MICROTUBULES IN THE MICROSPIKES AND CORTICAL CYTOPLASM OF ISOLATED CELLS

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ABSTRACT

In thin sections through microspikes extending from the surface of isolated cells, a core has been seen which may contain microtubular elements. The differences between these and microtubules seen elsewhere in the cytoplasm are attributed to their rapid growth and exposed location which make them especially vulnerable to injury by preparative treatment. In support of this view it is shown that cytoplasmic microtubules may be altered or even destroyed by exposing the cells to changes in osmotic pressure. Associated with these straight microtubules in the cytoplasm were also found solid microfilaments. The form of these components and their location and alignment in portions of cells which are under tension or in motion suggest that they function in the structural support of the cell and its microspikes and in the transmission of tension in the cytoplasm. A second type of microtubule, smaller in diameter and tortuous in form, was also seen in certain cells and is presumed, from its shape, to have little to do with cytoplasmic support.

INTRODUCTION

In an earlier publication (23), attention was called to the rapid formation, great activity, and short life span of the fine microextensions that may be seen on the surface of many isolated tissue cells in explantation. Although these surface extensions have been included under the name microvilli since Lewis and Lewis (10) and Gey (4) first observed and described them, it seems that there is sufficient difference in morphology and behavior to distinguish between these and the uniform and slow-growing (13) microvilli of intestinal and kidney epithelium cells. The term microspike, which has already been applied to them in the past (23, 25), seems an appropriate designation to indicate their rigid spinelike aspect while in active motion.

On the basis of their straight axes, the occasional occurrence of angular bends, and their evident rigidity while in motion, it was suggested that microspikes may be stiffened by rodlike central cores. Support for this assumption has been furnished by electron micrographs of shadowed unsectioned cells that showed an axial condensation extending through each microspike (23). However, no core structure was detectable in thin sections of cells that had been fixed by the conventional osmium tetroxide technique.

Subsequently, in sections of cells fixed with glutaraldehyde before fixation with osmium tetroxide, a bundle of fibrillar and tubular elements was seen running through many of the microspikes. These cores extended back into the cortical portion of the cells where their filaments mingled with similar elements in the cytoplasm. These elements are briefly described in the following section.

METHODS

The observations were made on cells from cultures of human conjuctiva and HeLa strains as well as on cells

from primary dissociates of embryonic chick liver and kidney tissues. The cells, suspended in Eagle's Basal Medium supplemented with 10% beef embryo serum, were permitted to spread on cover glasses that had been coated with a carbon film. The procedure of preparation for electron microscope examination was similar to that used by Robbins and Gonatas (18). Fixation was carried out for 15 min in 2% glutaraldehyde in Earle's balanced salt solution to which had been added 0.01% $CaCl_2$. This was followed by a 30 min postfixation in 1% OsO_4. Infiltration and embedding with Epon were carried out with the cells still attached to the cover slip. When it had harden, the Epon containing the cells and the carbon film was removed from the glass slide. Cells thus embedded were sectioned, mounted on grids, and stained with uranyl acetate (24) and lead citrate (17).

OBSERVATIONS

In these preparations the limiting membrane of the microspikes was seen to be continuous with the plasmalemma of the cell and, in favorable sections, showed clearly the characteristic unit membrane pattern of its structure. The microspikes had relatively uniform diameters between 100 and 200 mμ and showed no appreciable taper throughout their length. The cytoplasm within the microspikes appeared finely granular and contained none of the larger structures found in the cytoplasm of the cell body that would be excluded by the restriction of space. Even smaller structures, however, such as ribosomes and smaller components of the endoplasmic reticulum, were absent.

The only structure found in the microspike was a fascicle of strands running throughout its length. In sections in which some of these cores were cut longitudinally, the strands appeared to consist of pairs of dark lines separated by lighter gray spaces (Figs. 1, 2, and 5). When visible in cross-section, the core sometimes had the appearance of a cluster of about 8 to 20 dark rings that measured approximately 150 A in diameter and had light or hollow centers. These rings were more densely packed near the axis of the microspike (Figs. 3 and 4). The individual strands of such a core thus appeared to be minute tubules having electron-opaque walls.

In some cell processes the tubulelike strands had a nearly uniform diameter and, when their courses lay in the plane of sectioning, they could be traced through a considerable portion of the length of the microspikes. In others, the course of the strands of the core was so erratic or undulating that in the micrographs the tubules appeared discontinuous and irregular or, they disappeared from the plane of the section. In comparison with the straight microtubules of the cell cytoplasm, to be described in the following paragraphs, the microtubules of these cores were less distinct, were not so straight and uniform, and had a smaller and less regular diameter.

There were also microspikes in which the tubular character of the core elements was not obvious (Fig. 6). In these the cores appeared to be composed of fine electron-opaque strands, each running an irregular longitudinal course unrelated to that of its neighbors. It was not clear whether the appearance of irregularity and discontinuity of the strands accurately depicted the true character of the core structure in these microspikes or resulted from either degeneration of the core elements or from disruption of them in preparation.

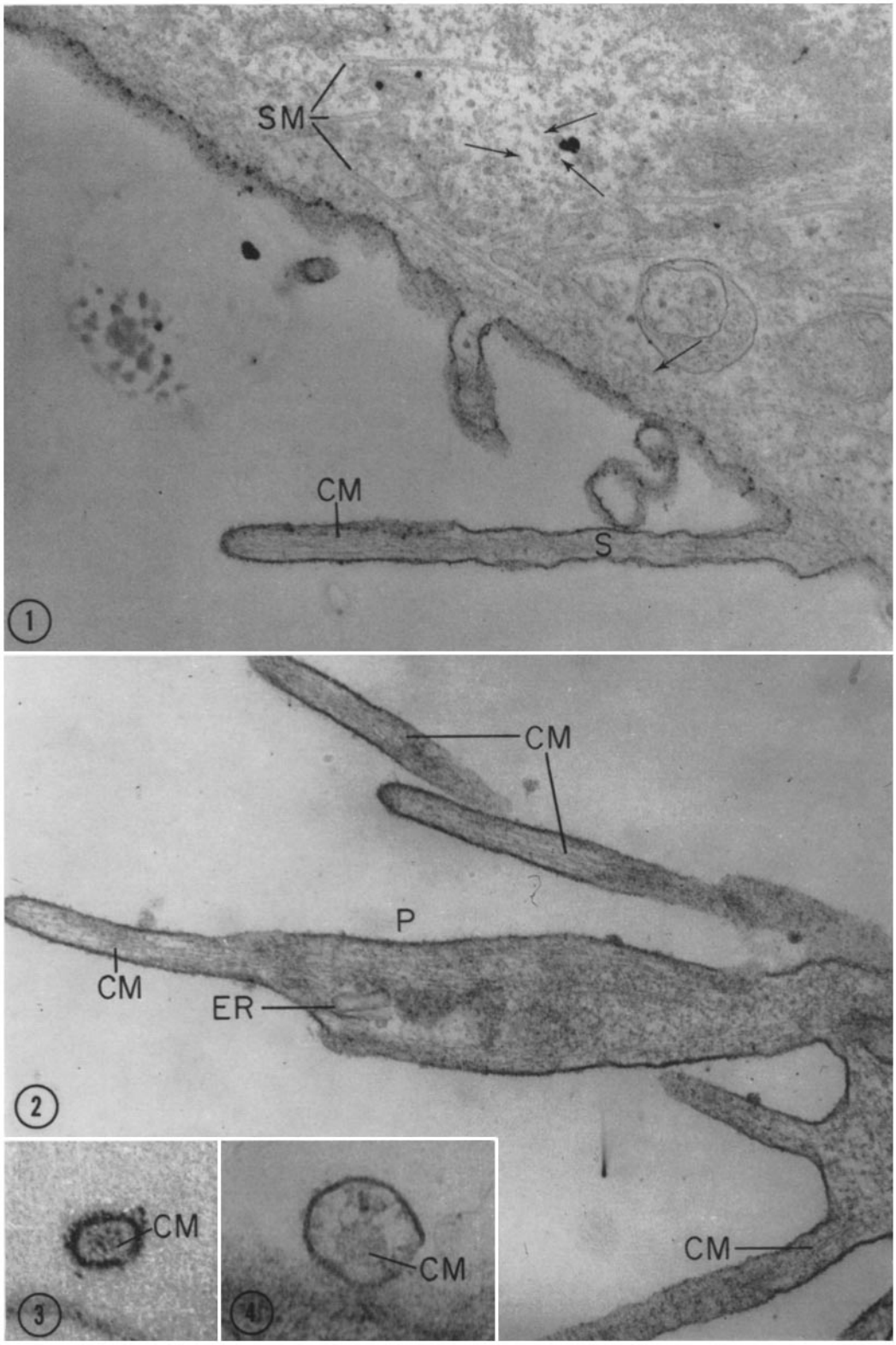
In some microspikes no core could be discerned. Whether this was due to failure of the core elements present to take stain, or to the breakdown of the microtubules during manipulation before they could be preserved by fixative, or whether

FIGURE 1 Margin of a human conjunctiva cell showing a microspike (*S*) containing core microtubules (*CM*). Seen also are straight microtubules (*SM*) and tortuous microtubules (arrows). × 47,500.

FIGURE 2 Microspikes of chick embryonic kidney cell showing core microtubules (*CM*). Core elements of the microspike on the left extend back through a large cell process (*P*) that contains cytoplasm with endoplasmic reticulum (*ER*). × 48,500.

FIGURE 3 Cross-section through a microspike from a human conjunctiva cell showing cluster of core microtubules (*CM*). × 59,000.

FIGURE 4 Cross-section through a cell process similar to that at *P* in Fig. 2, showing cross-section through core microtubules (*CM*). × 77,000.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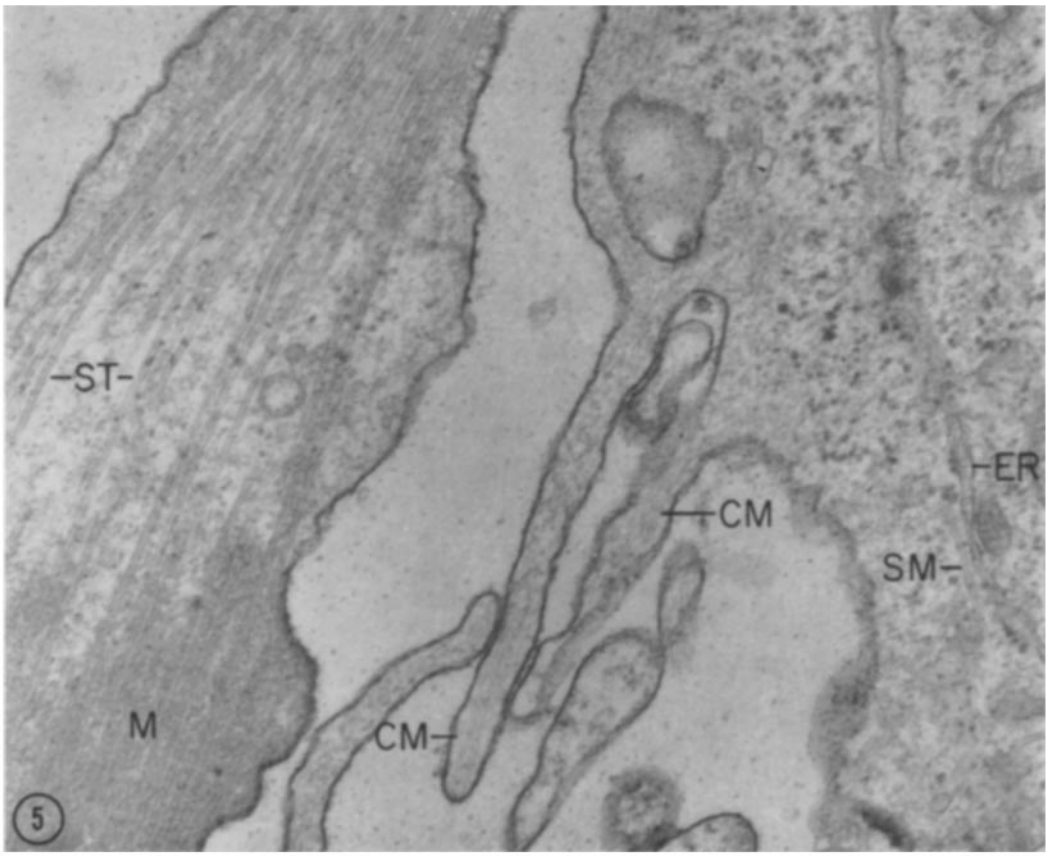


FIGURE 5 Human conjunctiva cell in late telophase showing spindle tubules (*ST*) and midbody (*M*) at the left. Core microtubules (*CM*) are seen in 2 microspikes on 1 daughter cell. Straight microtubules (*SM*) and endoplasmic reticulum (*ER*) appear in the cytoplasm. × 47,500.

microspikes may exist for periods of time without a core, was not determined.

The core running through the microspike had its root in the cytoplasm of the cell. Here the core elements joined with and became lost among similar filamentous and tubular components of the cytoplasm. These were of three distinct types.

1. Most conspicuous of these were the straight microbules, so designated because of their generally straight or gently curved course as they extended for relatively long distances through the cell. Portions of tubules could be traced for as far as 5 μ in a single section. These appeared in longitudinal section as parallel double lines, the space between the lines being more electron-opaque than the surrounding ground material (Figs. 5, 7, and 8). In cross-section these tubules were dark circles enclosing less dark central areas. The diameter of these microtubules was remarkably uniform along their length, measuring 250 ± 30 A. The thickness of their walls was about 60 A. In appearance and dimensions, these tubules were identical with mitotic spindle fibers in the same preparations (Fig. 5) and with microtubules described by a number of workers in a great variety of cells (1, 2, 8, 20).

In the cells examined, straight tubules were never observed to branch or to anastomose with each other, nor were they seen to be continuous with or to open into any membrane-enclosed space. At certain places, such as the region of attachment of the cell surface to the substratum or to

other cells, the straight microtubules became aligned vertically or obliquely with respect to the area of attachment and appeared to end close to the cell membrane (Figs. 9 and 10). Their actual relationship to the plasma membrane here was obscured by the many microfilaments which also converged upon these areas and appeared to be anchored to the cell membrane.

2. Microtubules of a second type were distinguished by their smaller diameter and their tortuous course. These were abundant in cells from strains of human origin but were not seen in the freshly isolated embryonic chick cells (Figs. 1 and 11). The hollow cylindrical structure of these tortuous tubules was evidenced, as with the straight microtubules, by their appearance in side view as shaded bands bordered by parallel dense lines, and by their ring-shaped appearance in cross-sections. They had a uniform diameter of about 150 A. The meandering course of each tubule carried it repeatedly through the planes of sectioning, and thus a characteristic feature of these microtubules in the micrographs was the frequent appearance of traversely cut profiles. Since the tubules in these sections were so interrupted, it was impossible to determine their length without making reconstructions from serial sections. This was not done. Their small caliber and uniform diameter and the lack of branching, or anastomoses, clearly distinguished these microtubules from the endoplasmic reticulum. Intermediate, or transitional forms between the straight and tortuous microtubules were not seen.
3. Besides these tubular structures, the cytoplasm also contained microfilaments that were electron-opaque throughout (Figs. 7 and 8). These measured about 80 A in diameter and showed no distinct periodicity along their length. They were seen more frequently in the freshly prepared chick liver and kidney cells than in the cultured strain cells.

The microfilaments were generally loosely interspersed with the straight microtubules and, like the latter, often extended through the cytoplasm for several microns without sharp bends. However, these two com-

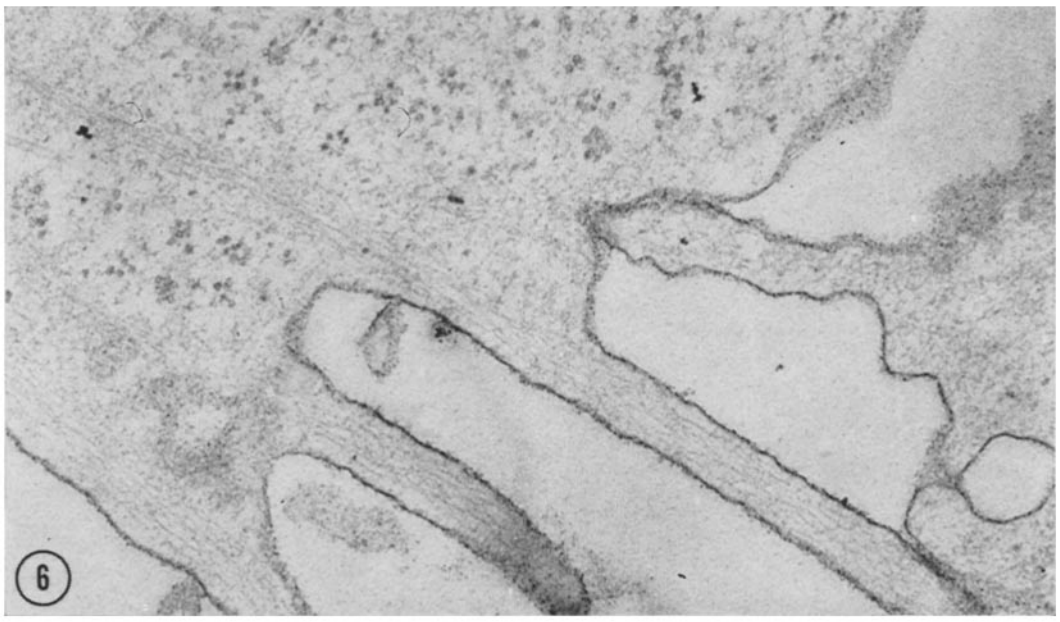


FIGURE 6 Microspikes from chick kidney cell that do not show tubular character of core elements. × 57,000.

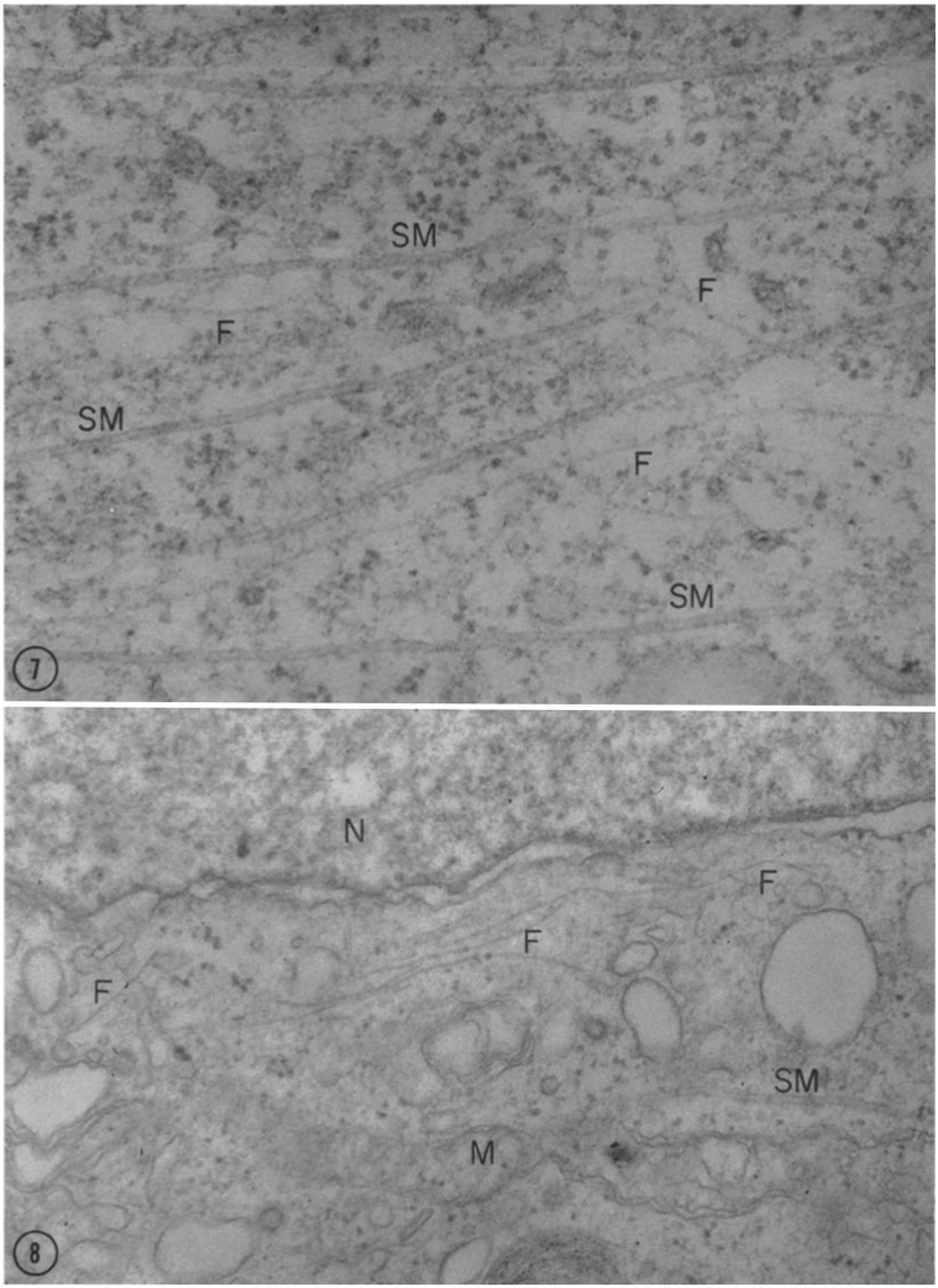


FIGURE 7 Cytoplasm of human conjunctiva cell showing straight microtubules (*SM*) and microfilaments (*F*). × 65,000.

FIGURE 8 Cytoplasm of chick kidney cell, showing straight microtubule (*SM*), microfilaments (*F*), nucleus (*N*), and mitochondria (*M*). × 60,000.

ponents were sometimes grouped together into more or less dense sheets that were frequently situated just beneath the plasmalemma, or packed into bundles that extended inward from the cell membrane at areas of its attachment to the substratum (Figs. 9 and 10). Sections through spread cells cut normal to the plane of the substratum showed that some of these bundles passed almost vertically completely through the cytoplasm. These bundles appeared to be anchored to the cell membranes, above and below, and would thus assist in maintaining the extremely flattened condition of the cell margins.

The form and arrangement of these cytoplasmic elements were shown to be affected by certain changes in environmental conditions of the cells. Some of these effects were observed when chick kidney cells in monolayer were placed, for a period of 20 sec to 2 min before fixation, into a medium made hypotonic by dilution with an equal volume of distilled water. In cells that had been affected only mildly by this treatment, straight microtubules and microfilaments sometimes became arranged alternately in regularly packed bands whose straight course through a randomly aligned milieu indicated that these structures either had a rigidity that resisted deformation or were being held taut by stress in an axial direction (Fig. 12). Other cells that were more strongly hydrated exhibited, to varying degrees, evidence of swelling: vacuoles became enlarged, mitochondria became distended, and ribosomes came together into polysomal clusters separated by spaces devoid of structure. In these cells nearly all straight microtubules had disappeared from the cytoplasm and only the microfilaments remained. Although the latter were very abundant, they were scattered about in disorderly array (Fig. 13). At some points the filaments appeared to branch, but it was difficult to tell whether the branching represented true branching or the unraveling of multistranded cords.

When the osmotic pressure of the cell environment was increased by transferring the cells, just before fixation, into standard medium containing 3% glucose, the filamentous and tubular elements clustered together so that they lay in close contact with each other, side by side. They became thus aggregated into spindle-shaped wisps that assumed a characteristic arcuate configuration (Fig. 14). The contrast in the electron micrographs of these cells was considerably reduced by this treatment, and it became difficult to distinguish between microfilaments and microtubules in the wisps, especially since they were so closely packed.

DISCUSSION

Improved electron microscope techniques and particularly the use of glutaraldehyde as a fixative have revealed the presence of minute tubulelike components of the cytoplasm in a wide variety of cells (1, 2, 8, 20). At first these components were called microtubules, on the basis of their appearance in electron micrographs, despite the argument that solid filaments with a surface deposit of osmium, or other stain, would give similar images in the electron microscope. More conclusive evidence (Pease, reference 14), that some of these components actually are tubules with a true lumen, has come from electron micrographs of sperm flagella prepared with negative staining. These micrographs showed that, when the axial filaments were broken, a fluid substance exuded from the ruptured ends, and also that stain could penetrate through such breaks to replace the contents of the inner spaces. Further evidence of the hollow nature of microtubules has recently been presented in electron micrographs of tubules which had been isolated and shadowed with metal (26).

A characteristic microtubule, then, has the appearance of a straight or slightly curved structure with an unvarying outer diameter (generally between 150 and 250 A) and uniform inner caliber and wall thickness. It extends for relatively long distances and does not branch or interconnect with other tubules.

Such ideal microtubules have now been seen in nearly all cells in which they have been sought, and it might be expected that if they are present in the cores of microspikes they could be recognized readily and identified without ambiguity. There is, however, the possibility that peculiar circumstances in the development of these microtubules or their special location in projections from the cell surface might render them less clearly visible and more vulnerable to conditions capable of distorting and altering their form, if not destroying them altogether. A knowledge of variations in the structure of microtubules during their development and of modifications resulting from exposure to

adverse conditions would therefore facilitate identification of atypical forms.

Some clues regarding the formation and growth of microtubules have come from observations on the behavior of tubules of the mitotic apparatus. These tubules are thought to originate from, or attach to, the centrioles or kinetochores and increase rapidly in length by coalescence or polymerization of units considered by different investigators to be protein molecules (5), micelles (11), or granules (7). Their growth and their persistence in a dynamic "state of flux" (5) appear to be dependent upon their relationship to the center from which they arise. The rate at which a damaged portion of the tubule is replaced (3), or the rate of advance of a chromosome attached to the tubule (12), both measured at about 1 μ per min, is a possible indication of the rate of tubule formation.

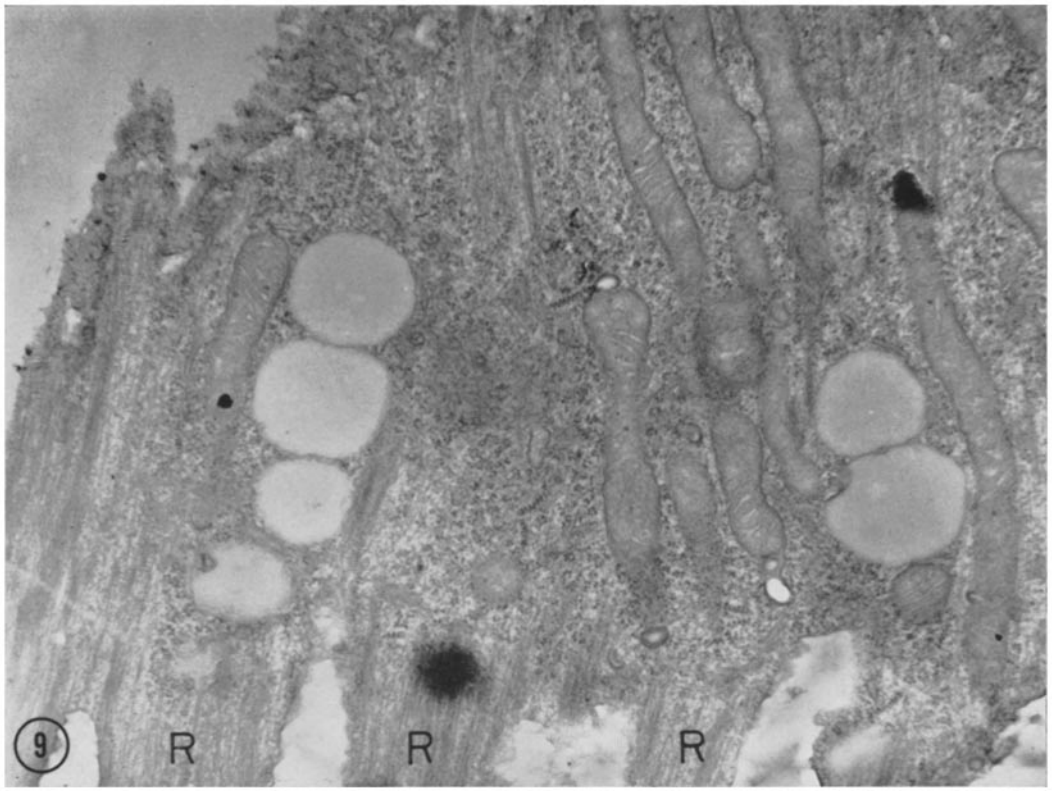
Tubular axial filaments in cilia and flagella originate from centriolar basal bodies (15, 21), and their growth may progress at a rate of approximately 2 μ per min, according to calculations based on illustrations presented by Renaud and Swift (16). Sotello and Trujillo-Cenóz (21) have observed separate vesicles situated distal to the tips of tubules in newly forming cilia and have concluded that growth of the tubules in length occurs by the confluence of these vesicles with the already formed portion of the tubule. This method of growth seems unlikely for the tubules in the developing flagella pictured by Renaud and Swift (reference 6; see Fig. 18) or for tubules in the microspikes described in the present paper, since in both instances the already formed microtubules extend completely to the end of the cell processes and abut tightly against the cell membrane at their tips, leaving no space for unincorporated vesicles (Figs. 1 and 5). In these cases, growth may occur by the addition of substances along the length of the tubule (19) or at its proximal end.

Core microtubules of the microspikes resemble those of microvilli and differ from the axial filaments of cilia and flagella in not having basal bodies at their proximal ends from which they appear to arise. The core elements of microspikes can be traced far back into the cortical cytoplasm where they become lost by intermingling with the filaments and tubules of the cytoplasm. If these microtubules do originate from organizing or initiating molecular configurations, such centers may reside in the surface membrane of the cell, and growth of the microtubules may be one of extension in a proximal direction. Since time-lapse studies (22) have shown that the projection of a microspike from the cell surface may occur at the rate of about $\frac{1}{2}$ μ per sec, we may assume that microtubules taking part in its formation would have a similarly rapid growth rate. Tubules formed at this rate and fixed within a few minutes' time may be more tenuous and more fragile than those which are permitted to mature, as in the case of mitotic spindle fibers and flagellar filaments.

The breakdown of microtubules occurs both in normal physiological processes, such as the disappearance of spindle tubules after completion of mitosis, and as a result of certain experimental interventions, such as ultraviolet irradiation, treatment with some mitotic poisons, or use of conventional fixatives. The lability of most microtubules is demonstrated by their susceptibility to destruction or degradation by procedures of fixation which will preserve most other cytological structures. There is, however, variation among microtubules in their resistance to these disruptive forces. While the microtubules in the highly specialized and more permanent organelles, such as cilia or flagella, will withstand the usual osmium tetroxide fixation, those that are distributed through the cytoplasm or that form the mitotic spindle apparatus are not generally preserved by the same treatment (18). The present study indicates, furthermore, that microtubules present in the cores of the rapidly forming and short-lived microspikes may be particularly unstable so that even with special precautions they may be distorted or partially destroyed and sometimes become entirely unrecognizable as microtubules.

FIGURE 9 Oblique section through chick kidney cell showing ridges of attachment (*R*) to carbon film substratum at bottom of micrograph. Ridges are completely filled by bundles of microfilaments and microtubules. × 16,000.

FIGURE 10 Several ridges of attachment at higher magnification, showing microtubules (*SM*), and microfilaments (*F*). × 50,000.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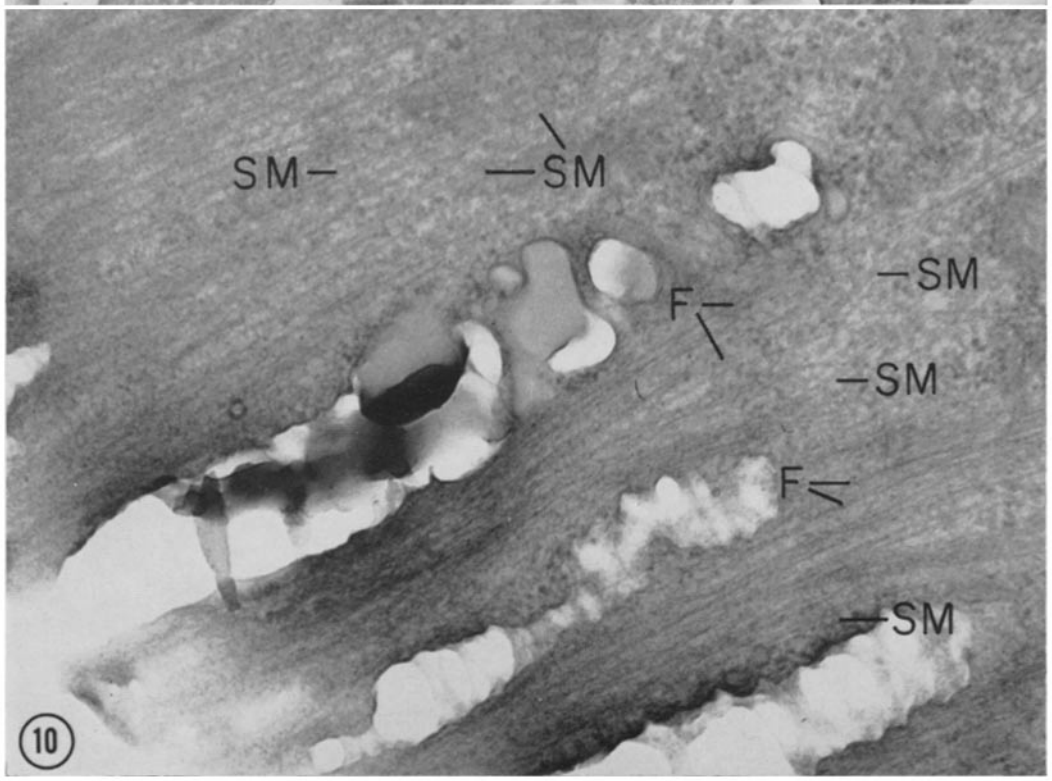
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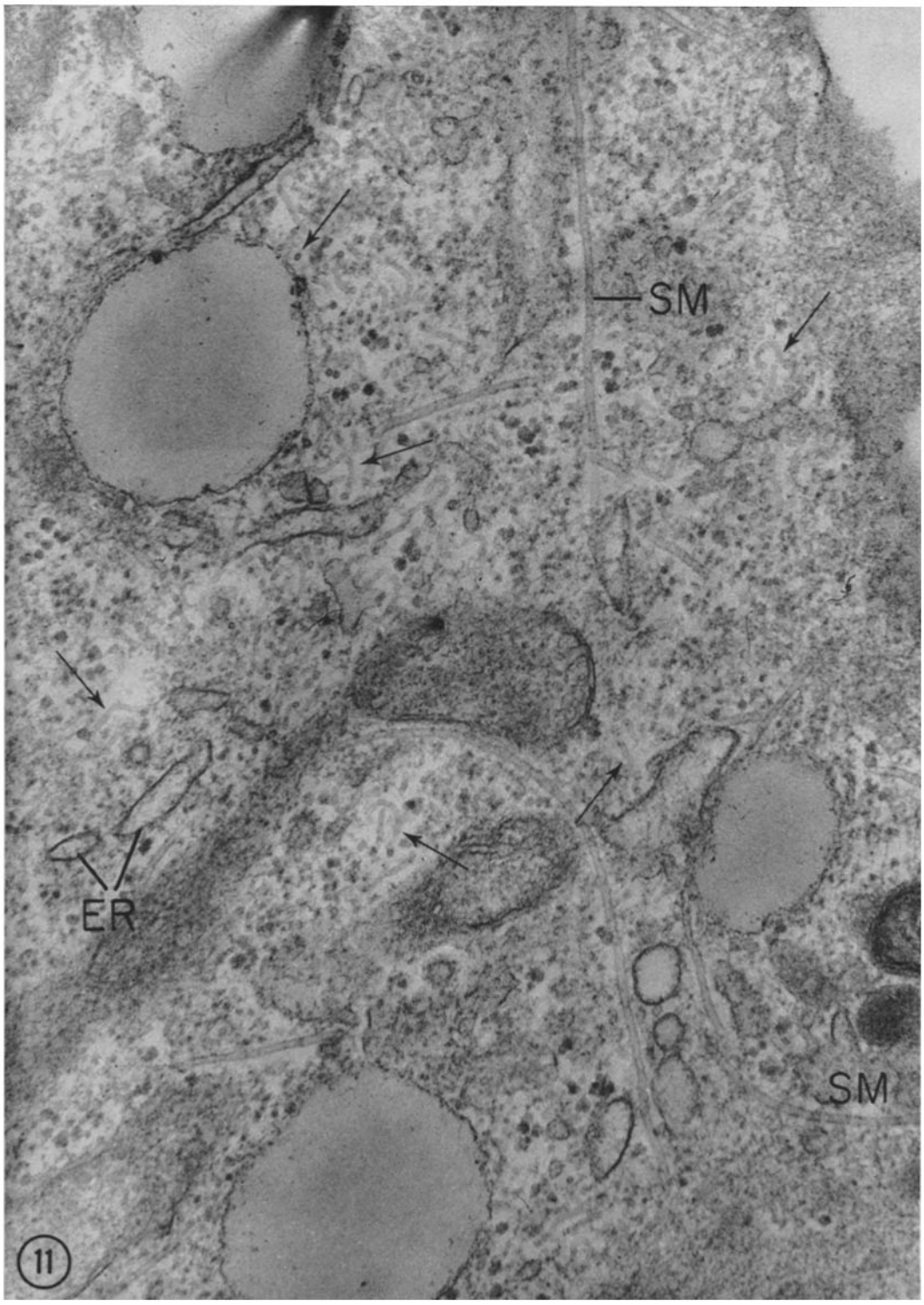

FIGURE 11 Cytoplasm of human conjunctiva cell showing straight microtubules (*SM*), some of which are curved. Many tortuous microtubules are seen in side view and cross-section (arrows). Endoplasmic reticulum at (*ER*). × 62,000.

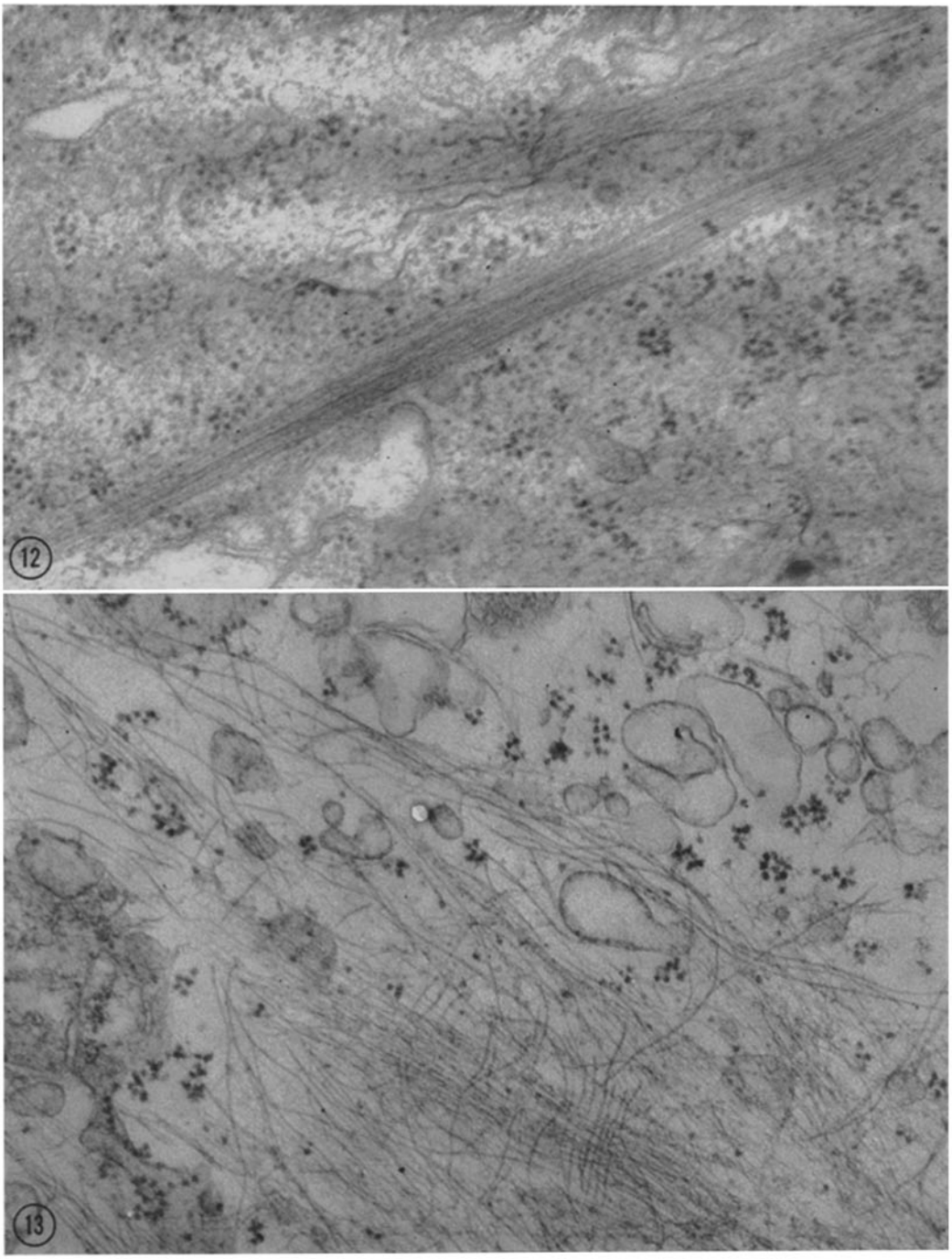

FIGURE 12 Alternate parallel alignment of microtubules and microfilaments in chick kidney cell after short exposure to hypotonic medium. × 48,000.

FIGURE 13 Appearance of cytoplasm in chick kidney cell more strongly affected by hypotonic medium. Note that distinct microtubules are absent and that the microfilaments are randomly oriented. × 48,000.

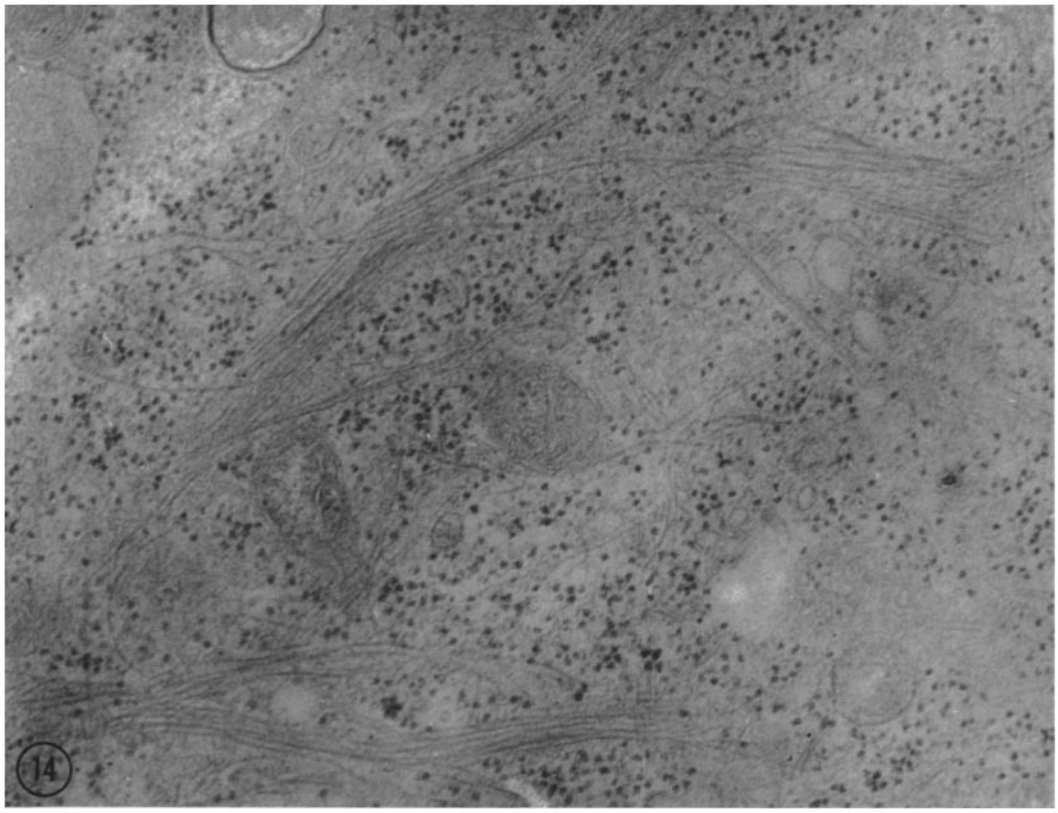

FIGURE 14 Cytoplasm of chick kidney cell treated with hypertonic medium, showing microspikes and wisps of aggregated microfilaments. × 46,000.

Finding identifiable tubules in some of the microspikes seems acceptable evidence, however, that microtubules may take part in the core structure and could be the major component of microspike cores in the living cells.

The apparent structural rigidity of straight microtubules suggests that a major function of these elements is one of support and maintenance of shape in the cell and in its microspike extensions. A close relationship between microtubules and microfilaments was observed. The apparent attachment of the ends of the microfilaments to the cell surface membrane, where the latter was anchored to objects in the environment, and their intimate lateral relationship and fasciculation with the microtubules in sheets and bundles which are correctly oriented to bear or transmit tension suggest that these elements function cooperatively in transmitting stress which could result in movement in the cell.

While there is no evidence that these structures do more than transmit tension, the frequent presence of microtubules in many cells at locations where movement is occurring has led to the suggestion that these elements may be involved in a primative mechanism of motion (8, 15). Ledbetter and Porter (9) found that certain microtubules were composed of longitudinal parallel subunits and have suggested than an undulatory motion of the tubule might result from sequential rhythmic shortening of these units. Kavanau (6) has proposed for tubulelike structures a type of "jet propulsion" through the matrix of the cytoplasm. The association of fine filaments and larger, more rigid, sometimes tubular components of the moving cytoplasm that has so frequently been seen in cells of plants, protozoa, and mammals, favors a speculation that in this association there may exist an interaction that causes the displacement of one filament with

respect to the other by some still unknown mechanism, possibly homologous to the highly specialized interaction between actin and myosin filaments in myofibrils.

The tortuous microtubules of the cytoplasm appear to be nonelastic elements whose structure may be unsuited to play a role in the support or movement of cells. The marked differences between tortuous and straight microtubules that exist side-by-side, and the absence of any evidence of transition or intergrade forms suggest that these microtubules are two quite distinct and unrelated structural types. Slautterback (20) has previously made such a distinction between microtubules. He has suggested that microtubules having a smaller diameter are possibly associated with metabolic and synthetic processes, whereas large tubules may have a supporting function.

The distinction between core microtubules and cytoplasmic straight microtubules is less clear. Their differences in diameter, in regularity of contour, in resistance to breakdown, and in electron-scattering power might indicate the existence of a third type of microtubule in the cell, especially if these differences are basic to, or inherent in, the structure of these elements. If, on the other hand, the differences represent only variations in expression of the same forces of molecular organization induced by local conditions in various parts of the cell, then both types of microtubules may have similar characteristics and similar functions and may be considered modifications of the same type of microtubule. While not conclusive, the results of these experiments indicate that the latter alternative could be true.

This investigation was carried out at The Rockefeller University and was supported in part by Grant No. CA-06375 from the National Cancer Institute (National Institutes of Health of the United States Public Health Service) to Dr. Paul Weiss as principal investigator.

Received for publication 28 July 1965.

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