MICROTUBULAR ORGANIZATION IN ELONGATING MYOGENIC CELLS

ROBERT H. WARREN

From the Department of Biological Structure, University of Miami School of Medicine, Miami, Florida 33152

ABSTRACT

Microtubule organization has been studied in serially sectioned myogenic cells in the tail muscle regeneration blastema of *Rana pipiens* tadpoles. In mesenchymal cells and in some premyoblasts, microtubules radiate from centriolar satellites in a cell center, while in more mature myoblasts and myotubes the centrioles no longer appear to serve as organizing centers for microtubules. In all elongate, fusiform myogenic cells, the microtubules are predominately oriented in the longitudinal axis of the cell. Counts of microtubules in transverse sections spaced at regular intervals along the cells show that the absolute number of microtubules is greatest in the thickened midregions of the cells and decreases relatively smoothly toward the tapered ends of the cells. Close paraxial association of microtubules (within 40 nm surface-to-surface) occurs along the entire lengths of cells but appears with greatest frequency in their tapered ends. In two myoblasts, serial sections were used to trace all microtubules in 8-μm long segments of the cells located about midway between the nucleus and one end of the cell. Since tracings show that as many as 50% of the microtubules terminate within the 8-μm long segment, it seems unlikely that any microtubules extend the entire length of the cell. It is proposed that lateral interactions between paraxial microtubules stabilize the overall microtubular apparatus and contribute to maintenance of the bipolar form of the cells. A three-dimensional model of the complete microtubular array in one of the 8-μm long segments of a myoblast has been constructed. The model reveals that a few microtubules within the segment are bent into smooth curves and loops that could be generated by sliding interaction between paraxial microtubules.

Microtubules are recognized as cytoskeletal elements that play an important role in the maintenance of cell shape and in the organization of cellular cytoplasm (29). The organizational patterns of microtubules in cells vary with the shape of the cell and with its function. A common microtubular pattern in cells is a radial dispersion of microtubules from the cell center, a region near the nucleus which usually contains a centriolar pair. Such radially arranged microtubules may be associated with movements of cytoplasmic particles towards or away from the cell center (2, 10, 22, 28, 44) or with secretion (21, 45). A good deal of study has been focused on the roles that microtubules

play in supporting cell shape, particularly with regard to cell elongation (4, 7, 12, 8), and the formation and maintenance of long processes from the cell surface (36, 41), and it has been shown with the use of antimicrotubule agents that disruption of microtubules in anisometric cells results in reversible cell shortening or withdrawal of cell processes (5, 20, 37, 38, 40). In the systems studied, the common feature of microtubule organization is an orientation of microtubules in the axis of the cellular anisometry, although there may be varying degrees of more complex interactions among the aligned microtubules.

It is generally assumed that microtubules support cellular anisometry by virtue of an intrinsic rigidity. Beyond that, however, there are many unanswered questions about the cytoskeletal functions of microtubules. On the structural level, one would like to know more of the detailed organization of the microtubular apparatus in elongating cells in order to determine, for example, whether microtubules extend the entire length of the cell. Do microtubules actively participate in producing cell elongation, or do they passively support an elongation produced by another mechanism? How is the organization of the microtubular apparatus controlled in the cell? In order to begin to arrive at answers for these questions, a detailed structural study of the organization of the microtubular apparatus has been undertaken in a cell that undergoes a relatively great elongation during the course of its differentiation. This cell is the myoblast that appears in the tail muscle regeneration blastema of the *Rana pipiens* tadpole after tail tip amputation. In previous studies of this and similar cells, it has been shown that colchicine induces the rounding of elongate myoblasts (3), and that microtubules in the cells are disrupted by the colchicine (43). In the present study, the organizational patterns of the microtubular apparatus have been examined using serial sections of myogenic cells in progressive stages of differentiation and elongation. The results of this examination suggest that: (*a*) as myogenic cells differentiate; there is a progressive reorientation of microtubules from a radial focus on the centrioles in the cell center to a longitudinal distribution in the cell cortex; (*b*) the microtubular apparatus in elongate myoblasts consists of overlapping microtubules which are individually shorter than the length of the cell; and (*c*) some degree of sliding interaction between paraxial microtubules occurs.

MATERIALS AND METHODS

R. pipiens tadpoles (from adult frogs obtained from J. M. Hazen, Alburg, Vermont) were cultured in the laboratory at 20°C and fed boiled lettuce or powdered nettle leaves. Tadpoles 1–2 cm in length were used in this study. Regeneration of the skeletal muscle of the tadpole tail was induced by amputation of the distal half of the tail, and regenerating tails were fixed at intervals of 3–10 days after amputation. Fixation was done at room temperature for 1 h in 3–6% glutaraldehyde in a 0.05–0.1 M cacodylate buffer (pH 7.3) with 1 mm $CaCl_2$ added. After a .5-h rinse in the same buffer with 10% sucrose, the tissues were postfixed for another .5 h in 1% OsO_4 in 0.1 M cacodylate. The tissues were rinsed briefly in 0.05 M maleate buffer (pH 5.2) and stained en bloc with 0.5% uranyl acetate in the same buffer for .5 h at about 5°C. After a distilled water rinse, the tissues were dehydrated in ethanol and embedded in Epon resin. Thin sections were further stained with uranyl acetate and lead citrate.

Regeneration of the muscle fibers of the tadpole tail of *R. pipiens* has been described previously (33, 43). In brief, the muscle regeneration blastema forms within 3–5 days (at 20°–22°C) after tail amputation and appears as a thin layer of cells just underneath the tail fin epithelium along the midline. As the blastema matures, overtly undifferentiated mesenchymal cells and elongate myogenic cells may be found within it. The chief advantage of the tadpole for the study of microtubular organization in elongating cells is that the cells are essentially all oriented in the axis of the tail. A number of cells may be examined conveniently at the same orientation in any one thin section of the regenerating tail tip. In the present study, all information concerning the organization of microtubules comes from transverse serial sections of cells within the tail tip. For general evaluation of microtubular organization in entire cells, thin sections were cut only at 2–3-μm intervals along the lengths of the cells and the intervening thick sections were saved for light microscope orientation. Since continuities of individual microtubules cannot be followed over 2–3-μm intervals, sets of consecutive serial sections were cut along 8-μm long segments of two myogenic cells. Microtubule continuities were then traced by direct comparison of adjacent sections. For construction of a three-dimensional model of the microtubular apparatus in one of the segments, transparent templates of every third serial sections were made and holes were drilled in the templates at the locus of each microtubule in the templates. String was used to connect the appropriate holes in the templates and to represent the microtubules. The model itself was constructed to scale, at a ratio of 58,000:1, and the spaces between templates in the model represent as accurately as possible the distances between the thin sections from which the templates were made. Although only every third section actually appears in the model, microtubule

continuities were traced where necessary during construction by comparison of microtubule loci on the intervening sections, as well as on the templates.

RESULTS

Cell Types Present in the Muscle Regeneration Blastema

Two basic patterns of cell shape are displayed in the differentiating muscle blastema. Initially, the blastema is dominated by overtly undifferentiated mesenchymal cells that are mononucleate and display no apparent axis of anisometry. As myogenesis proceeds, the mesenchymal cells give rise to the elongate, fusiform cells characteristic of the early stages of skeletal muscle formation (Figs. 1 and 2). It must be emphasized that cell shape alone is not a sufficient criterion by which to distinguish the spectrum of cells that differentiate within the regeneration zone (18). Thus, any given mesenchymal cell may or may not be committed to a pathway of muscle or connective tissue differentiation. Among the elongate myogenic cells, three types will be distinguished in the present study. Of the fusiform mononucleate cells, one type is 50–150 μm in length and contains no visible myofibrils, while the other is greater than 150 μm in length and contains at least a few developing myofibrils. Using current terminology (18), the former will be termed "premyoblasts" and the

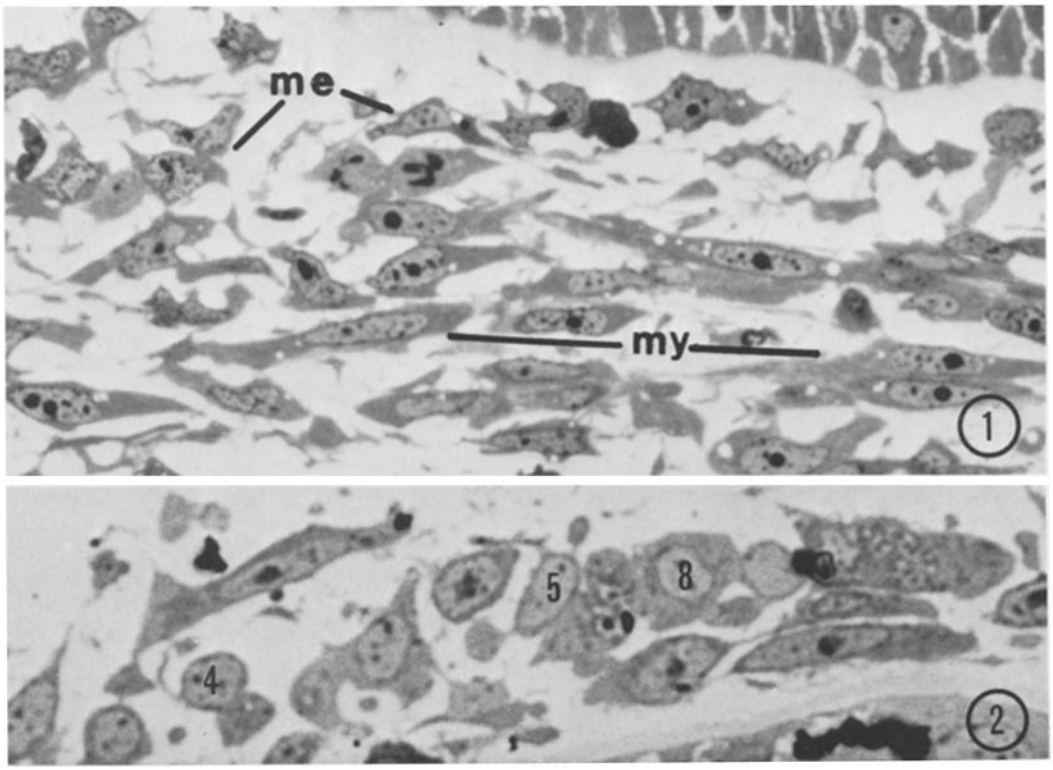


FIGURE 1 Longitudinal section of a regenerating tadpole tail at 7 days after tail amputation. The plane of section passes through the muscle regeneration blastema just below the surface epithelium of the tail. Elongate, spindle-shaped cells that appear to be mononucleate myogenic cells (*my*) are oriented in the axis of the tail (the tip of the tail is towards the left). Stellate mesenchymal-like cells (*me*) appear at the top of the field. × 580.

FIGURE 2 One of a set of transverse serial thick sections of a 7-day muscle regeneration blastema just beneath the surface epithelium (bottom right). The numbered cells are myogenic cells in which microtubule distribution along the lengths of the cells was analyzed in corresponding thin serial sections in the electron microscope. Cell *4*, premyoblast; cell *5*, myoblast; cell *8*, myotube. × 800.

latter "myoblasts." The third type of elongate cell is the multinucleate myotube that arises by fusion of the myoblasts. While it is recognized that no predictions can be made concerning the ultimate fate of any of the mesenchymal cells or premyoblasts examined in the present study, it is assumed that these cells are representative of the transitional stages leading to overtly differentiated muscle fibers, and observations made with regard to changing patterns of cell shape and microtubular organization will be interpreted within this context.

Organization of Microtubules in Myogenic Cells at Progressive Stages of Differentiation

MESENCHYMAL CELLS: These cells are irregularly shaped with no apparent axis of elongation (Fig. 1). Microtubules in these cells radiate in all directions from a generally prominent cell center containing a Golgi apparatus, numerous vesicles, centrioles, and dense centriolar satellites. It has been reported that centrioles may function as ciliary basal bodies in similar mesenchymal cells in other systems (9), but cilia have not been observed on mesenchymal cells in the present study. Microtubules radiating from the cell center usually appear singly in the cytoplasm, and paraxial association between microtubules is not often observed.

BIPOLAR MYOGENIC CELLS: A total of four premyoblasts lacking myofibrils (referred to subsequently by number in this report as cells 1–4), three myoblasts (cells 5–7), and one multinucleate myotube (cell 8) have been examined in sets of serial transverse sections (Fig. 2) covering a total axial distance of 150 μm. This distance is sufficient to include the entire lengths of the premyoblasts, most of the lengths of the myoblasts, but only the midregion of the myotube.

Microtubular organization in the elongate myogenic cells is characterized by certain general features. Microtubules are somewhat more prominent in the cortical regions of the cells (Fig. 3) and for the most part are longitudinally aligned. Occasional microtubules may appear in other orientations, especially in the vicinity of centrioles or basal bodies in less mature cells. For all elongate cells, without exception, the absolute number of microtubules is greatest in the midregion of the cells and decreases with relative smoothness toward the ends of the cells. A more detailed analysis of the organization and distribution of microtubules and the changes that occur with differentiation of the myogenic cells follows.

THE RELATIONSHIP OF MICROTUBULES TO CENTRIOLES: Microtubules have been observed to originate from a cell center containing a centriole or a basal body in two of the premyoblasts examined. In one of these cells (cell 3), a prominent cell center with a large Golgi apparatus appears in transverse section near the nucleus (Fig. 4). A centriole is not visible in this section nor in adjacent sections 3 μm to either side, but the existence of a ciliary basal body lying nearby within a distance of less than 3 μm may be inferred from the presence of an aberrant cilium projecting from the cell surface (Fig. 4). The numerous microtubules in the cell appear to radiate in all directions from electron-dense satellites. This radial pattern of microtubular organization in cell 3 is very local, however, since, on the other side of the cell, away from the cell center, microtubules are longitudinally oriented. There is a sharp peak in microtubule number at the level of the cell center and basal body (graph, Fig. 5), which indicates that many of the microtubules originating in the cell center must be relatively short. The apparent overall organization of the microtubules in cell 3 is summarized in a diagram in Fig. 5.

In another, somewhat longer (96 μm) premyoblast (cell 4, Figs. 6 and 7), a centriole was observed in one section taken at the level of the nucleus. In this premyoblast, the cell center is less well-defined than in cell 3. Only one or two dense satellites appear in the section in the vicinity of the centriole. Microtubules in the immediate vicinity of the centriole are oriented in all axes, whereas the microtubules distributed peripherally around the nucleus are longitudinally oriented. Microtubule number is at a maximum around the nucleus, but there is no sharp peak in microtubule number associated with the centriole as was seen in cell 3 (Fig. 8).

No obvious cell centers or centrioles have been detected in the two other premyoblasts (cells 1 and 2) or in the three myoblasts (cells 5–7). Any centrioles or basal bodies present in these cells must have lain in between levels where thin sections were taken.

Since the multinucleate myotubes are formed by fusion of myoblasts, they may potentially contain

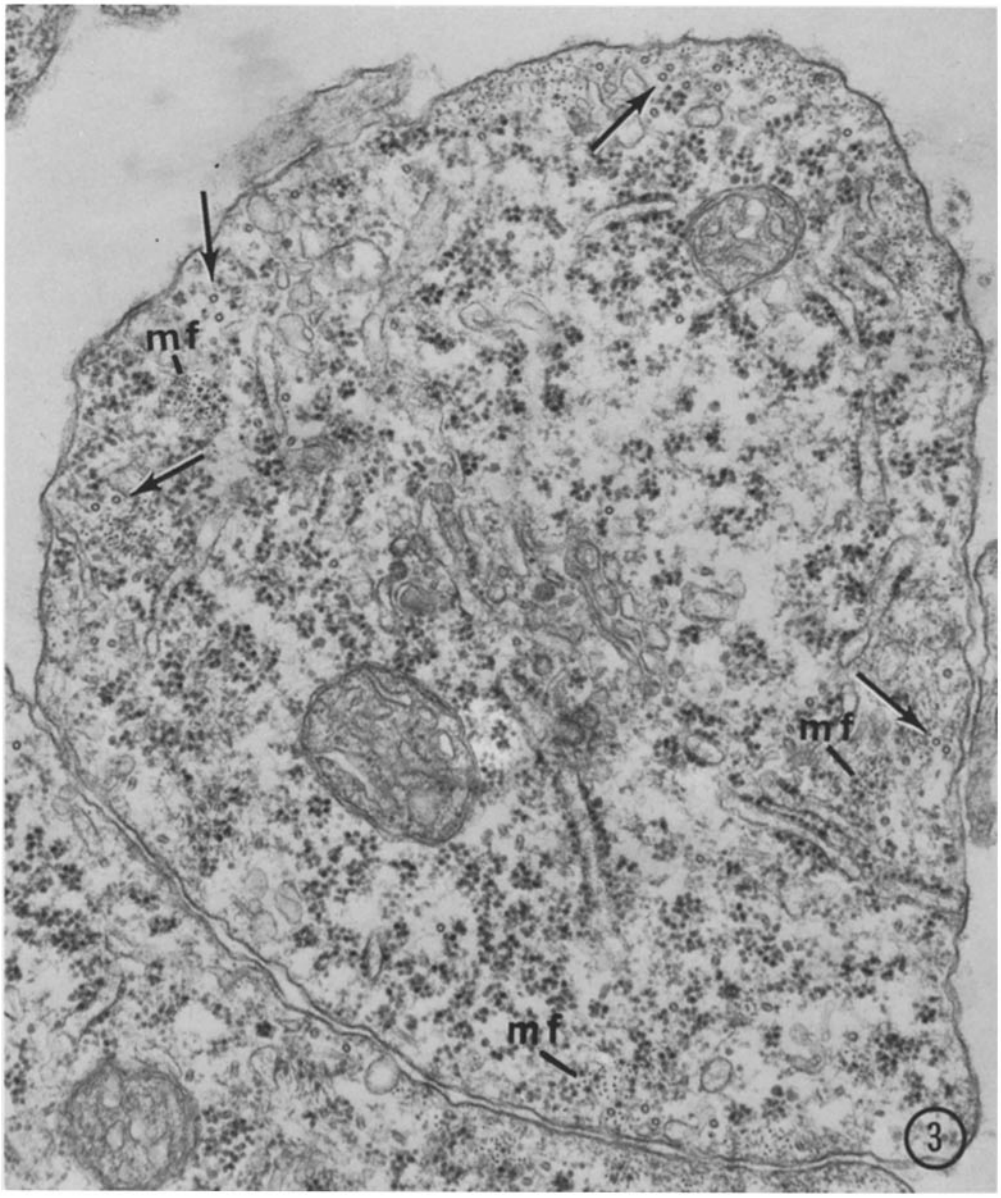


FIGURE 3 One of a set of 76 transverse serial thin sections of a myoblast from which a model of the microtubular apparatus in a portion of the myoblast was constructed. Microtubules are found singly, in pairs (arrows), and small bundles around the periphery of the cell. A few microtubules are also associated with the Golgi apparatus in the center of the cell. A few very slender myofibrils (*mf*) with thick and thin filaments are present. × 38,000.

several centrioles or basal bodies. One basal body observed in the nuclear region of cell 8 is not associated with an obvious cell center or with satellites. Microtubules are numerous in the nuclear region of the myotube. At the level of the section containing the basal body they are evenly distributed around the entire periphery of the cell in longitudinal orientation. Microtubule number fluctuates significantly in the myotube nuclear region (Fig. 8), with the basal body occurring at a

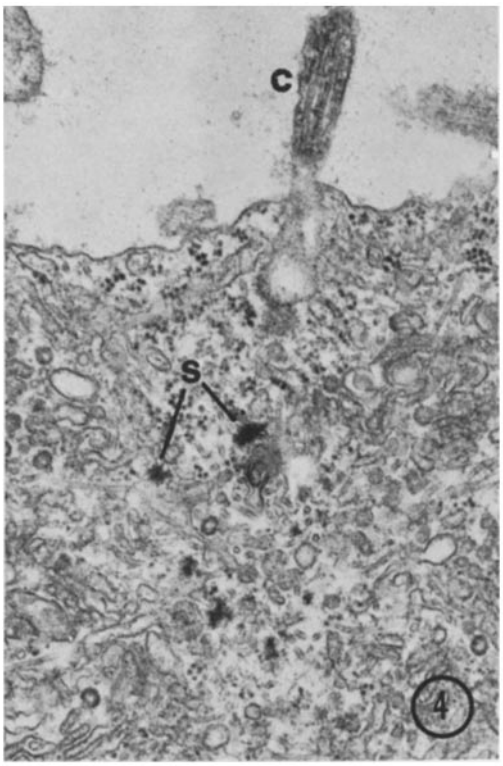


FIGURE 4 Transverse section through the periphery of a young myogenic cell (cell 3) in its midregion near the nucleus, showing a residual cell center at one side of the cell. Electron-dense satellites (S) are associated with the Golgi apparatus that fills the field, and microtubules radiate in all directions within this region. An aberrant cilium (C) projects from the cell surface. × 33,000.

level of reduced microtubule number, rather than at a peak.

MICROTUBULE COUNTS: Cells appear to contain the largest number of microtubules in their midregions as may be seen in Fig. 8, which displays counts of microtubules from four cells (Nos. 1, 4, 5, 8) of increasing size and apparent level of differentiation. It may also be noted that microtubule number increases with increasing level of differentiation. Cell 3 (Fig. 5) is exceptional in that its maximum number of microtubules (138) is greater than the maximum number of microtubules (130) observed in any of the more mature myoblasts, as for example cell 5 (Fig. 8). It has been noted, however, that cell 3 is unique in displaying a sharp peak in microtubule number at the level of the basal body, while all other cells display a broad maximum number of microtubules around the nuclei. Another way of comparing microtubule numbers among the cells is to consider the areas under the curves in the graphs as an index of the total quantity of microtubules in the cells. If these areas are compared and the cell with the least area under its curve is arbitrarily assigned a value of 1, then the values for all eight cells may be ordered in the sequence appearing in Table I. In this sequence, cell 3 falls within the same range as cells 1 and 2, which are premyoblasts of comparable length to cell 3. In comparing the values for cells 5–8, it should be noted that it was not possible to count microtubules in the extreme ends of these cells, as they lay beyond the limits of the 150-μm long sets of serial sections. The values for the areas under the curves of microtubule number for these cells accordingly contain correction factors derived by estimating the slopes of the curves at either end and extrapolating to a zero baseline. It may be observed that the values for both maximal microtubule number and relative total quantity of microtubules for cell 8, which contains either two or three nuclei, fall within a range that is two to three times the maximum values obtained for the myoblasts.

PARAXIAL MICROTUBULE INTERACTIONS: Longitudinally oriented microtubules in all myogenic cells are commonly found in paraxial association along the entire lengths of the cells, as may be observed in cell 4 (Figs. 6 and 7). At the level of the nucleus, microtubules appear in pairs and triplets spaced at approximately 30 nm (surface-to-surface) from one another. Near the end of the cell (Fig. 7), the microtubules are clustered together in larger bundles, but the minimum spacings between individual microtubules remain about the same as at the level of the nucleus. In these and other micrographs, there is an apparent lack of bridge interconnections between microtubules in close paraxial association.

An attempt to quantify the extent of paraxial association between microtubules at different levels in the same cell and between different myogenic cells has been made by counting numbers of microtubules lying within about 40 nm (surface-to-surface) of one another in transverse sections. The ratios of paraxially associated microtubules to total numbers of microtubules in consecutive transverse sections have been plotted as a function of cell length for five cells (4, 5, 6, 7, 8) of progressive stages of differentiation, three of which

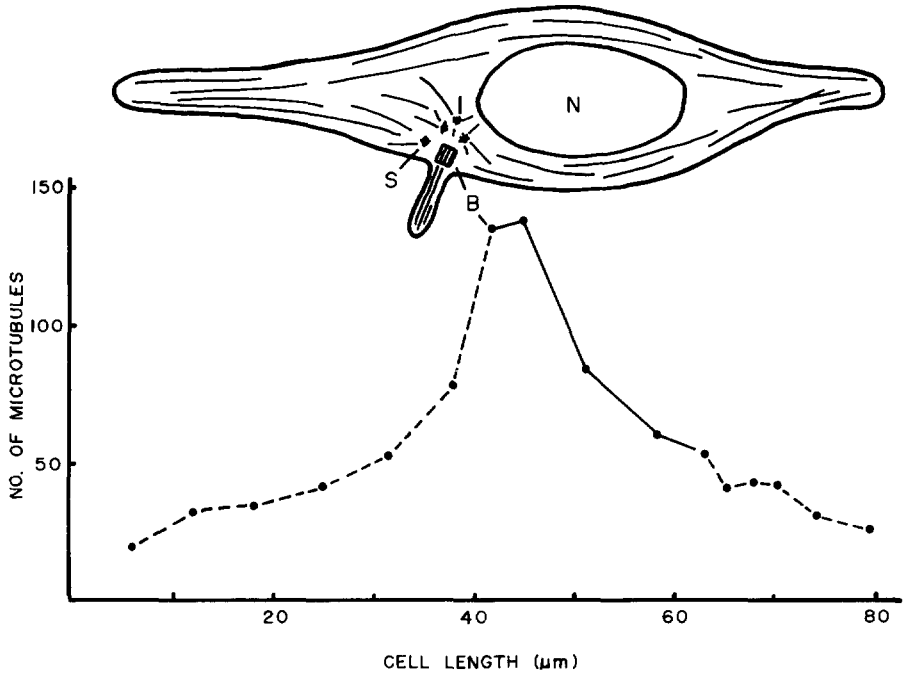


FIGURE 5 The graph indicates numbers of microtubules appearing in transverse sections spaced at about 3-μm intervals along the length of cell 3. The solid line indicates the region in the cell at which the nucleus was sectioned. The diagram above the graph is an interpretation of microtubule organization within the cell as determined from analysis of microtubule distribution in the thin sections. B indicates the inferred position of a basal body corresponding to the cilium seen in Fig. 4. A sharp peak in microtubule number appears at the level of the basal body and satellites (S) in the cell center.

(4, 7, 8) are presented in a graph (Fig. 9). The percentages of microtubules showing paraxial association vary considerably along the lengths of the premyoblast (cell 4) and the myoblast (cell 7), but are fairly constant over the portion of the myotube (cell 8) that is included within the set of serial sections. For all cells, however, there is a tendency for the percentage to increase from a value of 20% to 25% at the level of the nucleus to a higher value in the tapering ends of the cells. The maximum value observed of 70% occurs in one end of cell 4, the least elongate of the three.

It is conceivable that the higher percentages of microtubule association in the ends of the cells are simply a function of crowding in the restricted cytoplasmic space available. In order to weigh this possibility, the extent of crowding of microtubules at different levels (nuclei, tapering portions, and ends) in myogenic cells has been roughly determined by measuring the cytoplasmic area (in μm^2) of the cells in transverse sections and counting the number of microtubules appearing within that area (Table II). In determining areas of cytoplasm, no attempt was made to exclude mitochondria or other organelles (except the nucleus) since they appeared to be uniformly distributed along the entire lengths of the cells. For cells 4 and 7, it appears that microtubules are more crowded in the extreme ends of the cell, where the data of Fig. 9 indicate paraxial association of microtubules to be at a high level. It should be noted, however, that there is also an increase in microtubule density in the rim of cytoplasm around the nucleus, and in this region paraxial association of microtubules reaches the lowest level. In cell 8, there is a negative correlation between microtubule density and paraxial association in the midregion, but the ends of the cell are not included within the set of serial sections. On the basis of these observations, it would appear that other factors besides crowding may be involved in determination of the extent of microtubule association in different regions along the cell.

A Model of the Microtubular Apparatus in a Portion of an Elongating Myoblast

A three-dimensional reconstruction of the microtubular apparatus was made from one of two

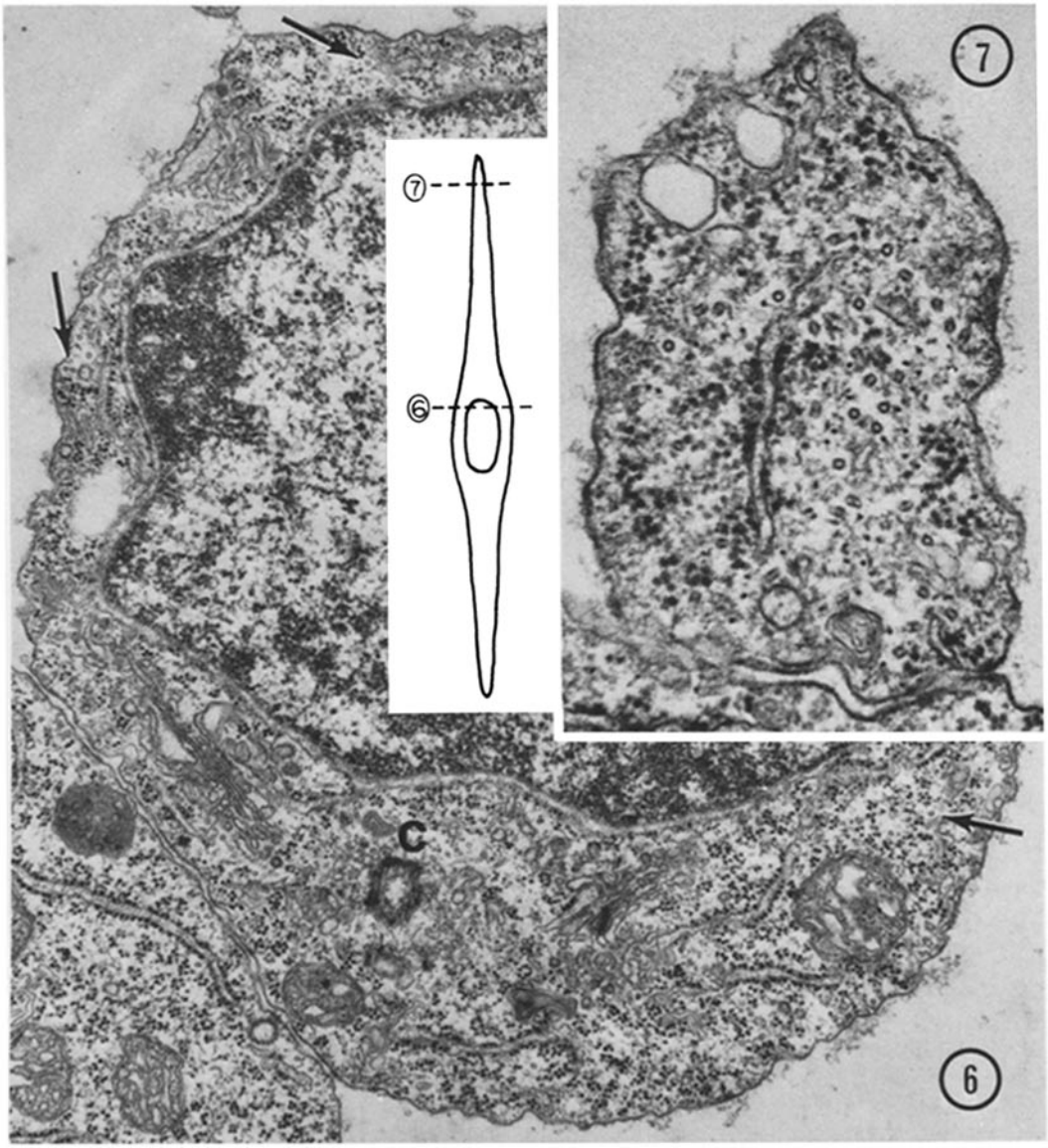


FIGURE 6 One of a set of transverse sections spaced at 2–3-μm intervals along the length of an elongate myogenic cell (cell 4). The *inset* shows the position of this section (6) along the length of the cell. Note the microtubules surrounding the centriole or basal body (C). Transverse profiles of microtubules also appear around the entire periphery of the cell away from the centriole (arrows). × 26,400.

FIGURE 7 Transverse section of the tapering portion near the end of cell 4. The position of this section along the cell is indicated in the *inset* of Fig. 6 (7). Microtubules display extensive paraxial association in pairs and groups. × 53,000.

8-μm long sets of serial sections along two myoblasts in order to provide additional insight into the following questions. (*a*) Do individual microtubules extend the entire length of the cell? (*b*) Where do microtubules originate in the myogenic cells in which there is not a prominent cell center? (*c*) What morphology is observed at the end of a microtubule? (*d*) How do the microtubules interact with each other and with other organelles along the length of the cell?

One transverse section belonging to the set of serial sections from which the model was con-

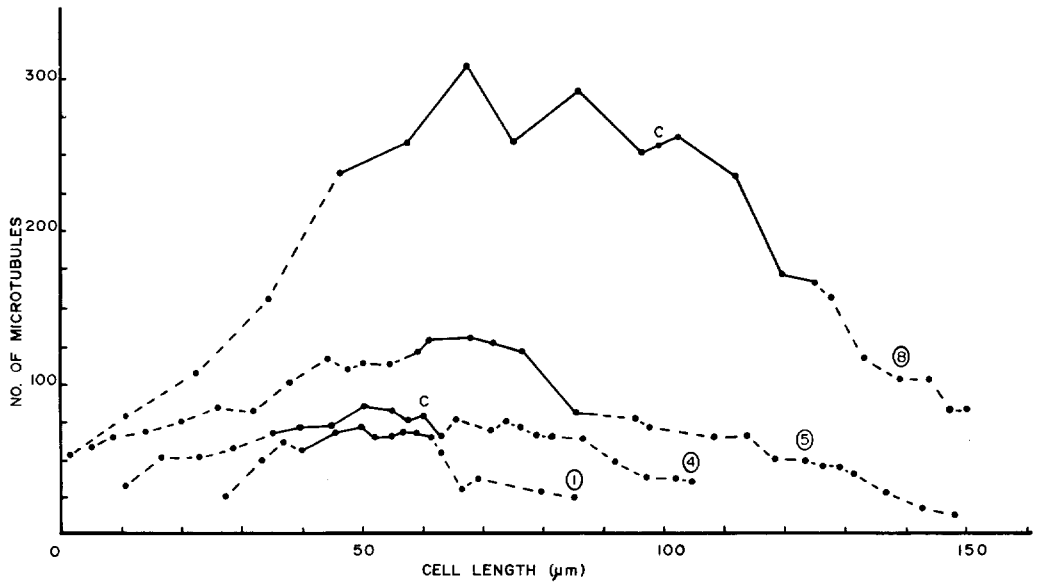


FIGURE 8 The graph shows counts of microtubules appearing in transverse sections taken along the lengths of four cells, two premyoblasts (1, 4), a myoblast (5), and a myotube (8). The solid lines indicate levels at which nuclei appeared in the sections. C indicates positions of centrioles or basal bodies that were observed in sections.

TABLE I

Numbers of Microtubules in Myogenic Cells of Increasing Length

Cell no.	Relative quantities of microtubules per cell*	Maximum no. of microtubules in midregion of cell	Cell length	No. of nuclei	Myofibrils present
			μm		
1-Premyoblast	1.0	72	59	1	–
2-Premyoblast	1.3	96	61	1	–
3-Premyoblast	1.4	138	69	1	–
4-Premyoblast	2.3	86	96	1	–
5-Myoblast	4.1	130	> 150	1	+
6-Myoblast	4.5	105	> 150	1	+
7-Myoblast	5.4	110	> 150	1	+
8-Myotube	11.0	310	≫ 150	2 or 3	+

* These quantities were estimated by measurement of the areas under the curves representing numbers of microtubules at consecutive intervals along the lengths of the cells.

structed appears in Fig. 3. The myoblast at this level contains a few slender, peripheral myofibrils and a central Golgi apparatus. Microtubules are found predominately in the cell cortex, often as pairs or in small groups, but they may also appear in the center of the cell, particularly at the interface between cytoplasm and Golgi apparatus.

Observation of the model in stereoscopic lateral view (Fig. 10) emphasizes the organizational pattern of microtubules that is generally apparent in individual transverse sections. The majority of microtubules are oriented in the longitudinal axis and are commonly aligned in pairs or in small bundles. Within these groupings, microtubules may approach to within 20 nm (surface-to-surface) or less of one another. A slight kinkiness in the

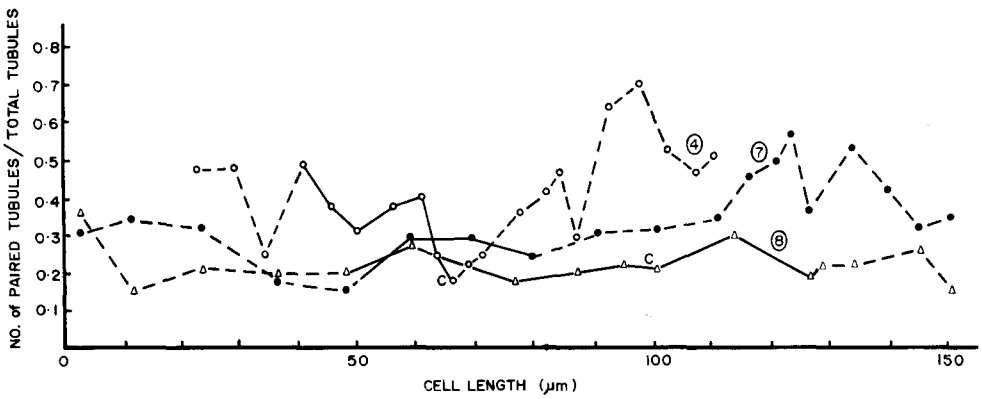


FIGURE 9 Graph indicating the extent of paraxial association of microtubules along the lengths of a premyoblast (4), a myoblast (7), and a myotube (8). Solid lines indicate levels at which nuclei appeared in transverse sections. C indicates positions of centrioles or basal bodies along the cell.

TABLE II

Microtubule Density (MT/μm²) at Midregion, Tapering Regions, and Ends of Myogenic Cells as Determined by Counts of Microtubules in Measured Areas of Cytoplasm in Transverse Sections

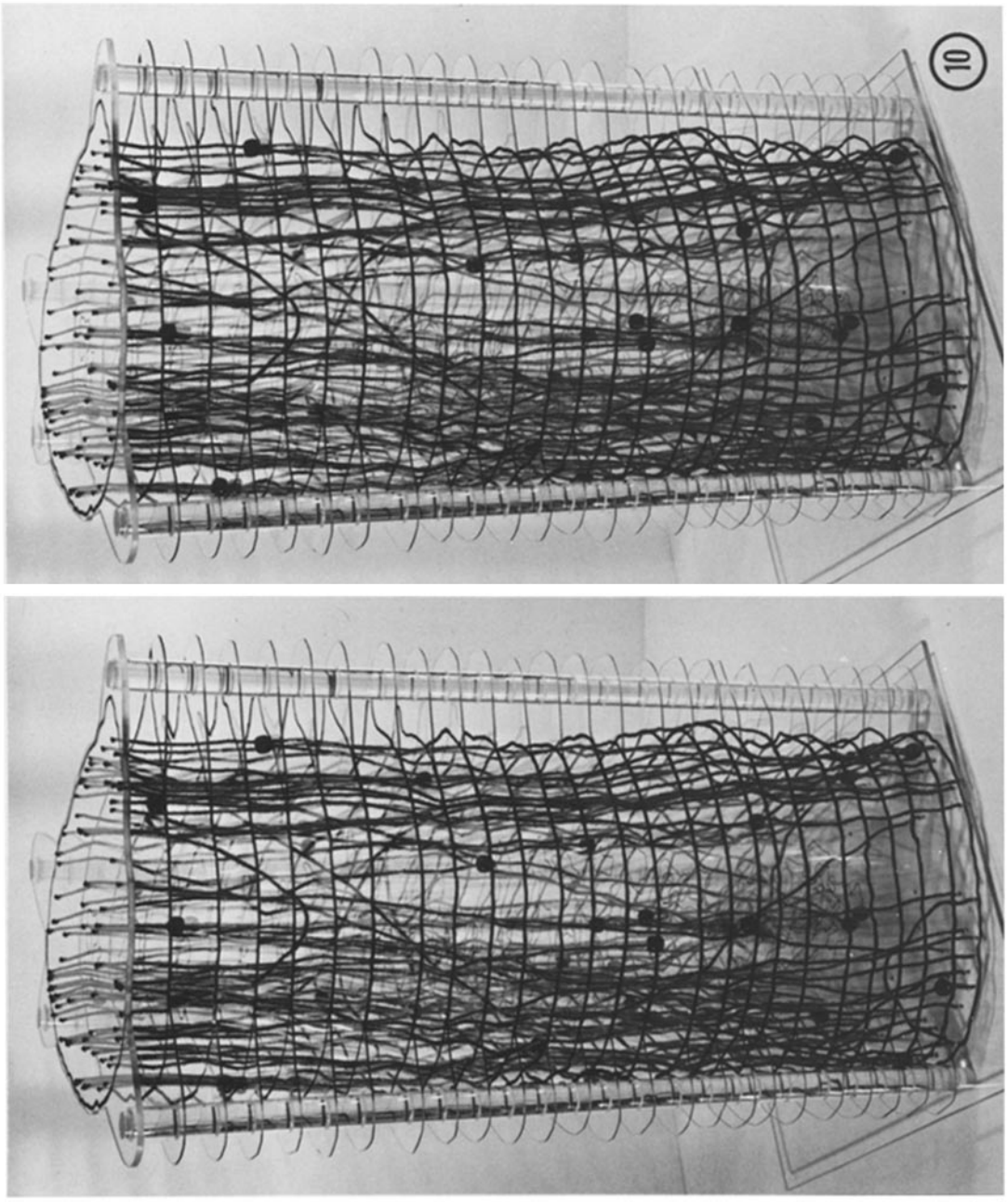
Cell no.	Position of section along cell				
	End	Taper	Nucleus	Taper	End
4 (premyoblast)	12.5 MT/μm²	3.3 MT/μm²	11.5 MT/μm²	5.5 MT/μm²	22 MT/μm²
7 (myoblast)	11.0	3.5	10.6	5.4	16.0
8 (myotube)	—	4.0	6.6	3.0	—

microtubules is believed to arise primarily as an artifact of distortions within the original electron micrographs and of small errors in registration of the templates from which the model was made.

The model reveals an aspect of microtubular organization in the myoblast that is not apparent in thin sections: the existence of individual microtubules that are curved and which run obliquely across the cell for variable distances before resuming a longitudinal course. Several such curving microtubules may be observed in the lateral view of the model. Three microtubules have been observed that are bent into pronounced sigmoid loops, and one of these may be observed at the top of the model in lateral view (Fig. 10).

The average number of microtubules remains nearly constant along the length of the model, with 79 microtubules appearing at one end and 76 at the other. Within the model, however, 46 microtubules are observed to terminate, and these terminations are represented for clarity by the black beads affixed to the ends of the strings in the model. The length of the myoblast from which the model was constructed is estimated to be greater than 150 μm. Thus, if such a large percentage of microtubules terminate within the 8-μm segment represented by the model, it seems highly unlikely that any microtubules extend the entire length of the cell. In the set of serial sections from the second myoblast in which microtubules were traced individually, but for which no model was constructed, only 12 microtubules terminated out of an average of 62 microtubules entering the 8-μm long segment from either end. Overall cell length for the second myoblast is estimated to be greater than 150 μm, and thus, while it appears that the frequency of microtubule termination is lower in this segment from the second cell, the probability of any microtubules extending the length of the cell also seems very low. In both cell segments, microtubule terminations appear to be randomly distributed throughout the lengths of the segments.

Are the observed terminations of microtubules representative of the in vivo situation or are they created during tissue processing? If breaks are induced during fixation, one might expect that


10

broken ends would be found in close proximity to one another in the model. In only a very few instances, however, can microtubule ends of opposite polarity in the model be matched reasonably to produce one linear microtubule. It may also be noted that in the second cell examined, 11 out of the 12 microtubule ends in the segment are of the same polarity and thus cannot be matched.

Microtubular Terminations and Bridges

Microtubular terminations are abrupt, and all structural traces of the microtubule wall usually disappear within the thickness of one thin section or about 100 nm. Microtubules have not been observed to unfold into C-shaped sheets or to break up into protofilaments, although detection of the latter would be difficult in a 100-mm thick section. Occasional ends of microtubules appear to lie completely free in the cytoplasm (Fig. 11), but more frequently the ends are associated with a membranous vesicle (Fig. 12), with cisternae of the endoplasmic reticulum, or with the plasma membrane. Amorphous, electron-dense material resembling a centriolar satellite has been found at the end of a microtubule in only a few instances (Fig. 13). It may be noted in connection with the microtubule illustrated in Fig. 12 that the wall of the microtubule becomes indistinct as the microtubule ends, and similar modification of the tubule wall has been observed in several other instances at the ends of tubules in this study.

Bridging between closely associated microtubules in bundles has not been observed, although there is an abundance of rather amorphous material in the background cytoplasm that often fills spaces between microtubules (Fig. 13). Discrete bridges have been observed in only a few instances between microtubules and membranes such as endoplasmic reticulum (Fig. 14).

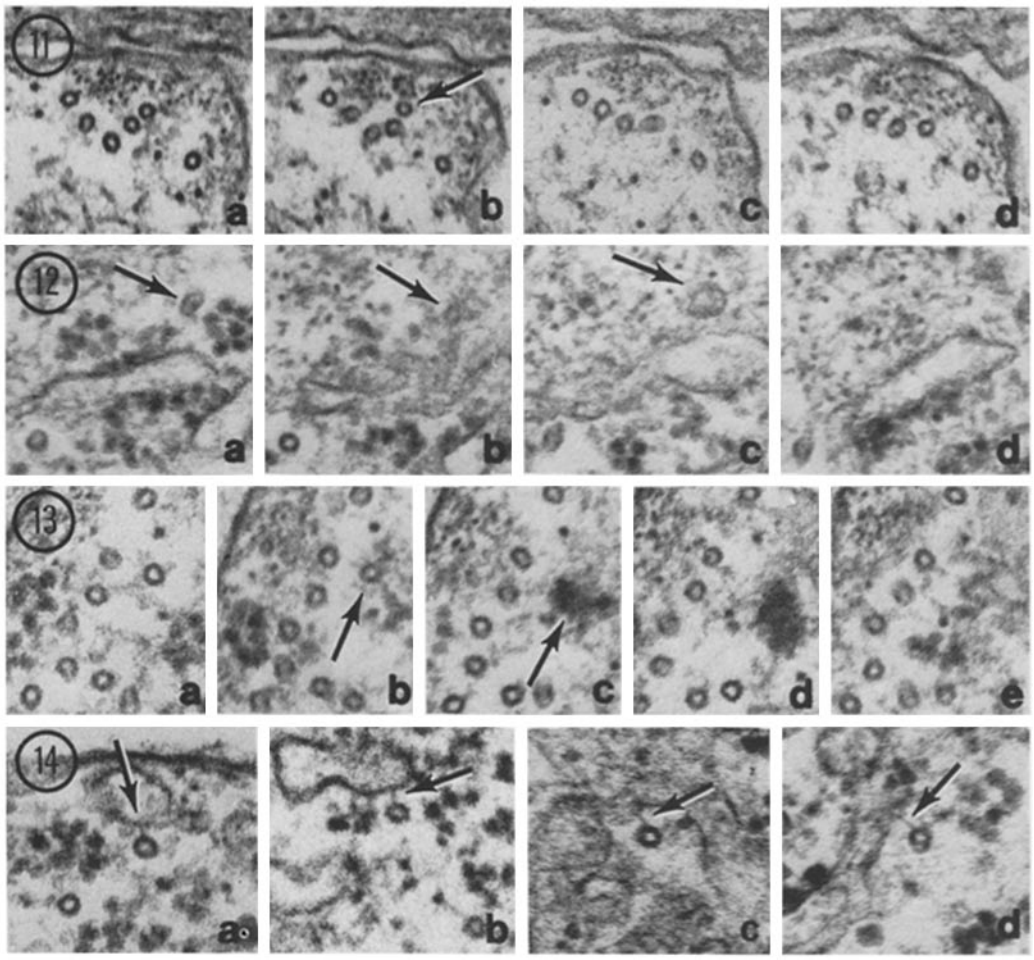
DISCUSSION

Control of Microtubular Organization

During muscle differentiation in the tadpole tail, the radial pattern of cellular organization in the mesenchymal cell is converted to the bipolar pattern of the myoblast. This change in overall cellular organization involves a concomitant shift in arrangement of the microtubules from a radial focus on the centriolar-satellite complex in the cell center of the mesenchymal cell to a cylindrical dispersion around the periphery of the elongate myoblast. The shift in microtubular organization appears to take place gradually, as a few premyoblasts have been found in which some microtubules continue to focus radially upon a centriole or basal body in the cortex. In the oldest myoblasts and in the myotubes that have been examined, no evidence of radial patterning of microtubules in a cell center has been observed. These basic features of the change in microtubule organization from mesenchymal cell to myotube are summarized in a diagram (Fig. 15).

The function of centriolar-satellite complexes as organizing centers for microtubule polymerization has been demonstrated by Tilney and Goddard (39), who showed that satellites associated with a ciliary basal body in ectodermal cells of sea urchin blastula served as foci for microtubule regeneration after the microtubules were first disrupted by cold treatment. Although similar experiments have not yet been done with the tadpole cells, it is assumed that the radiation of microtubules from the centriolar-satellite complexes in mesenchymal cells is indicative of a concentration of microtubule-initiating sites in those regions. One is immediately led to consider the question of the extent to which centriolar-satellite complexes in myogenic cells continue to function as microtubule-organizing centers as the cells elongate. In support of such continued function are the observations that some microtubules are found to radiate from basal body satellites in a few young myogenic cells, and that microtubule number in myogenic cells is always greatest in the midregions of the cells where the basal bodies and satellites are located. On the other hand, large numbers of microtubules in the midregions of myogenic cells appear to pass directly by the centriolar-satellite complexes, and in most cells there is no sharp peak

FIGURE 10 Lateral view of a model of the microtubular apparatus in an 8-μm long segment along the length of a myoblast. Stereoscopy may be achieved by eye convergence. The majority of microtubules are aligned longitudinally in the periphery of the cell, but a few are seen to course obliquely from one region of the cell to another. At the top center and bottom left of the model, looping configurations of microtubules are discernible. Note the random distribution of microtubule terminations within the model as indicated by the dark beads. The faint line tracings in the core of the model are outlines of the Golgi apparatus. × 58,000.



FIGURES 11–13 Sets of consecutive serial sections in which longitudinally oriented microtubules in myogenic cells are observed to terminate.

FIGURE 11 Of six microtubules present in section *a*, the one indicated by the arrow in *b* terminates abruptly in *c*. × 88,000.

FIGURE 12 The somewhat obliquely oriented microtubule indicated by the arrow in *a* becomes indistinct in *b* and is replaced by a vesicle in *c*. No trace of the microtubule is seen in *d* next to the cisterna of endoplasmic reticulum. × 103,000.

FIGURE 13 Of nine microtubular profiles visible in *a*, the one indicated by the arrow in *b* is replaced by a discrete electron-dense mass (arrow) in *c* that resembles a satellite. The dense mass is visible in *d* but disappears in *e*. × 103,000.

FIGURE 14 Four examples of microtubules that are linked to cisternae of the endoplasmic reticulum by distinct bridges (arrows). × 112,000.

in microtubule number that might be associated with a centriole or basal body. Microtubule number in myogenic cells apparently continues to increase somewhat even as the number of microtubules specifically associated with a centriole or basal body becomes very low. Finally, one must also consider the fact that in older myoblasts the model reveals that many microtubules terminate in the cell some distance away from any centrioles or basal bodies in the midregion.

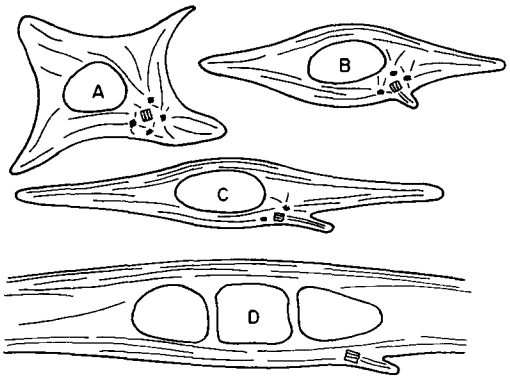


FIGURE 15 A summary of the organizational changes in microtubules believed to occur as mesenchymal cells (A) differentiate into myoblasts (C) and myotubes (D). Microtubules in the mesenchymal cells radiate from a cell center containing centrioles and satellites. In premyoblasts (B), the centrioles appear to move to the cell surface to function as ciliary basal bodies. A residual cell center with microtubules radiating from satellites may appear around the basal body. In myoblasts and myotubes, almost all microtubules are longitudinally oriented and few, if any, appear to radiate from the basal bodies.

There are at least two interpretations for these observations. One possibility is that microtubules continue to be initiated in the general vicinity of the centrioles or basal bodies, but that they are then displaced from those regions toward either end of the cell. A second possibility is that the cell center in myogenic cells ceases to function as a primary microtubule-organizing region and that microtubule-initiating sites, such as satellites, become more widely dispersed in the cell cortex with cell elongation. In this regard, special attention has been paid to terminating microtubules as they have been observed in the serial sections from which the microtubule model was made, but, as noted, only a very few microtubules have been observed to terminate in material of sufficient density and discreteness to resemble a satellite. Except for these few instances, satellites have not been observed to occur away from the centriolar region in myogenic cells, although a great deal of relatively amorphous, moderately dense material does appear in the background of cytoplasm throughout the cell. The common occurrence of cytoplasmic membranes or vesicles in association with microtubule terminations is intriguing and may be compared with the occurrence of numerous membranous vesicles in the Golgi apparatus surrounding the microtubules in the cell center in mesenchymal cells. The association of microtubule terminations with vesicles has been noted in other studies, and images suggestive of microtubule continuity with vesicles in the vicinity of the Golgi apparatus (32) and centrioles (30) or of terminal swelling of microtubules (17, 47) have been presented. In the present study, however, continuity of microtubules with vesicles has not been noted.

Microtubule and Cell Elongation

The observations made in the present study strongly indicate that the microtubules in the elongate myogenic cells do not extend the entire length of the cell. How, then, does the microtubular apparatus contribute to the maintenance of cell elongation as is known from previous studies of the effects of colchicine on myoblast elongation? This might occur through individual interactions of microtubules with other elements in the cell cortex, such as the plasmalemma or the ubiquitous microfilaments underlying the plasmalemma. The net result of all such local interactions could be a stiffening of the cortical region of the cell along its entire length. Alternatively, the entire microtubular apparatus could be stabilized by interactions between individual overlapping microtubules. This second alternative is favored for the reason that the paraxial associations of microtubules occur regularly along the entire length of the cell, in contrast to the lack of a consistent pattern of microtubular interactions with the plasmalemma or other organelles.

The analyses of the extents of paraxial microtubule association as a function of cell length have indicated that such association occurs at a minimum value of 20–25% at any one level in all the cells examined. If microtubular associations are responsible for stabilizing the overall microtubular apparatus, then this value may represent the minimum amount of microtubular interaction necessary to stabilize cell form as well. It seems reasonable that the increased microtubular association near the ends of the cells, whether or not the association arises from crowding of microtubules, might provide additional structural stabilization of the slender end regions of the cells.

The regularity of paraxial microtubular associations suggests the existence of physical linkages between the microtubules such as have been observed in other, more highly organized parallel microtubular arrays (13, 16, 23, 25, 46). From the paucity of bridging elements actually observed,

one must conclude at present that any elements serving to link microtubules are ill-defined by nature or are not adequately preserved and stained with the methods employed in the present study.

Within the context of microtubular associations in the myoblasts, the bending and looping microtubules are of interest. Profiles of all microtubules showing significant bending in the model have been traced from lateral view, and five representative profiles are reproduced to scale in Fig. 16. Although these microtubules appeared at different levels within the cell, the order in which they have been placed in the diagram is intended to show that the sigmoid loops could arise from more gently curving microtubules if the ends of the latter were displaced in opposite directions as indicated by the arrows in the diagram. How might such displacements arise? For almost all microtubules displaying bends in the model, the longitudinally oriented straight portions of the microtubules are observed to interact paraxially with other straight microtubules. If sliding interaction of opposite polarity were to take place along these regions of paraxial interaction, then individual microtubules could be bent into the sigmoid loops that are observed. Sliding interactions between paraxial microtubules have now been defined in such microtubular apparatuses as cilia (31, 35) and protistan axostyles (19, 24). It is also conceivable that displacements of microtubules might occur as a passive response to forces in the cytoplasm external to the microtubular apparatus, as, for example, flow of cytoplasm itself. Whatever the actual mechanism, it is difficult to conceive how the families of bent microtubules could be produced other than by shearing forces operating at some level within the cell. It seems very unlikely that the microtubules, presumably being fairly rigid structures, could spontaneously polymerize or bend into the very smooth profiles observed.

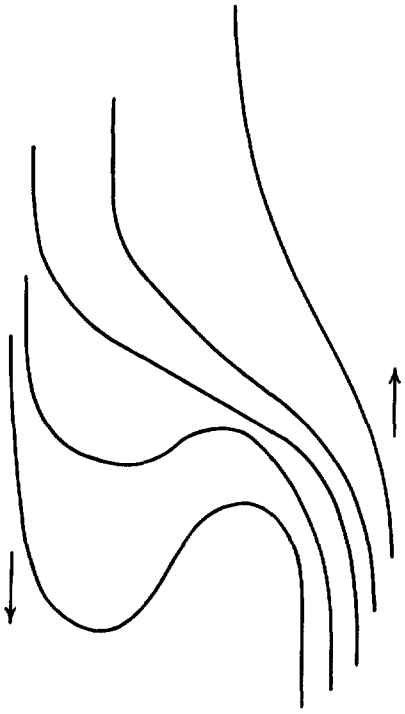


FIGURE 16 The lines in the diagram represent tracings, approximately to scale, of curving microtubules as they appear in lateral view of the model from different positions around the model. The lines are grouped in an order meant to indicate that the more pronounced sigmoid loops could be generated from the more gently curved microtubules if the ends of the latter are displaced in opposite directions as indicated by the arrows.

Among the various ways in which microtubules might contribute actively to cellular elongation is the possibility that ordered sliding interactions between microtubules could produce a force of extension within the cell (7). Although the results of the present study suggest that sliding might occur, there is no evidence to indicate that it occurs in an ordered manner such that the polarities of the sliding would lead to an overall net elongation of the microtubular aparatus. Sliding, even if disordered, might be of some significance with regard to establishing the patterns of distribution of microtubules along the length of the cell. If, for example, microtubule-initiating sites were not uniformly distributed throughout the length of the cell, then sliding of microtubules could lead to a movement of some microtubules to the regions of the cell where initiating sites were less numerous.

From studies of cellular elongation in other systems, it now appeard that various microfilament networks associated with the plasmalemma play a prominent role in active extension of some cells (1, 6, 27, 34, 48). In systems such as the elongating neurite in which both microtubules and microfilament networks are present, there is evidence that microfilaments are most directly involved in cellular extension at the leading edge, whereas microtubules seem to be involved in the stabilization of the axis of the neurite behind the growth cone (6, 48). This interpretation is reinforced by studies of the effects of colchicine or Colcemid upon fibroblast movement and elonga-

tion (11, 15, 42). Normally, the cells are bipolar and display surface membrane ruffling at the leading edge, but after colchicine treatment the cells cease moving, round up, and display ruffling around their entire periphery. The function of microtubules in these cells may thus relate most directly to stabilization of an axis of anisometry rather than to extension of the cell surface in the direction of movement.

Microfilaments are present in the elongating myogenic cells both in longitudinally oriented bundles and in less discrete subplasmalemmal networks, and could well participate in cellular elongation. If, as seems reasonable, the microtubular apparatus in these cells is more directly involved in stabilizing the axial anisometry, then it would be of great interest to learn more concerning the nature of the elusive elements that may bind the microtubules together. Attempts to characterize such elements in the more highly organized microtubular apparatuses (14, 26) have been rewarding, but as yet there has been little information forthcoming related to bridges or other binding elements in the relatively primitive cytoplasmic microtubular bundles. It has been noted that in the radially oriented microtubular apparatus of the mesenchymal cells, paraxial association of microtubules is uncommon, whereas it is extensive in the bipolar myogenic cells. If the reorganization of microtubules into paraxial bundles is a factor contributing to the initial establishment and stabilization of the bipolar axis, then one way in which the cell might control this change could be the synthesis or release into the cytoplasm of factors that encourage the association of microtubules into bundles. Thus, in terms of characterization of microtubular binding elements, it would also be of interest to determine whether the amounts and kinds of such elements vary in the cell according to its basic plan of organization.

I would like to thank Drs. Beth Burnside and Gary E. Wise for their very helpful discussions and evaluation of the manuscript, Mr. Charles Vick for assistance in building the model, and Ms. Kathy Davidson for her expert help in preparing the illustrations.

This work was supported by National Science Foundation Research Grant GB-32029.

Portions of this work were presented as a demonstration at the meeting of The American Society for Cell Biology in Miami Beach in November of 1973.

Received for publication 28 March 1974, and in revised form 19 July 1974.

REFERENCES

1. ABERCROMBIE, M., J. E. M. HEAYSMAN, and S. M. PEGRUM. 1971. The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. *Exp. Cell Res.* **67**:359.
2. BIKLE, D., L. G. TILNEY, and K. R. PORTER. 1966. Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus*. *Protoplasma.* **61**:322.
3. BISCHOFF, R., and H. HOLTZER. 1968. The effect of mitotic inhibitors on myogenesis *in vitro*. *J. Cell Biol.* **36**:111.
4. BOUCK, G. B., and D. L. BROWN. 1973. Microtubule biogenesis and cell shape in *Ochromonas*. I. The distribution of cytoplasmic and mitotic microtubules. *J. Cell. Biol.* **56**:340.
5. BROWN, D. L., and G. B. BOUCK. 1973. Microtubule biogenesis and cell shape in *Ochromonas*. II. The role of nucleating sites in shape development. *J. Cell Biol.* **56**:360.
6. BUNGE, M. B. 1973. Fine structure of nerve fibers and growth cones of isolated sympathetic neurons in culture. *J. Cell Biol.* **56**:713.
7. BURNSIDE, B. 1971. Microtubules and microfilaments in newt neurulation. *Dev. Biol.* **26**:416.
8. BYERS, B., and K. R. PORTER. 1964. Oriented microtubules in elongating cells of the developing lens rudiment after induction. *Proc. Natl. Acad. Sci. U. S. A.* **52**:1091.
9. FONTE, V. G., R. L. SEARLS, and S. R. HILFER. 1971. The relationship of cilia with cell division and differentiation. *J. Cell Biol.* **49**:226.
10. FREED, J. J., and M. M. LEBOWITZ. 1970. The association of a class of saltatory movements with microtubules in cultured cells. *J. Cell Biol.* **45**:334.
11. GAIL, M. H., and C. W. BOONE. 1971. Effect of colcemid on fibroblast motility. *Exp. Cell Res.* **65**:221.
12. GIBBINS, J. R., L. G. TILNEY, and K. R. PORTER. 1961. Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. I. The distribution of microtubules. *J. Cell Biol.* **41**:201.
13. GIBBONS, I. R., and A. V. GRIMSTONE. 1960. On the flagellar structure of certain flagellates. *J. Biophys. Biochem. Cytol.* **7**:697.
14. GIBBONS, I. R., and A. J. ROWE. 1965. Dynein: A protein with adenosine triphosphatase activity from cilia. *Science (Wash. D. C.).* **149**:424.
15. GOLDMAN, R. D. 1971. The role of three cytoplasmic fibers in BHK-21 cell motility. I. Microtubules and the effects of colchicine. *J. Cell Biol.* **51**:752.
16. GRIMSTONE, A. V., and L. R. CLEVELAND. 1965. The fine structure and function of the contractile axostyles of certain flagellates. *J. Cell Biol.* **24**:387.
17. HEPLER, P. K., and W. T. JACKSON. 1968. Microtubules and early stages of cell-plate formation in

the endosperm of *Haemanthus katherinae* Baker. *J. Cell Biol.* **38**:437.
18. HOLTZER, H. 1970. Myogenesis. *In* Cell Differentiation. O. Schjeide and J. De Vellis, Editors. Van Nostrand Reinhold, New York. 476–503.
19. HUANG, B., and D. R. PITELKA. 1973. The contractile process in the ciliate, *Stentor coeruleus*. I. The role of microtubules and filaments. *J. Cell Biol.* **57**:704.
20. KARFUNKEL, P. 1971. The role of the microtubules and microfilaments in neurulation in *Xenopus*. *Dev. Biol.* **25**:30.
21. LACY, P. E., S. L. HOWELL, D. A. YOUNG, and C. J. FINK. 1968. New hypothesis of insulin secretion. *Nature (Lond.)*. **219**:1177.
22. MALAWISTA, S. E. 1971. The melanocyte model. Colchicine-like effects of other antimitotic agents. *J. Cell Biol.* **49**:848.
23. MCINTOSH, J. R. 1968. Periodic projections from the surface of microtubules in developing chicken sperm cells. *J. Cell Biol.* **39**(2, pt. 2): 89 a (Abstr.).
24. MCINTOSH, J. R. 1973. The axostyle of *Saccinobaculus*. II. Motion of the microtubule and a structural comparison of straight and bent axostyles. *J. Cell Biol.* **56**:324.
25. MCINTOSH, J. R., E. S. OGATA, and S. C. LANDIS. 1973. The axostyle of *Saccinobaculus*. I. Structure of the organism and its microtubule bundle. *J. Cell Biol.* **56**:304.
26. MOOSEKER, M., and L. TILNEY. 1973. Isolation and reactivation of the axostyle. Evidence for a dynein-like ATPase in the axostyle. *J. Cell Biol.* **56**:13.
27. PERDUE, J. F. 1973. The distribution, ultrastructure, and chemistry of microfilaments in cultured chick embryo fibroblasts. *J. Cell Biol.* **58**:265.
28. MURPHY, D. B., and L. G. TILNEY. 1974. The role of microtubules in the movement of pigment granules in teleost melanophores. *J. Cell Biol.* **61**:757.
29. PORTER, K. R. 1966. Cytoplasmic microtubules and their function. *In* Principles of Biomolecular Organization. G. E. W. Wolstenholme and M. O'Connor, Editors. Churchill, London. 308.
30. ROBBINS, E., G. JENTZSCH, and A. MICALI. 1968. The centriole cycle in synchronized Hela cells. *J. Cell Biol.* **36**:329.
31. SATIR, P. 1968. Studies on cilia. III. Further studies on the cilium tip and a sliding filament model of ciliary motility. *J. Cell Biol.* **39**:77.
32. SLAUTTERBACK, D. B. 1963. Cytoplasmic microtubules. I. Hydra. *J. Cell Biol.* **18**:367.
33. SPEIDEL, C. C. 1937. Studies of living muscles. I. Growth, injury, and repair of striated muscle, as revealed by prolonged observations of individual fibers in living frog tadpoles. *Am. J. Anat.* **62**:179.
34. SPOONER, B. S., K. M. YAMADA, and N. K. WESSELLS. 1971. Microfilaments and cell locomotion. *J. Cell Biol.* **49**:595.
35. SUMMERS, K. E., and I. R. GIBBONS. 1971. Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. *Proc. Natl. Acad. Sci. U. S. A.* **68**:3092.
36. TAYLOR, A. C. 1966. Microtubules in the microspikes and cortical cytoplasm of isolated cells. *J. Cell Biol.* **28**:155.
37. TILNEY, L. G. 1968. Studies on microtubules in Heliozoa. IV. The effect of colchicine on the formation and maintenance of the axopodia and the redevelopment of pattern in *Actinosphaerium nucleofilum*. *J. Cell Biol.* **3**:549.
38. TILNEY, L. G., and J. R. GIBBINS. 1969. Microtubules in the formation and development of the primary mesenchymal in *Arbacia*. *J. Cell Biol.* **41**:227.
39. TILNEY, L. G., and J. GODDARD. 1970. Nucleating sites for the assembly of cytoplasmic microtubules in ectodermal cells of blastulae of *Arbacia punctulata*. *J. Cell Biol.* **46**:564.
40. TILNEY, L. G., Y. HIRAMOTO, and D. MARSLAND. 1966. Studies on microtubules in Heliozoa. II. A pressure analysis of the role of these structures in the formation and maintenance of the axopodia of *Actinosphaerium nucleofilm*. *J. Cell Biol.* **29**:77.
41. TILNEY, L. G., and K. R. PORTER. 1965. Studies on the microtubules in Heliozoa. I. Fine structure of *Actinosphaerium* with particular reference to axial rod structure. *Protoplasma*. **60**:317.
42. VASILIEV, J. M., I. M. GELFAND, L. V. DOMNINA, O. Y. IVANOVA, S. G. KOMM, and L. V. OLSHEVSKAYA. 1970. Effect of colcemid on the locomotory behavior of fibroblasts. *J. Embryol. Exp. Morphol.* **24**:625.
43. WARREN, R. H. 1968. The effect of colchicine on myogenesis in vivo in *Rana pipiens* and *Rhodnius prolixus*. *J. Cell Biol.* **39**:544.
44. WIKSWO, M. A., and R. R. NOVALES. 1969. The effect of colchicine on migration of pigment granules in the melanophores of *Fundulus heteroclitus*. *Biol. Bull. (Woods Hole)*. **137**:228.
45. WILLIAMS, J. A., and J. WOLFF. 1970. Possible role of microtubules in thyroid secretion. *Proc. Natl. Acad. Sci. U. S. A.* **67**:1901.
46. WILSON, J. H. 1969. Arms and bridges on microtubules in the mitotic apparatus. *J. Cell Biol.* **40**:854.
47. WISE, G. E. 1969. Origin of amphibian premelanosomes and their relation to microtubules. *Anat. Rec.* **165**:185.
48. YAMADA, K. M., B. S. SPOONER, and N. K. WESSELLS. 1971. Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49**:614.