ON THE ROLE OF MICROTUBULES IN MOVEMENT AND ALIGNMENT OF NUCLEI IN VIRUS-INDUCED SYNCYTIA

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ABSTRACT

Infection of baby hamster kidney (BHK21-F) cells with the parainfluenza virus SV5 causes rapid and extensive cell fusion. Time-lapse cinematography shows that when cells fuse, their nuclei migrate straight to the center of the syncytium at rates of 1–2 μ/min. Nuclei are often arranged in long, tightly packed, parallel rows in syncytia derived from the fibroblastic BHK21-F cells. Polarization microscopy shows birefringent material between and parallel to these rows of nuclei, and electron microscopy shows bundles of cytoplasmic microtubules, ~250 A in diameter, and filaments, ~80 A in diameter, parallel to and between the rows of nuclei. Colchicine treatment causes disappearance of microtubules from BHK21-F cells and an apparent increase in the number of 80-A filaments. Although colchicine-treated, SV5-infected cells fuse, their nuclei do not migrate or form rows but remain randomly scattered through the syncytial cytoplasm. Incubation at 4°C does not disrupt microtubules in BHK21-F cells. Rows of nuclei have been isolated from SV5-induced syncytia, and the nuclei in them have been found to be intimately associated with microtubules but not with other cytoplasmic structures. These results suggest that microtubules demarcate cytoplasmic channels through which nuclei migrate and that they may also be involved in the mechanism of nuclear movement.

INTRODUCTION

Many animal viruses have been shown to cause cells to fuse together to form multinucleate syncytia (reviewed by Roizman, 1962). Although members of several of the major groups of animal viruses can cause fusion, the most effective cell-fusing viruses belong to myxovirus subgroup II, which includes parainfluenza, mumps, Newcastle disease, and measles viruses (Waterson, 1962; Warren et al., 1962). These viruses consist of a single-stranded helix of ribonucleoprotein coiled within a lipoprotein envelope with surface projections (Horne and Waterson, 1960; Choppin and Stoeckenius, 1964; Compans et al., 1966). Associated with the viral envelope are hemagglutinating, hemolytic, neuraminidase, and cell-fusing activities. Cells may be fused either by parainfluenza virus infection or in the absence of virus multiplication when high concentrations of parainfluenza virus are applied to the cell membrane (Henle et al., 1954; Enders et al., 1957; Okada et al., 1957; Okada and Murayama, 1966; Bader and Morgan, 1961; Kohn, 1965; Holmes and Choppin, 1966).

The BHK21-F line of hamster kidney fibroblasts has been shown to be extremely sensitive to the cell-fusing activity of the simian parainfluenza virus SV5 (Holmes and Choppin, 1966). Within 14–18 hr after SV5 infection, virtually all of the

cells in a BHK21-F monolayer have fused together to form a single huge syncytium. Time-lapse photomicrography has shown that the nuclei in BHK21-F giant cells migrate through the syncytial cytoplasm to form tightly packed, parallel rows. Nuclei are also found packed together in naturally occurring syncytia such as osteoclasts, foreign body giant cells, syncytiotrophoblasts, and striated muscle cells. Such grouping of nuclei in one part of the syncytium is the result of migration of nuclei toward each other after cell fusion (Klöne et al., 1963, 1966; Holmes and Choppin, 1966).

Nuclei in SV5-induced syncytia derived from the fibroblastic BHK21-F cells are often arranged in long, tightly packed, parallel rows. In contrast, nuclei are found in tight clusters in syncytia derived from epithelioid cells such as primary rhesus monkey kidney cells or the HKCC line of hamster kidney cells (Holmes and Choppin, 1966, and unpublished observations.) After a study of published micrographs (Henle et al., 1954; Endo et al., 1959; Bussell and Karzon, 1965), we were impressed by the fact that virus-induced syncytia derived from fibroblastic cells often have nuclei arranged in parallel rows, whereas syncytia derived from epithelioid cells contain round clusters of nuclei. Because microtubules are believed to have a cytoskeletal function (Porter, 1966), these findings suggested that cytoplasmic microtubules might play a role in the arrangement of nuclei in syncytia. This paper describes experiments which suggest that, in virus-induced syncytia, the alignment of nuclei, the direction and perhaps the motive force for nuclear migration may be provided by oriented bundles of cytoplasmic microtubules.

MATERIALS AND METHODS

Cells

BHK21-F cells (Holmes and Choppin, 1966), a subline of the fibroblastic BHK21 cell line derived by Macpherson and Stoker (1962) from baby hamster kidney, were propagated in reinforced Eagle's medium (Bablanian et al., 1965) supplemented with 10% tryptose phosphate broth and 10% calf serum. The cells were grown in plastic Petri dishes, on glass cover slips, or on 2-cm squares of Saran Wrap (Dow Chemical Co., Midland, Michigan) which had been sterilized by irradiation for at least 4 hr under a GE 15 watt ultraviolet lamp No. G15T8 at a distance of 15 cm.

Virus Inoculations

The growth and storage of the W3 strain of SV5 (Choppin, 1964) in primary rhesus monkey kidney cells have been described in detail elsewhere (Holmes and Choppin, 1966). Confluent monolayers of cells were washed with isotonic phosphate-buffered saline, pH 7.2 (PBS) (Dulbecco and Vogt, 1954), and inoculated with SV5 at a multiplicity of about 20 infective virus particles (PFU) per cell. After a 1 hr adsorption period at 37°C, the inoculum was washed off, growth medium was added, and the cells were incubated at 37°C. Under these conditions all cells in a monolayer are known to be infected approximately simultaneously (Holmes and Choppin, 1966).

Polarization Microscopy

Cells on cover slips were mounted in wet mounts and were observed through crossed polarizers in a Leitz Dialux-Pol polarizing microscope. For some experiments cells on cover slips were fixed in 1% glutaraldehyde in PBS with no antibiotics (PBS-A) for 10 min prior to examination with the polarizing microscope. Photomicrographs were made with a Zeiss Ultraphot II either with phase-contrast optics or through crossed polarizers.

Electron Microscopy

Monolayers of control or SV5-infected BHK21-F cells on Saran Wrap were placed for 4 min in 1% glutaraldehyde in PBS-A (Sabatini et al., 1962) at 37°C, washed three times in PBS-A, postfixed for 4 min in 1% osmium tetroxide in PBS-A (Palade, 1952), and dehydrated in a series of alcohols. The Saran Wrap bearing the cells was cut into strips about 2 mm wide, and stacks of five strips were embedded in capsules containing epoxy resin (Luft, 1961). The specimens were sectioned with a diamond knife in a plane parallel to that of the monolayer of cells. Sections were mounted on carbon-coated Formvar films on 1 mm slot grids so that a very large area of the syncytia would be visible, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined with a Hitachi HS-7S electron microscope.

Time-Lapse Photomicrography

SV5-infected BHK21-F cells in tissue culture chambers (Sykes and Moore, 1960) were photographed as previously described (Holmes and Choppin, 1966), using the phase-contrast optics of a Zeiss inverted microscope attached to a Cine-Kodak Special II camera.

RESULTS

BHK21-F cells infected with SV5 form huge syncytia (Fig. 1) containing hundreds of nuclei in

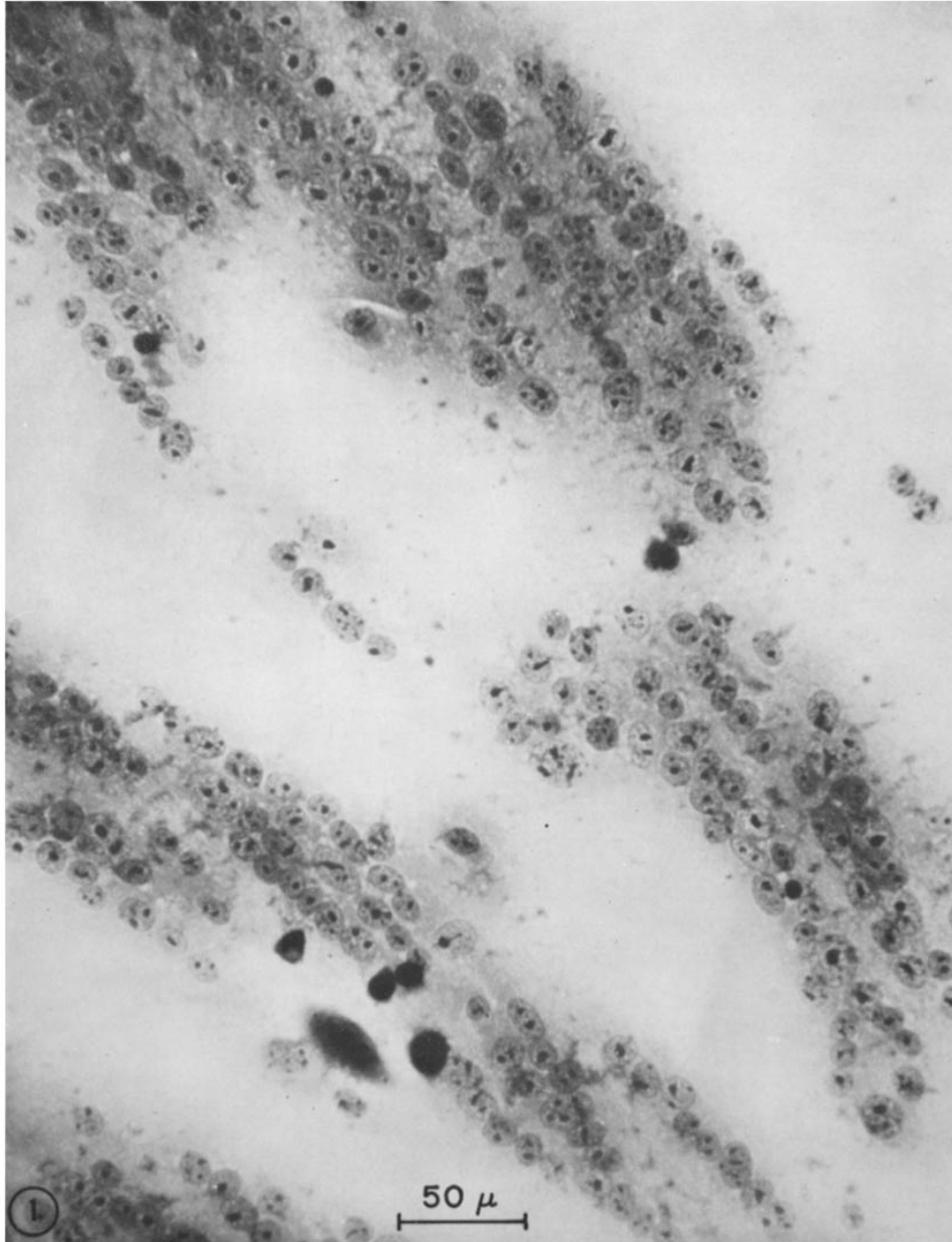


FIGURE 1 Syncytium formed from a monolayer of BHK21-F cells 16.5 hr after inoculation with the simian parainfluenza virus SV5 at a virus multiplicity of 20 PFU/cell. The nuclei are arranged in long, tightly packed, parallel rows in the thin sheet of syncytial cytoplasm. × 370.

long, parallel rows in a thin, flat sheet of cytoplasm (Holmes and Choppin, 1966). In contrast, when primary cultures of rhesus monkey kidney cells are infected with SV5, large amounts of virus may be produced for many days with only minimal cell fusion (Choppin, 1964; Holmes and Choppin, 1966; Compans et al., 1966). Thus, SV5 infection per se does not cause cell death, and different cell types may show great variation in sensitivity to the cell-fusing activity of SV5.

BHK21-F cells infected with SV5 seem to be well suited for the study of nuclear migration and alignment because (*a*) all cells in an SV5-infected BHK21-F monolayer are recruited into a huge syncytium, and (*b*) cell fusion occurs relatively rapidly in this system, at a time when cellular metabolism has not been greatly altered and cellular synthesis of RNA, DNA, and protein are still close to normal levels (Holmes and Choppin, 1966). This system has an obvious advantage over those systems in which virus-induced cell fusion has been studied in gradually expanding giant cells. When fusion occurs relatively slowly over several days, the center of the syncytium may be very old, beginning to degenerate at the time when the giant cell is studied.

Cytoplasmic Birefringence in Virus-Induced Syncytia

The possibility that the rows of nuclei in SV5-induced BHK21-F syncytia might be oriented by bundles of cytoplasmic microtubules was investigated. Oriented bundles of microtubules appear birefringent when observed through crossed polarizers (Porter, 1966; Inoué and Sato, 1967). Control and SV5-infected BHK21-F cells were examined with a polarizing microscope to determine whether birefringent material was present in the cytoplasm.

Uninfected BHK21-F cells, which tend to grow in oriented whorls, show very weak birefringence running parallel to the long axis of the cells. As cell fusion progresses, the cytoplasm of the giant cells flattens out, and parallel lines of tightly packed nuclei begin to appear. Wide bands of weakly birefringent material are always visible running between and parallel to the rows of nuclei in both fixed and unfixed preparations. Fig. 2 shows an area of a virus-induced syncytium containing parallel lines of nuclei photographed with phase-contrast optics (Fig. 2 *a*) and through crossed polarizers (Fig. 2 *b*). The wide bands of weakly birefringent material lie between the rows of nuclei. The fiber axis of the birefringence is parallel to the lines of nuclei, and the sign of birefringence appears to be positive. Broad bands of birefringence are visible in the cytoplasm of giant cells only when the nuclei are arranged in parallel rows. Late in infection when the nuclei in a giant cell become packed in a round cluster, birefringence is no longer visible.

These observations suggest that the cytoplasm of SV5-induced BHK21-F syncytia contains oriented bands of elongated material. Electron microscopic observations which will be described below suggest that the cytoplasmic birefringence is due to bundles of microtubules parallel to the rows of nuclei.

Fine Structure of Virus-Induced Syncytia

Monolayers of BHK21-F cells grown on Saran Wrap were fixed *in situ* and examined by electron microscopy 17–19 hr after infection with 20 PFU/cell of SV5, a time when the syncytial cytoplasm contained many long parallel rows of tightly packed nuclei and when the birefringence between and parallel to these rows of nuclei was maximal. In sections made parallel to the plane of the monolayer, long rows of nuclei were observed. Fig. 3 shows portions of several parallel rows of nuclei in a huge syncytium. No cell borders are visible between the nuclei. Running between the rows of nuclei are long pale bands of fibrous material. At higher magnification (Figs. 4 and 5) these areas are seen to consist of oriented bundles of cytoplasmic microtubules, about 250 A in diameter, and filaments, about 80 A in diameter. The bands of microtubules and filaments are in the same positions as the birefringent material seen with the light microscope. Throughout the cytoplasm of a given giant cell, nearly all of the microtubules and filaments are oriented parallel to the rows of nuclei.

Nuclear Migration

Time-lapse cinematography has demonstrated that SV5-induced syncytia form by cell fusion. Cells with single nuclei may fuse with each other or with multinucleate cells, and several multinucleate cells can fuse together (Holmes and Choppin, 1966). In time-lapse movies, cell fusion is first apparent as the disappearance of a small region of the border between two neighboring cells. The area of cytoplasmic continuity extends in both directions until no border is visible between the

two cells. At that moment, all of the contents of the two original cells rapidly move a short distance toward each other and toward the center of the newly formed giant cell. This movement, which apparently affects all components of the cytoplasm in the same way, may be caused by tension at the cell membrane as the giant cell assumes its new shape. The nuclei then migrate in very straight paths to the center of the giant cell, while the phase-dense granules of the cytoplasm become randomly dispersed through the cell. Nuclear migration appears to be a specific movement which affects nuclei and not other cytoplasmic components. Fig. 6 illustrates diagrammatically the events that are clearly visible in the time-lapse movies. As the nuclei migrate, they move relative to the phase-dense granules of the cytoplasm and push the granules ahead to form a little "cap" of granules, which points like the head of an arrow in the direction that the nucleus is migrating, and they leave behind a "wake" relatively free of phase-dense granules.

Fig. 7 shows sequential frames from a time-lapse film of migrating nuclei in an SV5-induced syncytium. The nuclei move at an average rate of 1–2 μ/min from the periphery of a giant cell straight toward the nearest nucleus in the center of the giant cell. They migrate over long distances, up to 150 μ. Occasionally, nuclei revolve end-over-end as they migrate in a straight line toward the center of a giant cell, or a nucleus may halt for a short time during migration. When several nuclei enter a giant cell at the same time, they often follow directly behind one another as if there were "channels" through the cytoplasm. The nuclei in a given channel do not necessarily move at the same rate, however. The results described above suggest that these cytoplasmic channels may be bundles of microtubules running parallel to the direction of the migrating nuclei.

Effect of Colchicine on Nuclear Migration and Alignment

To investigate whether intact microtubules are essential for movement and alignment of nuclei in virus-induced syncytia, experiments were performed using colchicine, which has been shown to bind to microtubule protein (Borisy and Taylor, 1967 *a* and *b*; Shelanski and Taylor, 1967) and to disrupt microtubules (Robbins and Gonatas, 1964; Tilney, 1965; Malawista and Bensch, 1967). Monolayers of BHK21-F cells were inoculated with SV5 at a multiplicity of about 20 PFU/cell. At the end of the adsorption period, some cells were placed in normal growth medium and others were placed in 10^{-3}–10^{-6} M colchicine in growth medium. At intervals the cells were either fixed in Zenker's solution and stained with hematoxylin and eosin (Holmes and Choppin, 1966) or fixed in 1% glutaraldehyde and examined with a polarizing microscope. SV5-infected, colchicine-treated BHK21-F cells were also studied by time-lapse cinematography and electron microscopy.

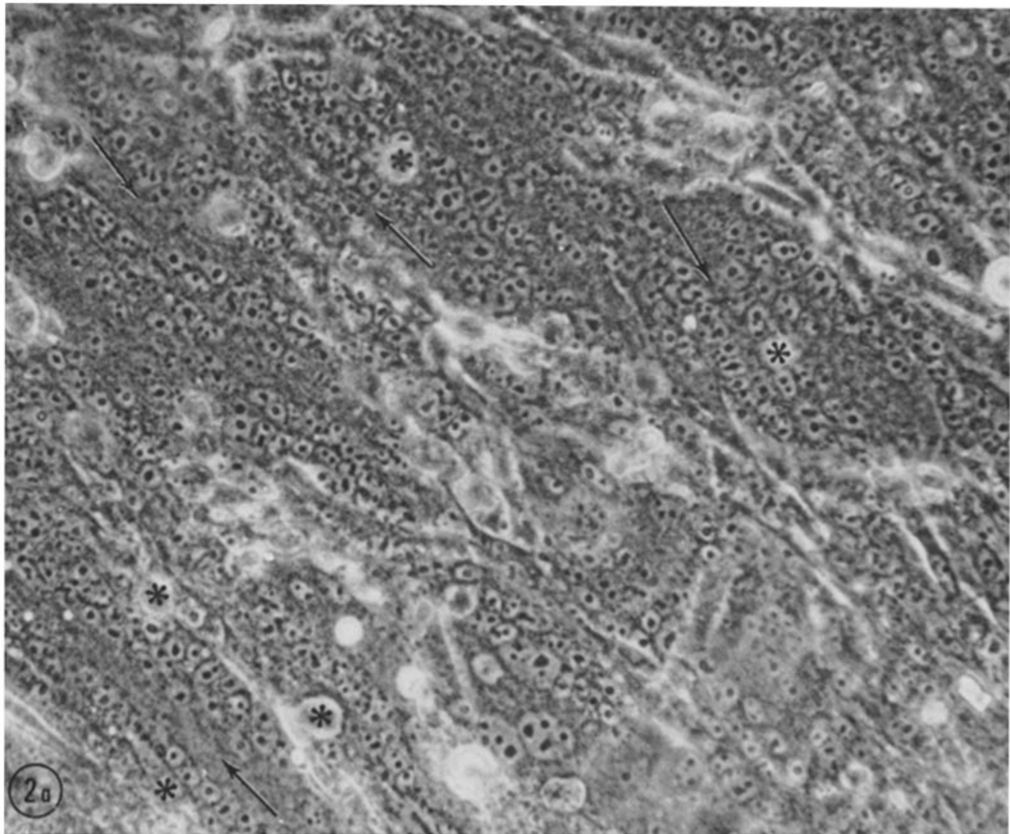
Untreated BHK21-F cells (Fig. 8 *a*) stained with hematoxylin and eosin are elongated and spindle shaped. Treatment with colchicine at 4×10^{-4} M for 17 hr causes many of the cells to become spherical and refractile in metaphase arrest and the remaining cells to lose their spindle shape as shown in Fig. 9 *a*. Fig. 8 *b* shows the many parallel lines of nuclei in a typical, SV5-induced BHK21-F syncytium. In contrast, when SV5-infected cells are incubated in the presence of 4×10^{-4} M colchicine, they fuse at the normal rate, but their nuclei remain randomly scattered through the cytoplasm of the syncytium (Fig. 9 *b*). Condensed bits of nuclear material from cells in metaphase arrest are found in the cytoplasm of the colchicine-treated syncytia.

Time-lapse photomicrographs of SV5-infected BHK21-F cells in 10^{-3}–10^{-5} M colchicine were made as described above. The infected cells began to fuse at the usual time, i.e. 5–6 hr after infection.

Figs. 2 *a* and *b* show a region of a monolayer of BHK21-F cells 17 hr after infection with SV5. Landmarks on the cell sheet are marked with asterisks. × 400.

FIGURE 2 *a* Under phase-contrast microscopy, long parallel rows of nuclei may be seen in the syncytia. Arrows point to areas of cytoplasm between rows of nuclei.

FIGURE 2 *b* The same area photographed through crossed polarizers shows wide bands of weakly birefringent material (arrows) in the cytoplasm between the parallel rows of nuclei. The fiber axis of the birefringence is parallel to the rows of nuclei.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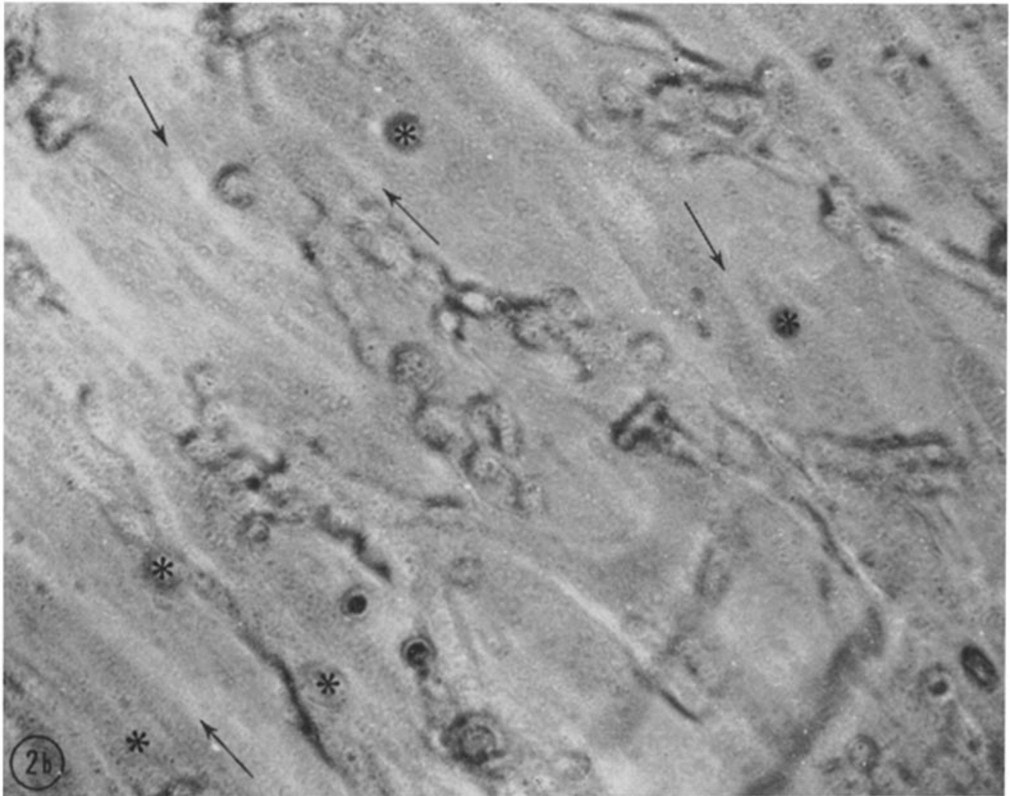
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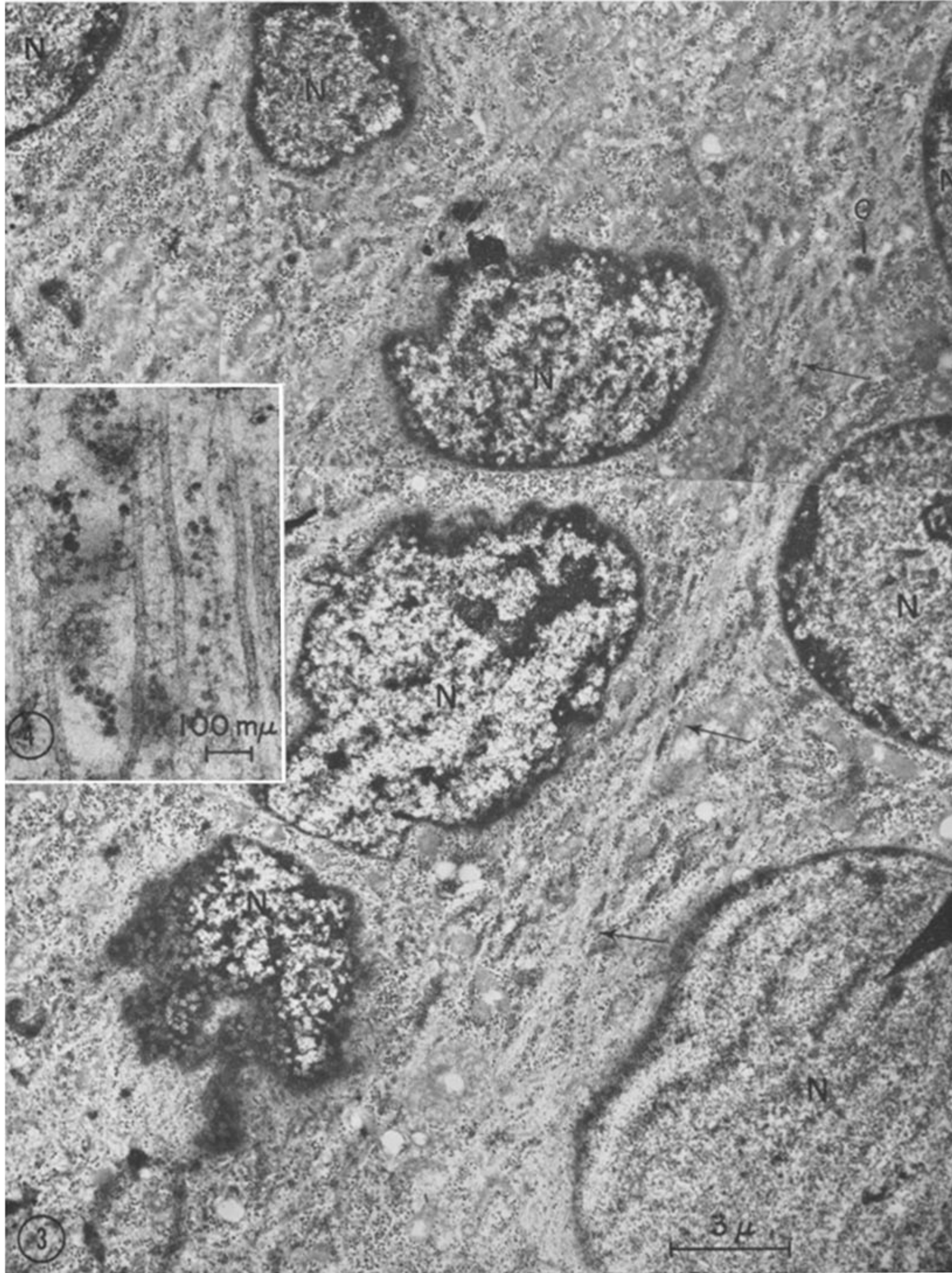


FIGURE 3 A BHK21-F syncytium 18 hr after SV5 infection. This composite photograph of a thin section in the plane of the monolayer of cells shows several parallel lines of nuclei in a syncytium. Arrows indicate long bands of fibrous material parallel to and between the rows of nuclei (*N*). Microtubules arise from a centriole (*C*). × 5700.

FIGURE 4. A higher magnification of a region between lines of nuclei showing that the "fibrous material" seen in Fig. 3 is composed of parallel microtubules about 250 A in diameter and filaments about 80 A in diameter. × 66,000.

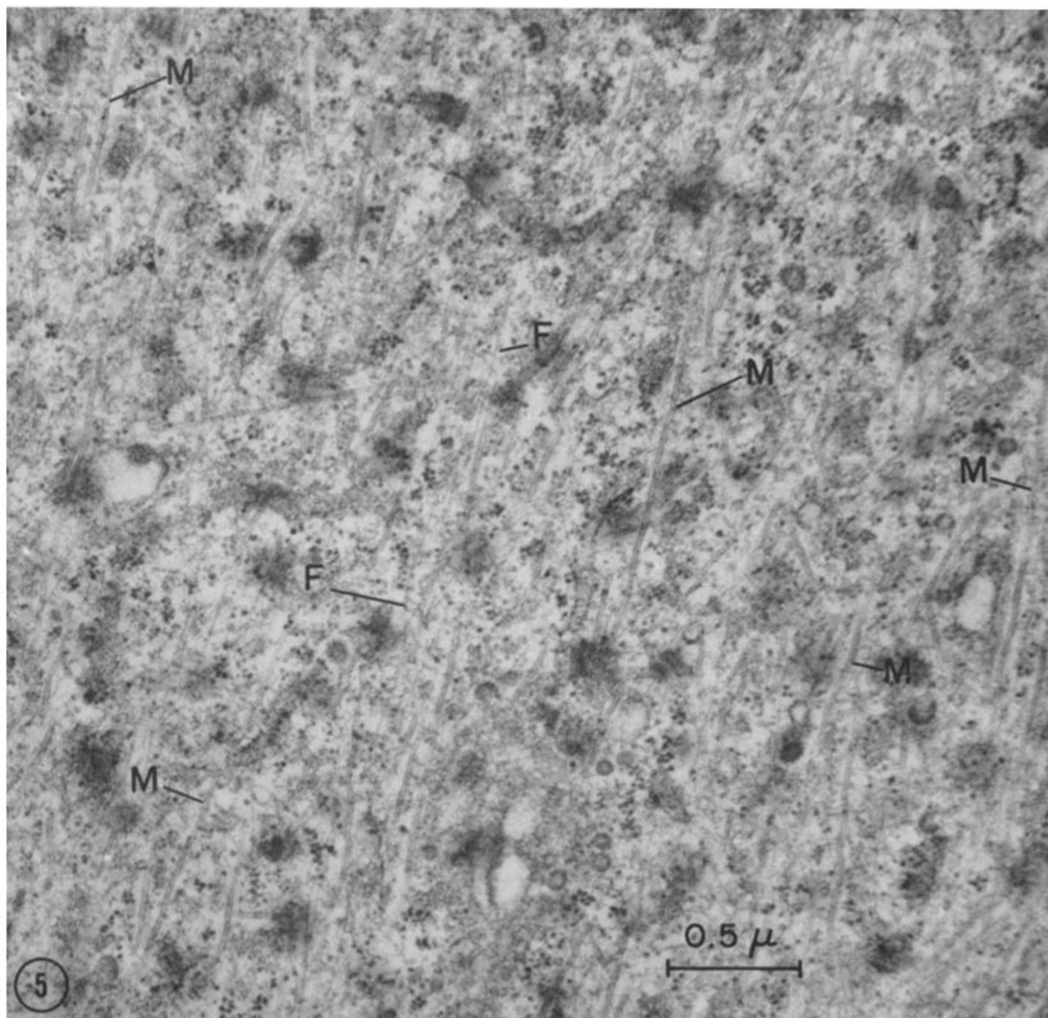


FIGURE 5 A region of syncytial cytoplasm between two parallel rows of nuclei in a syncytium, showing many parallel 250-A microtubules (*M*) and 80-A filaments (*F*). Several microtubules more than 2 μ long are visible in this thin section. × 35,000.

The first visible effect of the colchicine was the arrest of some cells in mitosis. The cells rounded up and became refractile as cells normally do when entering mitosis, but instead of dividing and flattening out into two fibroblastic daughter cells, they remained rounded and refractile. The nonmitotic cells withdrew their elongated cytoplasmic extensions, and these cells as well as the rounded, refractile cells in metaphase arrest were recruited into syncytia. As the cells fused into syncytia, all the contents of the cell, including nuclei, nuclear fragments, and cytoplasmic granules, moved a short distance toward the center of the newly formed giant cell. Colchicine dramatically inhibits the nuclear migration which normally follows this this cytoplasmic coalescence in untreated, fusing cells. In colchicine-treated, SV5-infected cells, the nuclei do not migrate at all. These observations suggest that the process of nuclear migration requires intact cytoplasmic microtubules.

SV5-infected BHK21-F cells treated with 4×10^{-4} M colchicine were examined by polarization microscopy. In marked contrast to untreated SV5-induced syncytia where bands of birefringent ma-

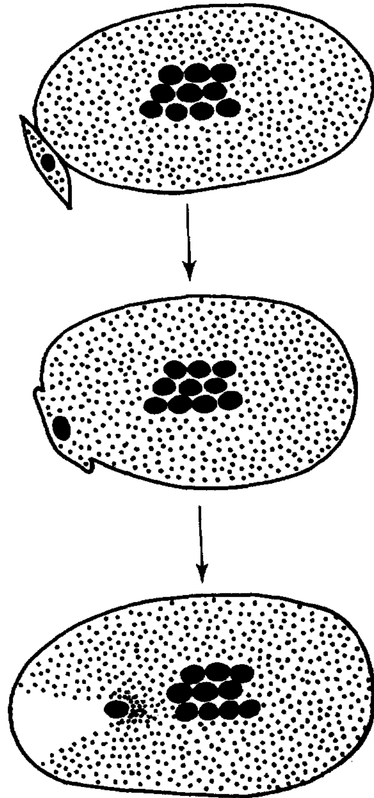


FIGURE 6 Diagrammatic representation of the migration of a nucleus into a virus-induced syncytium. The events shown here have been observed in many time-lapse movies of SV5-infected BHK21-F cells. The nucleus migrates in a straight path toward the nuclei clustered at the center of the giant cell. The moving nucleus accumulates a cap of cytoplasmic granules in front and leaves behind a wake relatively free of granules.

terial course through the syncytial cytoplasm, birefringent material was *not* visible in the cytoplasm at any time during the development of colchicine-treated syncytia. Thus colchicine also prevents the appearance of bands of birefringent material in the cytoplasm of syncytia.

Monolayers of BHK21-F syncytia, 18 hr after SV5 infection and after 17 hr in medium with 10^{-3} M colchicine, were examined by electron microscopy. Unlike normal BHK21-F cells (Fig 10), these colchicine-treated cells (Fig. 11) contain no cytoplasmic microtubules. Instead, many broad bands of filaments about 80 A in diameter run through the cytoplasm. The observation that no birefringence is observed in these colchicine-treated cells even though many filaments are present suggests that the cytoplasmic birefringence observed in untreated syncytia is primarily due to the oriented microtubules. Athough no microtubules have been seen in colchicine-treaed cells, centrioles are frequently observed. Thus colchicine, which prevents nuclear migration and alignment, destroys cytoplasmic microtubules in these cells and may cause an increase in the number of filaments.

Effect of Cold on Nuclear Alignment

Incubation of many different types of cells at low temperatures causes depolymerization of cytoplasmic microtubules and loss of cellular asymmetry associated with intact microtubules (Inoué and Sato, 1967; Tilney and Porter, 1967; Roth, 1967). To determine whether cold treatment would destroy cytoplasmic microtubules of BHK21-F cells, the following experiments were performed. Uninfected BHK21-F cells and cells

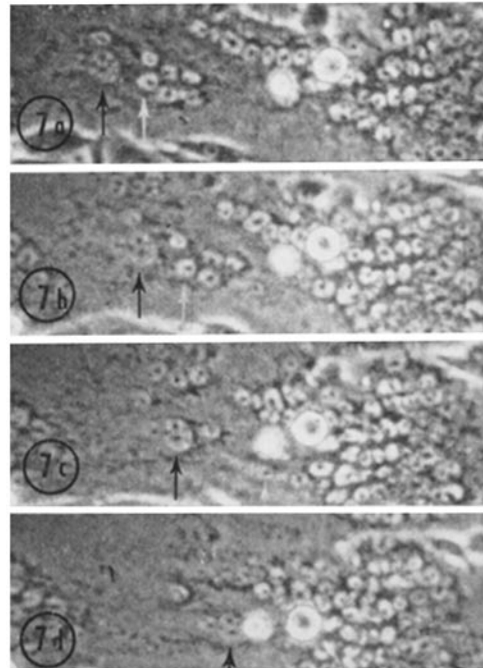


FIGURE 7 Sequential time-lapse, phase-contrast photomicrographs of nuclear migration in an SV5-induced BHK21-F syncytium. Arrows identify two nuclei which are migrating from left to right to join a large cluster of nuclei in the same syncytium. The nuclei move independently of each other at a rate of 1–2 μ/min. The interval between frames is 29 min. × 320.

infected with SV5 were incubated at 37°C until long parallel lines of nuclei had formed in the syncytia. At this time, some monolayers were fixed at 37°C in 1% glutaraldehyde in PBS-A, and other monolayers were transferred to incubators at 4°C. At intervals cells were removed from the cold, fixed with 1% glutaraldehyde, and examined with phase-contrast and polarization microscopy.

After 12 hr at 4°C, monolayers of uninfected BHK21-F cells were indistinguishable from those incubated at 37°C. These cells did *not* lose their elongated, fibroblastic shape at 4°C. After SV5-induced BHK21-F syncytia containing long parallel rows of nuclei were transferred to 4°C and incubated for as long as 12 hr, the nuclei remained in long parallel rows. Thus, cold treatment does not lead to disruption of lines of nuclei which have already formed. It was not possible to investigate whether low temperature would prevent the formation of oriented bundles of microtubules and rows of nuclei in syncytia if the cells were chilled before syncytia were formed, because cell fusion will not occur in the cold.

When cold-treated syncytia were examined by polarization microscopy, wide bands of weakly birefringent material, like those present in the syncytia before transfer to 4°C, were observed running parallel to and between the lines of nuclei. Thus treatment of the cells at 4°C for up to 12 hr does not destroy the cytoplasmic birefringence which is apparently caused by oriented bands of microtubules.

To determine the effect of low temperature on the structure of cytoplasmic microtubules in BHK21-F cells, syncytia, 18 hr after SV5 infection, were placed at 4°C for 12 hr before they were fixed for electron microscopy. The cold-treated syncytia were indistinguishable from control syncytia fixed 18 hr after SV5 infection without cold treatment. Bundles of cytoplasmic microtubules and filaments ran through the syncytial cytoplasm parallel to the rows of nuclei. The microtubules and filaments appeared to be present in normal numbers in the cold-treated cells, and the microtubules were found to be 250 A in diameter. These observations indicate that the cytoplasmic microtubules of baby hamster kidney cells are not depolymerized by cold treatment.

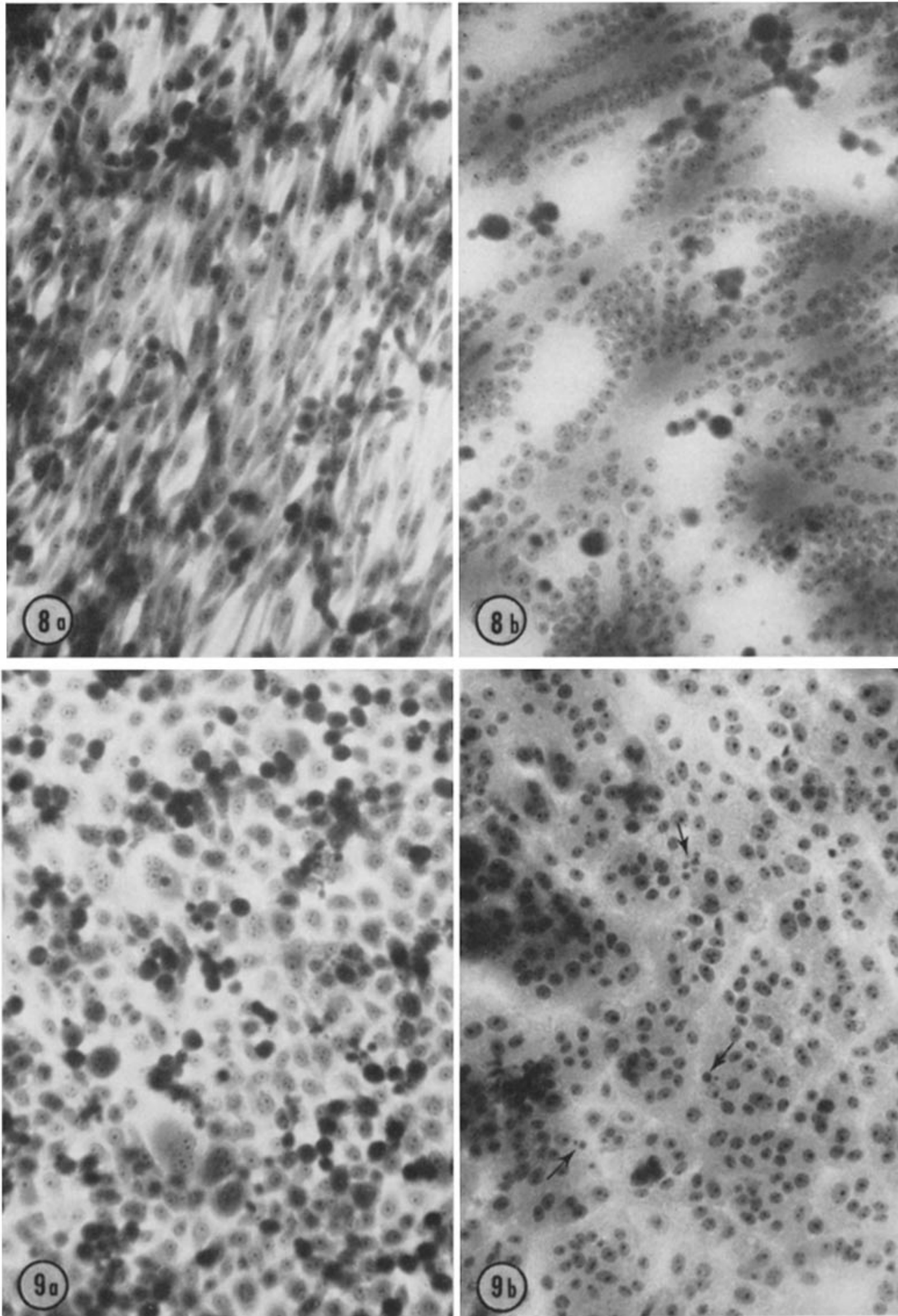
Association of Nuclei with Microtubules

Because microtubules are always found running parallel to the lines of nuclei in a syncytium, and because of the apparent involvement of microtubules in nuclear migration, a careful search was made for connections between microtubules and nuclei.

In thin sections of SV5-induced BHK21-F syncytia, microtubules are often seen in close association with interphase nuclei. Fig. 10 shows a bundle of microtubules very near a nucleus. The slender microtubules frequently pass out of the plane of section, however, and it has not been possible to observe any direct connection between microtubules and nuclei.

To investigate whether some connections between microtubules and interphase nuclei were present even though they could not be observed in thin sections of syncytia, we attempted to isolate lines of nuclei from SV5-induced BHK21-F syncytia. Cover slips containing monolayers of BHK21-F cells at various stages after infection with SV5 were inverted over drops of distilled, demineralized water. After several moments, the cells burst by osmotic shock. When cells containing long, tightly packed rows of nuclei were osmotically shocked, long rows of swollen nuclei were released and fell to the bottom of the drop of water. These rows of nuclei could be removed with a pipette and examined with phase-contrast microscopy. Fig. 12 shows two rows of nuclei isolated in this way from a BHK21-F syncytium 18 hr after SV5 infection. With phase-contrast microscopy, the nuclei appear to be relatively free of contaminating cytoplasm. To demonstrate that the linear association of these isolated nuclei reflects their alignment within the living giant cells, several control experiments were performed. Uninfected BHK21-F cells were osmotically shocked under the same conditions, and it should be emphasized that only single nuclei, never pairs or chains of nuclei, were released. This demonstrates that isolated BHK21-F nuclei do not spontaneously aggregate into rows. Furthermore *colchicine-treated*, SV5-infected BHK21-F cells were also osmotically shocked. These syncytia, which contain randomly scattered nuclei, release only separate nuclei, never nuclei in pairs or chains.

Rows of nuclei isolated from virus-induced syncytia were placed on carbon-coated Formvar films supported by copper mesh grids, negatively stained with 2% potassium phosphotungstate at pH 6.2, and examined in the electron microscope. For some experiments the isolated rows of nuclei were fixed in 1% glutaraldehyde in PBS before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negative staining. Fig. 13 shows an area between two nuclei from an isolated chain of four nuclei. Because of the great thickness of the nuclei, the films often broke under the electron beam. Stretching between the two nuclei and parallel to the row of nuclei are several extremely long microtubules. The area between the nuclei is relatively free of cytoplasmic components other than microtubules. Although the microtubules isolated under these conditions have a somewhat irregular appearance possibly due to partial disruption during preparation, it has been possible to measure the diameter of the microtubules, ~250 A. The fact that rows of nuclei can be isolated together with microtubules and relatively free of other cytoplasmic components suggests that there is indeed an association between microtubules and the interphase nuclei aligned in a giant cell and that microtubules are the cytoplasmic structures which keep the isolated nuclei aligned.

DISCUSSION

The observations described above have demonstrated that when BHK21-F cells are caused to fuse together by the simian parainfluenza virus SV5, the nuclei of the fusing cells migrate straight to the center of the syncytium in an orderly and directed manner. The nuclei are apparently moved by a specific mechanism and not by simple cytoplasmic streaming, since the phase-dense granules of the cytoplasm are not carried along with the nuclei. The nuclei in syncytia derived from the fibroblastic BHK21-F cells are frequently arranged in long, tightly packed, parallel rows. In time-lapse movies, migrating nuclei have been observed to follow one another as if there were channels through the cytoplasm. The nuclei within these channels move independently of each other and may revolve end-over-end as they migrate toward the center of the cell.

When SV5-induced syncytia containing rows of nuclei were fixed *in situ* and examined by electron microscopy, long bundles of cytoplasmic microtubules, ~250 A in diameter, and filaments, ~80 A in diameter, were observed running between and parallel to the rows of nuclei. An intimate association between the microtubules and the migrating interphase nuclei has been suggested by the isolation of microtubules with rows of nuclei from disrupted syncytia. That the microtubules may play an important role in nuclear migration in virus-induced syncytia is suggested by the observation that treatment of the cells with colchicine, which destroys cytoplasmic microtubules but *not* the 80 A filaments, prevents nuclear migration and alignment. All of these observations suggest that cytoplasmic microtubules may be responsible for directing nuclear movement and that they may be involved in providing the motive force for nuclear migration.

The weight of evidence from many different systems suggests that microtubules are involved in the movement of cytoplasmic particles (reviewed by Porter, 1966), although the mechanism for such a movement remains theoretical. Examples of movements of cellular organelles in which micro-

Figs. 8 *a* and *b* show monolayers of BHK21-F cells grown in normal growth medium, stained with hematoxylin and eosin. × 220.

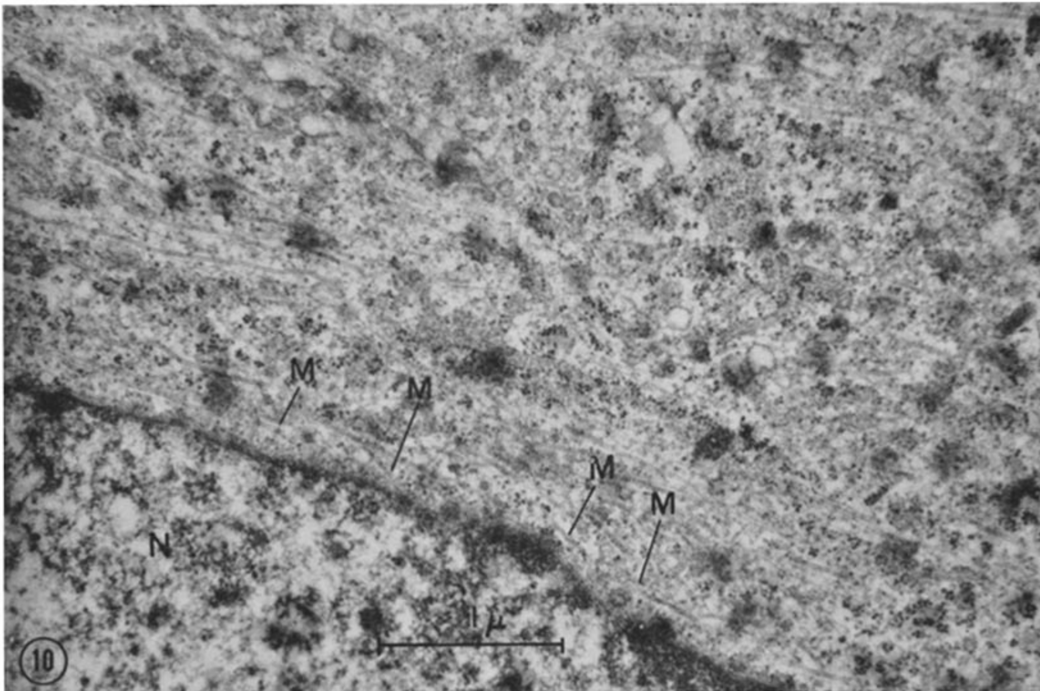
FIGURE 8 *a* Uninfected control cells. These elongated, fibroblastic cells tend to grow in oriented whorls and occasionally pile up on each other.

FIGURE 8 *b* A portion of a huge syncytium 18 hr after SV5 infection. The nuclei are in long, tightly packed, parallel rows in the syncytial cytoplasm.

Figs. 9 *a* and *b* show hematoxylin- and eosin-stained monolayers of BHK21-F cells after 17 hr in medium containing 4×10^{-4} M colchicine. × 220.

FIGURE 9 *a* Uninfected, colchicine-treated cells. The cells have lost their elongated, fibroblastic shape and appear epithelioid. Cells in metaphase arrest appear rounded and darkly stained.

FIGURE 9 *b* Colchicine-treated cells 18 hr after SV5 infection. The cells have fused into large syncytia. In contrast to the orderly rows of nuclei seen in Fig. 8 *b*, the nuclei in colchicine-treated syncytia are randomly scattered through the syncytial cytoplasm. Arrows indicate condensed fragments of nuclear material which probably arose from the incorporation of cells in metaphase arrest into syncytia, a phenomenon which has been observed by time-lapse photomicrography.



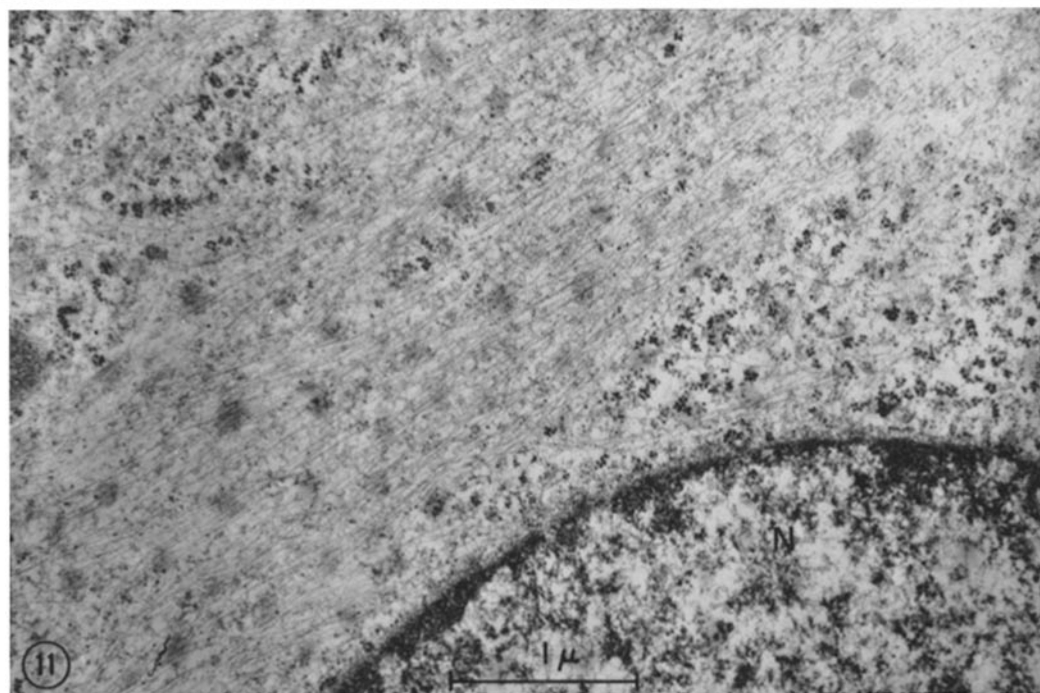


FIGURE 10 A BHK21-F cell in normal growth medium 18 hr after SV5 infection. Cytoplasmic microtubules (*M*) are visible in very close association with the nucleus (*N*), but because the microtubules may extend out of the section, it is not possible to determine whether the microtubules are directly connected to the nuclei. × 24,200.

FIGURE 11 A BHK21-F cell after 17 hr in 10^{-3} M colchicine, showing that cytoplasmic microtubules have disappeared. A broad band of filaments about 80 A in diameter lies in the cytoplasm near a nucleus (*N*). These filaments appear to be more numerous in colchicine-treated cells. × 24,200.

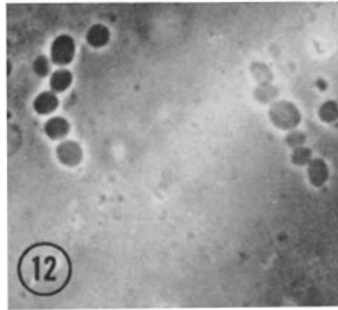


FIGURE 12 Several rows of nuclei isolated from an SV5-induced BHK21-F syncytium. The nuclei appear tightly bound together and are relatively free of cytoplasmic debris. ×184.

tubules have been implicated include: the movements of melanin granules through the cytoplasm of melanocytes (Bikle et al., 1966), movements of chromosomes during mitosis (Inoué and Sato, 1967), movements of cytoplasmic granules through the axopods of the heliozoan *Actinosphaerium* (Tilney et al., 1966; Tilney and Porter, 1967), movements of Golgi vesicles at the cell plate of plant cells (Whaley and Mollenhauer, 1963; Ledbetter and Porter, 1963), and movements of enzyme packets and food particles along the tentacles of the suctorian *Tokophrya* (Rudzinska, 1965). All of these particles move parallel to the long axis of the cytoplasmic microtubules, and disruption of the microtubules by colchicine, high hydrostatic pressure, or low temperature prevents the movements of the particles in those instances in which this has been studied. No connections have been visualized between microtubules and any moving cellular organelles with the exception of chromosomes (Roth et al., 1966; Barnicot, 1966). The movement of interphase nuclei through virus-induced syncytia described in the present report also occurs parallel to cytoplasmic microtubules and is inhibited by colchicine. In this system, however, some association between microtubules and migrating nuclei has been suggested by the isolation of nuclei with microtubules, although no direct connections between nuclei and microtubules have been seen in thin sections.

Effect of Colchicine or Cold on Microtubules

Large doses of colchicine are required to destroy the cytoplasmic microtubules of BHK21-F cells. For example, the elongated fibroblastic shape of the interphase cells is not lost until after about 10 hr of treatment with 4×10^{-4} M colchicine. That these microtubules may be more stable than microtubules in some other cell types is also demonstrated by the observation that the cytoplasmic microtubules of BHK21-F cells are not destroyed by incubation at 4°C for up to 12 hr.

Colchicine-induced disruption of microtubules in interphase HeLa cells has also been described by Robbins and Gonatas (1964). It is interesting to note that, in their preparations and in our own preparations, the 80-A filaments in the cytoplasm are not destroyed by colchicine treatment but appear to increase in number. Because these filamentous structures are numerous in cells which have lost all of their many microtubules, it is possible that they may be a second form of polymerization of microtubule protein monomers which is stable in the presence of colchicine. It has been suggested that the slender neurofilaments in nerve axons may be an alternate association of the protein subunits which comprise the neurotubules (Fawcett, 1966).

Direction of Migration and Position of Nuclei

Nuclei in fusing cells migrate toward the center of the giant cell whether it is round or elongated in shape. Since cytoplasmic microtubules appear to be responsible for directing the migration of nuclei in syncytia, it seems possible that the network of microtubules is able to distinguish between the central and peripheral regions of the cell. In BHK21-F cells, it appears that the cytoplasmic microtubules are arranged in a symmetric network running from dense "initiating sites" at the plasma membrane toward the nucleus which is located in the geometric center of the cell. This system of microtubules might possibly direct nuclear movement in the following manner. When two cells fuse, their outer borders remain intact, whereas the plasma membranes between the two cells disappear. The orientation of the cytoplasmic microtubules in fusing cells is apparently unchanged. The microtubule-initiating sites at the periphery of the newly formed giant cell are probably unchanged, while the initiating sites within the new giant cell where the plasma membranes have been lost would presumably be altered. The microtubule network around each nucleus would no longer be symmetric but would have acquired polarity from the edge of the cell toward the center. The nucleus might then migrate until it reached the center of

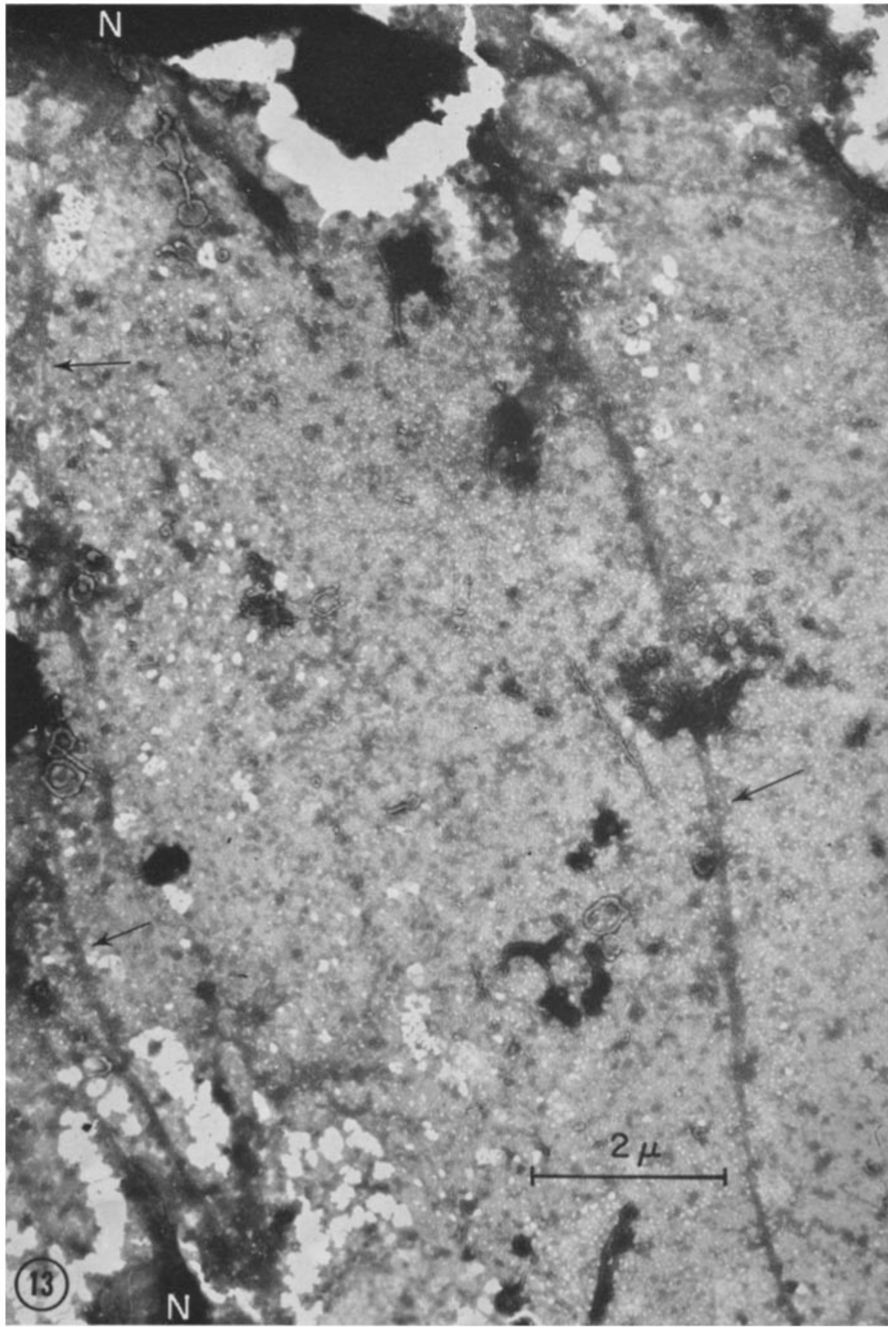


FIGURE 13 An area between two nuclei (N) in an isolated row of nuclei similar to those shown in Fig. 12. Long bundles of cytoplasmic microtubules about 250 A in diameter (arrows) extend between the nuclei (N) in an area which is relatively free of other cytoplasmic components. × 13,000.

the new giant cell and a symmetric network of microtubules had again been obtained.

The same processes which direct the placement of nuclei in normal mononucleate cells might cause the migration and alignment of nuclei observed in SV5-induced syncytia. In a great many different types of cells in vitro and in vivo, the nucleus is located at the geometric center of the cell, regardless of cell shape. The nucleus in this stable, central location may be surrounded by a symmetrical network of microtubules, although detailed observations on the orientation of cytoplasmic microtubules in many cell types have not been made and the present discussion cannot be extended to cells in which cytoplasmic microtubules have not been demonstrated. When the shape of the cell changes, the nucleus might migrate along the microtubules until it reaches the new center of the microtubule network. Specific migrations of nuclei in differentiating cells might be brought about by changes in the microtubule-initiating sites in specific areas of the cell periphery.

It seems clear that the migration of nuclei in virus-induced syncytia obtains its direction from the orientation of the cytoplasmic microtubules, but the evidence that the microtubules provide the motive force for nuclear movement is less conclusive. The nuclei seem to run along the bundles of microtubules, but whether the energy for nuclear migration is provided by some special feature of these bundles remains to be elucidated. What is obvious as a result of our experiments with colchicine is that nuclear migration stops when the cytoplasmic microtubules have been destroyed.

The movements of nuclei in syncytia normally present in vivo may closely resemble the migration and alignment of nuclei in SV5-induced BHK21-F syncytia. Embryonic or regenerating skeletal muscle is formed by the fusion of bipolar cells (Lash et al., 1957; Konigsberg et al., 1960; Stockdale and Holtzer, 1961). When these myoblasts fuse, their nuclei migrate to the center of the myotube where they often form long, tightly packed, parallel rows (Lash et al., 1957) which closely resemble those in BHK21-F syncytia. Later the muscle nuclei are displaced from their central location by large aggregates of actin and myosin. The nuclei of myoblasts fused in the presence of colchicine are not aligned in long parallel rows (Bischoff and Holtzer, 1968). A similar lack of nuclear alignment has been described above in virus-induced syncytia treated with colchicine. These observations suggest that cytoplasmic microtubules may be responsible for nuclear migration and alignment in different types of syncytia.

SV5-infected BHK21-F cells have been shown to be an excellent system for the study of cell fusion and nuclear migration and alignment. The cells fuse together rapidly and completely, and the nuclei migrate in very straight paths through the syncytial cytoplasm. Nuclear migration can be specifically inhibited by colchicine. In addition, it is possible to isolate microtubules in association with nuclei. Further study of migrating nuclei in virus-induced syncytia in vitro may help to elucidate the mechanism(s) by which certain cytoplasmic particles are specifically moved along microtubules.

We are grateful to Dr. George E. Palade for his helpful discussions of this work and for extending to us the use of the electron microscopic facilities of the Cell Biology Laboratory. We also wish to thank Dr. Samuel Silverstein for advice and suggestions and Miss Cathleen O'Connell for excellent technical assistance.

This work was supported by research grant No. AI-05600 from the National Institute of Allergy and Infectious Diseases. This paper was presented in part at the 7th Annual Meeting of the American Society for Cell Biology. 1967. *J. Cell Biol.* **35**:58A.

Received for publication 15 March 1968, and in revised form 10 July 1968.

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