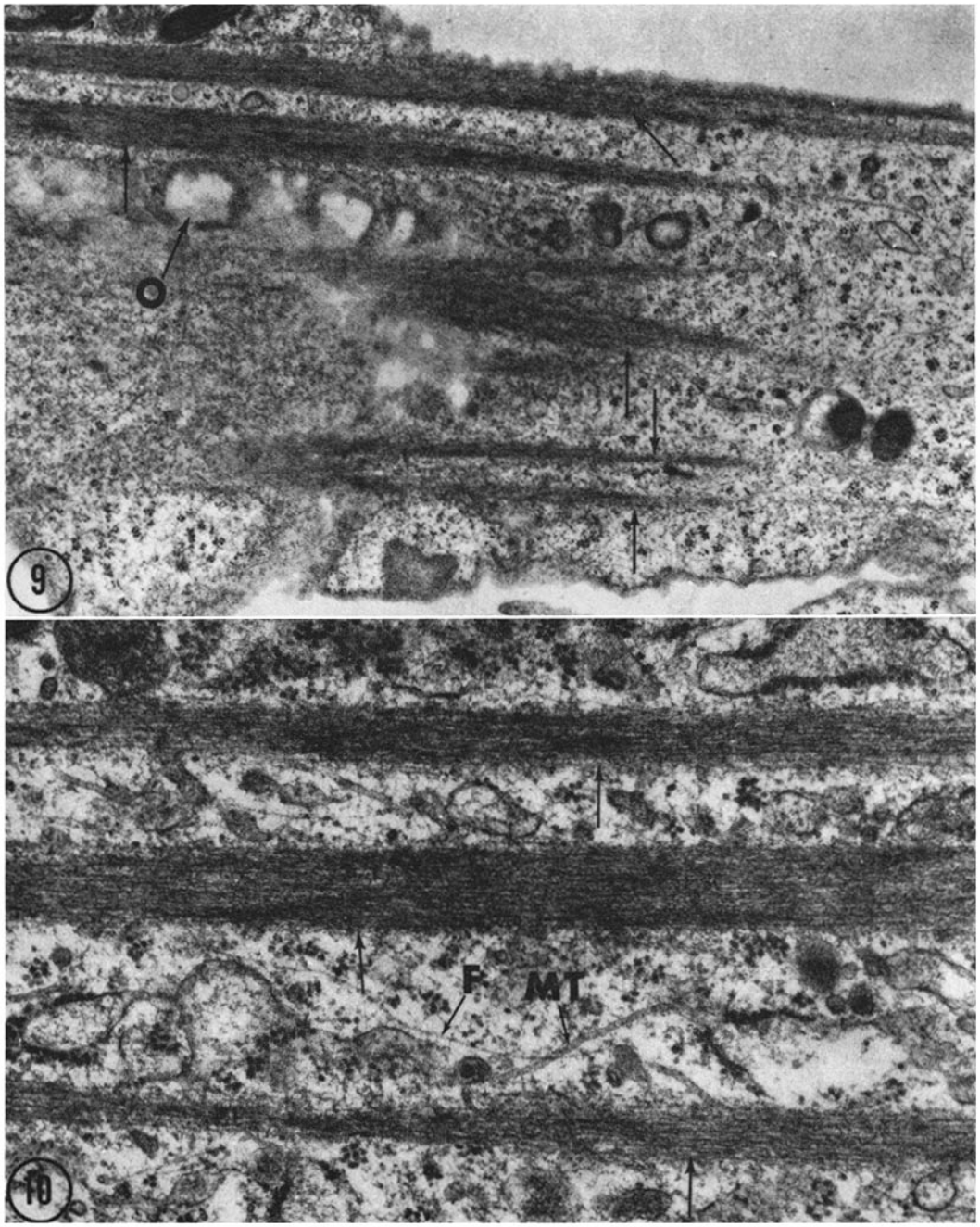



FIGURE 9 A longitudinal section just under the cell membrane of a major cytoplasmic process of a DMSO-treated cell (24 hr). The arrows point to bundles of 40–60 A microfilaments. The plane of section passes out of the cell at several places (O). × 16,000.

FIGURE 10 A higher magnification view of the same cell process as in Fig. 9. Note the presence of longitudinally oriented bundles of microfilaments (arrows), microtubules (*MT*), and filaments (*F*). × 40,000.

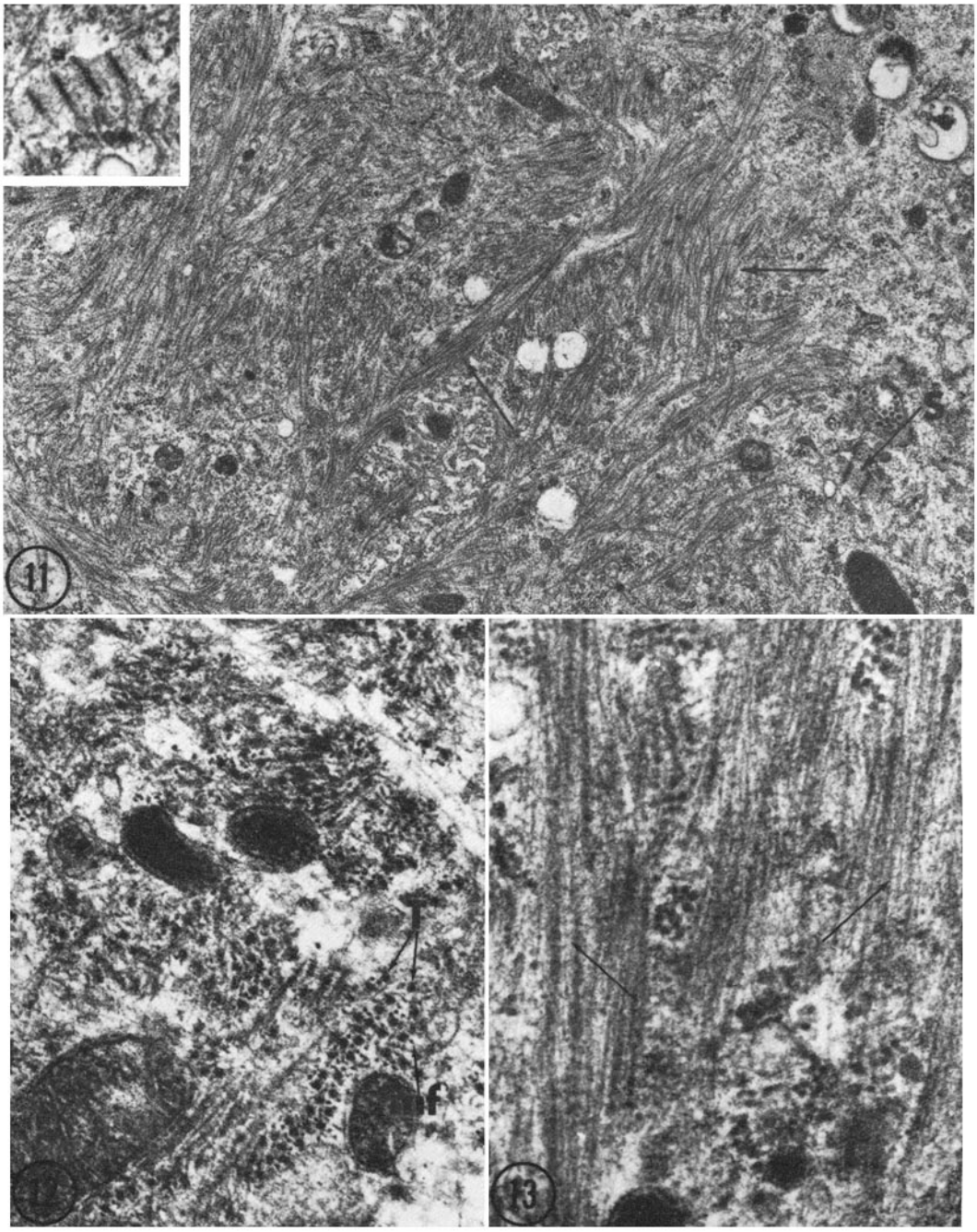



FIGURE 11 An electron micrograph of a rounded type of CB-treated cell. Bundles of fibers can be seen (arrows) as well as a striated body (*S*). The *insert* shows a similar striated body at higher magnification. × 13,000. *Insert*: × 30,000.

FIGURE 12 A higher magnification of a cross section through the bundles of fibers observed in Fig. 11. Thick filaments (*T*) are always surrounded by thin microfilaments (*mf*). × 55,000.

FIGURE 13 A higher magnification of a longitudinal section through the bundles of fibers observed in Fig. 11. Cross-links between thick and thin filaments are evident (arrows). × 55,000.

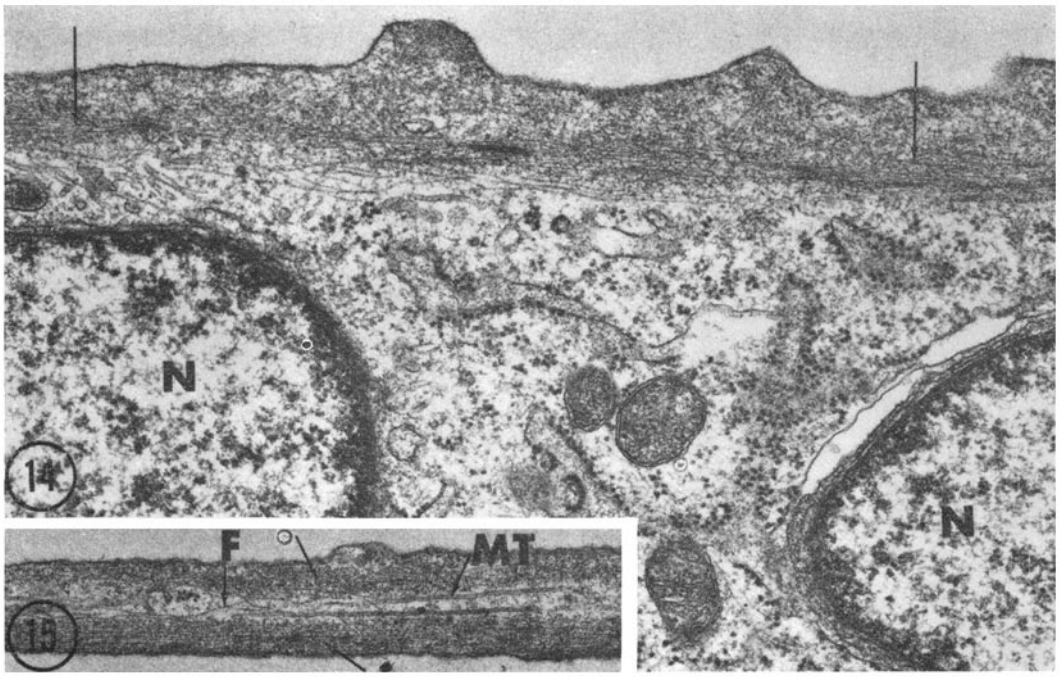



FIGURE 14 A binucleated cell (*N*) which has been in CB for 24 hr. Note the presence of microfilaments under the cell membrane (arrows). × 30,000.

FIGURE 15 A longitudinal section through a spiky cell process. Note the longitudinal distribution of microtubules (*MT*), filaments (*F*), and microfilament bundles (arrows). 24 hr, 10 μg/ml CB treatment. × 21,000.

filaments does not take place in BHK-21 cells. Furthermore, these results demonstrate that we cannot yet use this drug to analyze the role of microfilaments in BHK-21 cell motility (10), in a manner similar to our analysis of the roles of microtubules in BHK-21 cells treated with colchicine (17, 18).

The facts that some cells in some experiments extrude their nuclei and other cells form muscle-like configurations, and that still other cells respond differently, lead us to the conclusion that at least over a 24 hr period individual cells are affected in different ways by CB. Possible explanations for this differential action may lie in our use of freshly trypsinized unsynchronized cells. Thus, cells may react to CB in a manner which is dependent upon the stage of the cell cycle at which the cell is first exposed to the drug.

The 150–180 A fibers found in the muscle-like complexes resemble myosin of glutaraldehyde-fixed muscles in cross-sectional diameter (19–21). The 50–80 A microfilaments found in these complexes are also identical in dimensions and morphology to actin found in muscle (21). The fact that there are numerous microfilaments surrounding each thick fiber is also very similar to the packing of actin and myosin in muscle cells (19–22). The suggestion of links between microfilaments and thick filaments in CB-treated cells (Fig. 13) resembles the heavy meromyosin cross-links of striated muscle (22).

There is extensive evidence in the literature which suggests that microfilaments from a wide variety of nonmuscle cells are actin-like due to their binding of heavy meromyosin (23–25) and their chemical properties (26). There is also evidence that myosin-like proteins are present in such nonmuscle cells (e.g., 26). In light of all the rapidly accumulating evidence on muscle-like proteins in nonmuscle cells, it may not be so surprising to find muscle-like morphology in BHK-21 cells. The finding that these complexes are formed in some

CB-treated cells may reflect a change in the normal control mechanism involved in the configuration and distribution of contractile proteins in BHK-21 cells, allowing a more muscle-like association between actin and myosin-like molecules.

Some CB-treated preparations did not contain cells with muscle-like configurations, even though they contained rounded-up cells. Muscle-like configurations were only seen with two out of four different batches of CB, so the differential effects from one preparation to the next may possibly be due to differences in preparations of CB. At the present time, we have no positive method for determining which batches produce the muscle-like configurations, other than observing thin sections with the electron microscope.

The fact that CB does not act in a uniform and specific manner on microfilaments, is supported by the appearance of microfilaments which are not sensitive to CB in the muscle-like complexes and the maintenance of bundles of microfilaments in those cells which retain spiky cell processes. On the other hand, submembranous bundles of microfilaments cannot be found in the rounded up cell bodies, and microvilli which normally contain microfilaments (10) have not been found in CB-treated cells. These findings suggest either that there may be several types of microfilaments in BHK-21 cells, or alternatively that sensitivity of microfilaments to CB may depend on their physiological state (i.e., their degree of polymerization or aggregation). The former possibility has also been suggested by Schroeder (3) in studies of HeLa cells and by Spooner et al. (27), who find CB-resistant microfilaments in glial cells.

In light of the findings reported in this paper, it is difficult to determine which specific components of the BHK-21 cell's molecular architecture are affected in the presence of CB. Spooner et al. (27) have reported that the microfilament networks, which may be responsible for membrane ruffling are disrupted by CB; however we have not yet found similar networks in normal BHK-21 cells. It has also been suggested by some investigators (e.g., 28) that CB acts primarily on cell membranes, and this possibility should be taken into consideration in future investigations.

In conclusion, we feel that the action of CB in BHK-21 cells does not reflect a uniform change in the configuration of microfilaments. Thus, CB cannot be reliably used at the present time, to determine the possible roles of microfilaments in BHK-21 cell motility.

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