FORMATION OF ARROWHEAD COMPLEXES WITH HEAVY MEROMYOSIN IN A VARIETY OF CELL TYPES

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ABSTRACT

Heavy meromyosin (HMM) forms characteristic arrowhead complexes with actin filaments *in situ*. These complexes are readily visualized in sectioned muscle. Following HMM treatment similar complexes appear in sectioned fibroblasts, chondrogenic cells, nerve cells, and several types of epithelial cells. Thin filaments freshly isolated from chondrogenic cells also bind HMM and form arrowhead structures in negatively stained preparations. HMM-filament complexes are prominent in the cortex of a variety of normal metaphase and Colcemid-arrested metaphase cells. There is no detectable binding of HMM with other cellular components such as microtubules, 100-A filaments, tonofilaments, membranes, nuclei, or collagen fibrils. The significance of HMM-filament binding is discussed in view of the finding that arrowhead complexes form in types of cells not usually thought to contain actin filaments.

INTRODUCTION

Many questions relating to the assembly of myofibrils in embryonic muscle cells are difficult to approach owing to uncertainties of identifying the several classes of filaments observed (Ishikawa et al., 1968; Holtzer, 1970). Unambiguous identification in the cell of fully and partially assembled filaments, and ultimately of their presumed monomeric subunits, is required for a better understanding of the genesis of myofibrils.

In developing muscle cells there are at least five distinct classes of filamentous structures: (*a*) the definitive *thick* or myosin filaments approximately 160 A in diameter; (*b*) the definitive *thin* or actin filaments, approximately 60 A in diameter; (*c*) the *intermediate* filaments, approximately 100 A in diameter (Ishikawa et al., 1968; Kelly, 1969); (*d*) *microtubules*, approximately 240 A in diameter; (*e*) *subsarcolemmal* filaments ranging from 60 A down to dimensions difficult to measure accurately. The work reported in this paper was originally designed to probe the possibility that the intermediate-sized 100-A filaments are in some manner related to actin.

Huxley (1963) reported that isolated actin filaments form characteristic complexes (arrowhead structures) when treated with heavy meromyosin (HMM), and that these complexes could be visualized by negative staining. By adapting this technique to sectioned preparations we hoped to learn more of the distribution of actin within the cell, particularly with respect to the other filaments present in developing myotubes.

This report demonstrates that all thin filaments in developing muscle cells bind HMM in a man-

ner indistinguishable from that found with actin filaments in adult muscle. Other organelles, including the 100-A filaments, do not bind HMM. In addition, we found that HMM-filament complexes are formed in a variety of cell types other than muscle.

MATERIALS AND METHODS

Muscle Cultures

Breast muscle tissue from 10-day chick embryos was treated with trypsin to obtain a monodisperse suspension of mononucleated cells (Holtzer et al., 1958; Bischoff and Holtzer, 1968). The cells were cultured on thin fibrin clots or directly on plastic Petri dishes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). Cultures were fed daily with Eagle's Minimal Essential Medium, horse serum, and embryo extract (8:1:1). Cultures of various ages (4–14 days) were glycerinated *in situ* for 1–3 days with cold (4°C) 50% glycerol, prepared by diluting glycerol with an equal volume of standard salt solution composed of 0.1 or 0.05 M KCl, 0.005 M $MgCl_2$, and 0.006 M phosphate buffer, pH 7.0, as modified from the formula described by Allen and Pepe (1965). Glycerination was used to permit HMM to diffuse into the cells. The glycerol solution was replaced with fresh 25% glycerol and later by 5% glycerol (glycerol: standard salt solution, 1:3 and 1:19 respectively). The cultures were kept in each solution for 4–12 hr and were then incubated with HMM for varying periods of time.[1] Immediately before use, the original HMM solution was diluted with either 0.1 or 0.05 M KCl solution. Best results were obtained by incubating the materials with HMM for 12–24 hr and by using an HMM concentration (2–4 mg/ml) about 10 times higher than that which was found to give good binding in negatively stained preparations. All procedures prior to fixation were carried out at 4°C.

Other Cells

Pure populations of chondrogenic cells, all derived from a single chondrocyte (Abbott and Holtzer, 1968), were glycerinated and treated with HMM. To inspect various in vivo tissues, small pieces of skeletal and heart muscle, skin, small intestine, trachea, spinal cord, and smooth muscle from the gizzard were dissected from 10–19 day chick embryos, glycerinated, and treated with HMM. Lastly, cells arrested in metaphase by Colcemid were collected from myogenic and chondrogenic cultures and, after glycerination, were also treated with HMM.

Preparation of Sections

Cultured cells and pieces of tissue were rinsed with 0.1 or 0.05 M KCl for 1–2 hrs, then fixed with 3% glutaraldehyde followed by 1% OsO_4 as previously described (Ishikawa, 1968). Before dehydration, the materials were stained with 0.5% uranyl acetate in acetate-Veronal buffer, pH 5.0 (Farquhar and Palade, 1965). They were then dehydrated and embedded in Epon. Sections were cut on a Sorvall Porter-Blum MT-2 microtome (Ivan Sorvall Inc., Norwalk, Conn.) and stained with a saturated solution of uranyl acetate in 50% ethanol followed by lead citrate (Reynolds, 1963).

Negative Staining

For negative staining, the cell cultures were homogenized in the standard salt solution containing 0.001 M EDTA by using a 1 ml syringe without a needle (Allen and Pepe, 1965). A drop of the homogenate was placed on an electron microscope specimen grid coated with formvar and carbon and treated with a drop of the HMM solution for 1 min. After they had been washed with 0.1 M KCl, the specimens were negatively stained with 1% uranyl acetate (Huxley, 1963).

All specimens were observed with an AEI EM6B electron microscope. Measurements of the periodicity of the arrowheads in both sectioned and negatively stained materials were made on the printed micrographs enlarged to 160,000 diameters.

RESULTS

Interaction of HMM with In Vivo Muscle Fibers

When glycerinated mature skeletal muscle fibers are treated with heavy meromyosin (HMM) several changes can be observed in sections of the myofibrils. At low magnification, the density of the I bands is considerably greater than that of glycerinated controls (Figs. 1 *a* and *b*). A–I boundaries of the sarcomeres become less distinct. There is no increase in the density of the H zones. These findings are in agreement with observations of glycerol-extracted, HMM-treated myofibrils under the phase-contrast microscope. In this case, HMM binding in the I bands was detected by the enhanced phase density (Hanson and Huxley, 1955; Szentkirályi, 1961; Aronson, 1965).

The myofibrils bound with HMM swell, particularly at the level of the I–Z bands. HMM treat-

[1] HMM was kindly supplied by Dr. F. A. Pepe, Department of Anatomy, University of Pennsylvania. The original HMM solution was prepared from chicken breast muscle myosin and stored in 25% glycerol dissolved in standard salt solution.

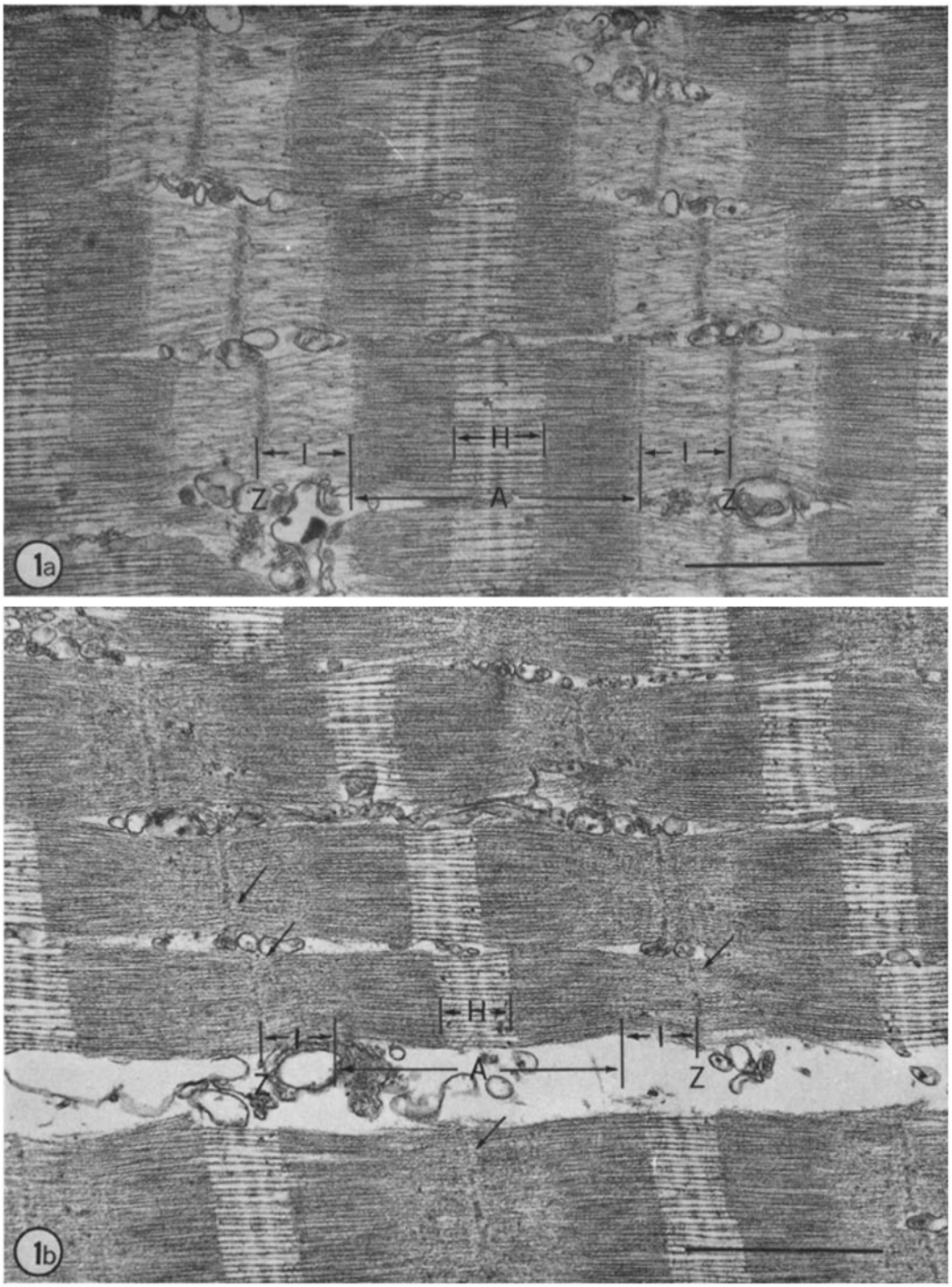

FIGURE 1 *a* and *b* Longitudinal section of glycerinated chick mature skeletal muscle fibers. *A*, A band; *I*, I band; *Z*, Z band; *H*, H zone. × 29,000. Fig. 1 *a* shows the typical pattern of the sarcomere as seen in the untreated control. In Fig. 1 *b* the HMM-treated fiber shows the increased density of the I bands of myofibrils as compared to that of the untreated control (Fig. 1 *a*). There is no increase in density in the H zones. The Z bands are less prominent than in the control and some of them almost disappear (arrows). Scale marks on electron micrographs equal 1 μ, unless otherwise indicated.

FIGURE 2 HMM-treated cardiac muscle cells. 10-day chick embryo. The thin filaments of the myofibrils complex with the HMM molecules. The density of the Z bands (*Z*) and of the interfibrillar regions of the intercalated disc (*id*) disappears (arrows). No binding of HMM can be detected in the H zones (*H*) of the sarcomeres. *A*, A band; *I*, I band. × 33,000.

ment appears to cause disruption of the regular structure of the Z bands. The Z bands become less prominent than in the controls (Figs. 1 *a* and *b*). It is not clear whether the Z band material is dispersed by the HMM or is simply obscured by HMM binding in the adjacent I bands. In some areas, however, the Z bands seem to disappear (see arrows, Fig. 1*b*).

At higher magnifications arrowheads are clearly observed along the entire length of the thin filaments. The arrowheads are present throughout the I band and often into the A band where the thick and thin filaments overlap. The arrowheads show a 367 A periodicity along the thin filaments (Table I). The arrowheads are often in register across the I band with the spacing corresponding to the arrowhead periodicity measured on individual filament complexes. The periodicity of the arrowheads is similar to the 365 ± 15 A measured by Huxley (1963) in negatively stained prepara-

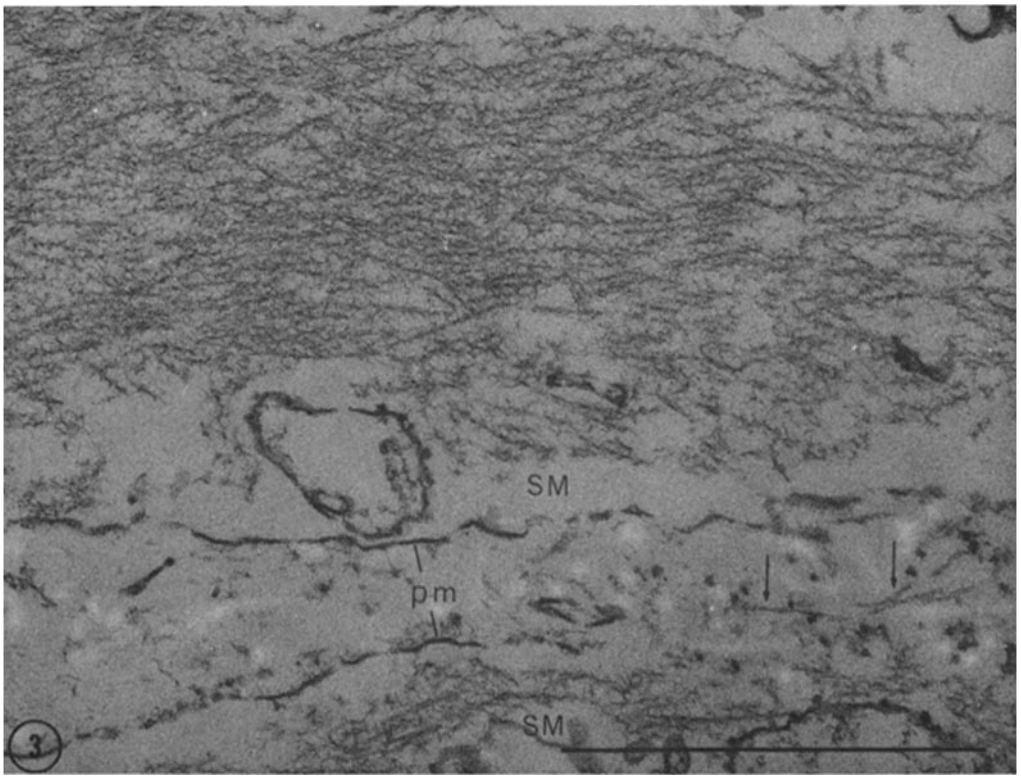

FIGURE 3 HMM-treated smooth muscle cells from the gizzard of an 18-day chick embryo. The thin filaments in the cells (*SM*) are bound with HMM. No binding of HMM can be seen with collagen fibrils (arrows) in the extracellular space. *pm*, plasma membrane of the smooth muscle cell. × 57,000.

tions. The arrowheads point away from the Z band as described for isolated I segments (Huxley, 1963).

Embryonic cardiac muscle and smooth muscle of the embryonic gizzard were also treated with HMM. Observations on cardiac muscle are consistent with those made on skeletal muscle. The thin filaments of the myofibril bind HMM to form characteristic arrowhead structures (Fig. 2). The relative density of the Z bands and intercalated discs (interfibrillar regions) is reduced after HMM treatment. There is no binding of HMM with the H zones of the sarcomeres. The thin filaments (60–75 A in diameter) of smooth muscle cells also bind HMM in a manner similar to that observed with the thin filaments of striated muscle (Fig. 3; Table I). Individual filaments are polarized with respect to arrowhead direction, but possible alternation of polarity in neighboring filaments remains to be analyzed.

Interaction of HMM with In Vitro Myotubes

When myotubes grown in vitro are glycerinated and treated with HMM, virtually all the thin filaments, including those interdigitating with myosin filaments, form arrowhead complexes (Figs. 4 and 5). Aggregates or skeins of thin filaments, believed to be nascent myofibrils, also react with the HMM. Filaments in these aggregates tend to be dispersed by the HMM when compared with the compact skeins found in untreated myotubes. All observations on sectioned myotubes are consistent with those made on mature fibers as described above. The arrowheads show a 371 A (SD = 35 A, n = 100) periodicity in sectioned preparations of glycerinated myotubes.

In cross-section, HMM-bearing filaments exhibit a fuzzy coat surrounding the core filament, and the whole thickness of these complexes ranges

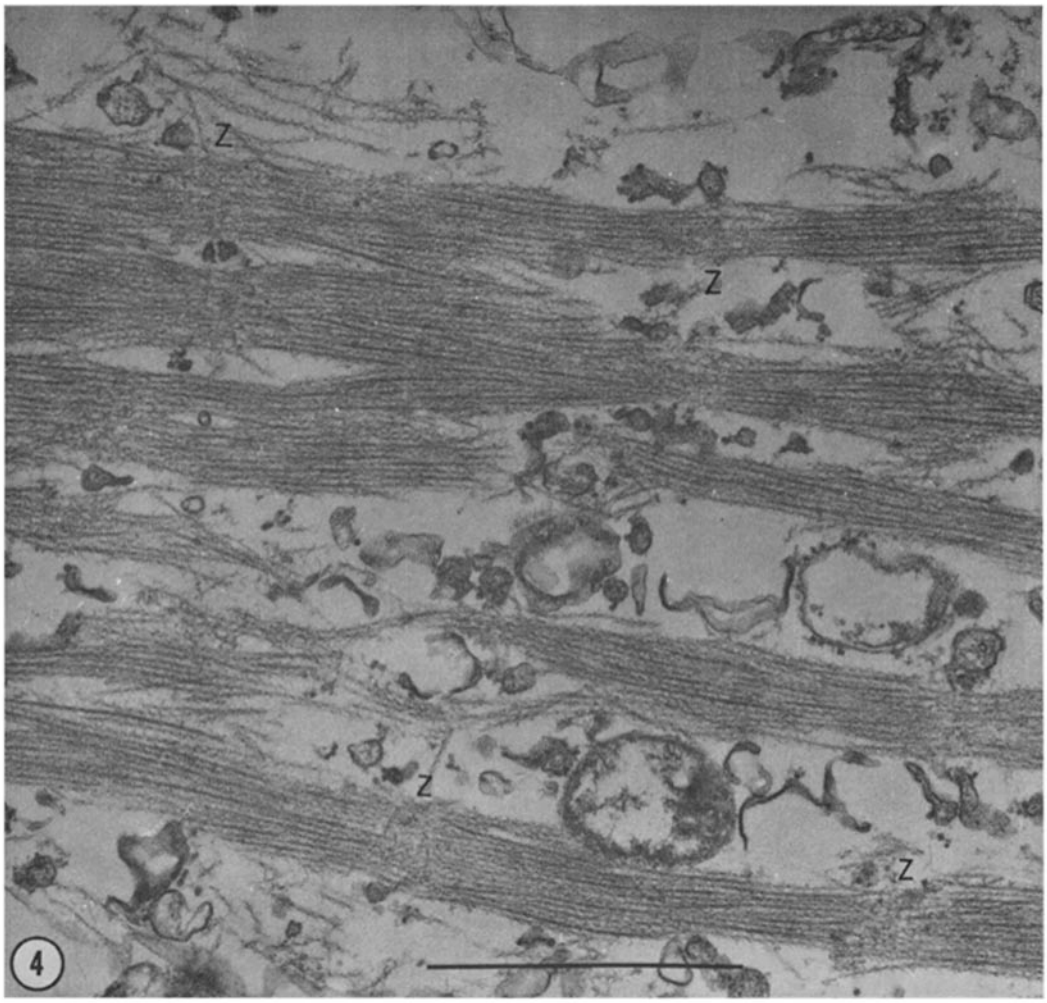

FIGURE 4 Longitudinal section of HMM-treated myotubes from a 14-day culture. All the thin filaments, including those interdigitating with myosin filaments, are seen bound with HMM and forming characteristic arrowhead complexes. Note that there is no detectable binding of HMM with the intracellular membranes. *Z*, the level of the Z bands. × 42,000.

usually from 200 to 250 A. Very often 4–6 filamentous projections can be seen extending radially from the core filaments (Fig. 6). Cross-sections through the I bands of HMM-treated myofibrils give the impression that the thin filaments are stacked in a more orderly array than they are in the controls. Bundles of the HMM-filament complexes often exhibit a highly ordered appearance. Very often adjacent filaments in these bundles appear bridged, about 240 A apart center-to-center (see Fig. 6). In longitudinal sections, as well, the parallel arrays of filaments appear to be connected by the arrowheads. Higher resolution microscopy will be required to determine whether single HMM molecules do in fact cross-link adjacent actin filaments by virtue of having two reacting sites to actin per molecule of HMM (Young, 1967).

The HMM-filament complexes are reasonably stable. They do not dissociate after repeated rinsing in 0.1 M KCl solution. They are dissociated, however, by rinsing in ATP (4 mM ATP, 2.5 mM $MgCl_2$, 6 mM phosphate buffer, pH 6.8) or in pyrophosphate (10 mM sodium pyrophosphate, 50

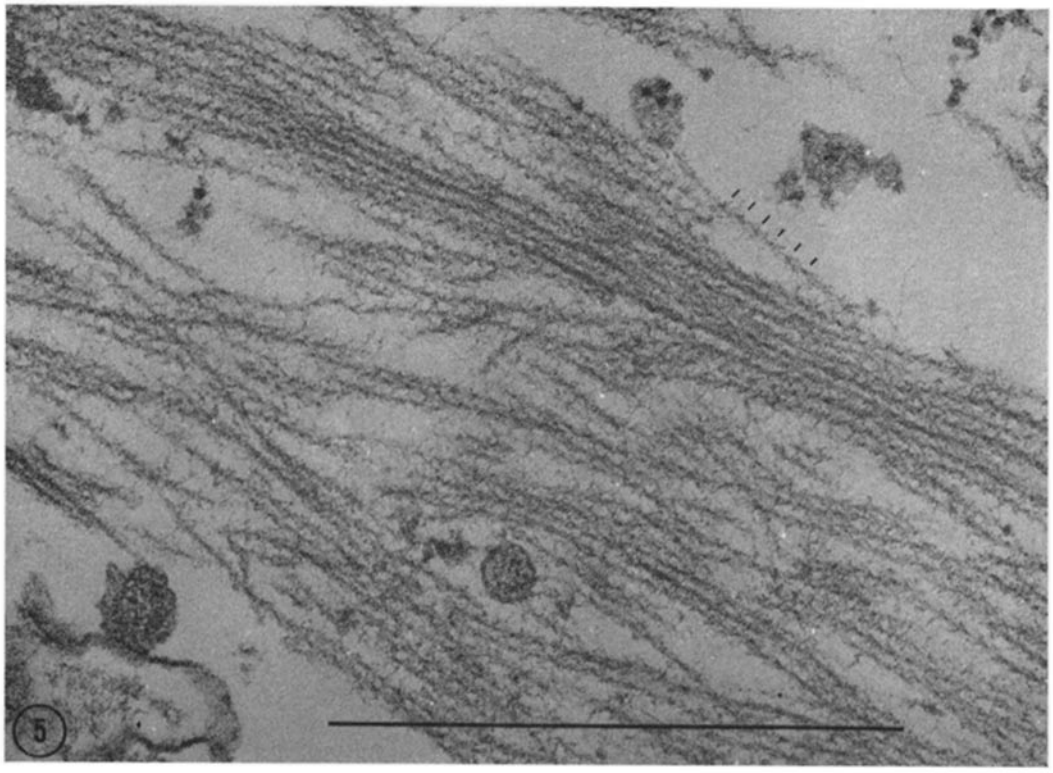

FIGURE 5 Higher magnification of the HMM-filament complexes formed in a myotube from 14-day culture. Arrowhead structures can be seen regularly spaced along the filaments (see dots). × 76,000.

mM KCl, 1 mM $MgCl_2$, 10 mM sodium phosphate buffer, pH 6.8) for 30 min.

There is no detectable binding of HMM by other cellular components in the myotubes, such as rough or smooth endoplasmic reticulum, transverse tubular system, sarcolemma, microtubules, ribosomes, nuclear membranes, or intranuclear components (Figs. 4 and 6). Collagen fibrils, regardless of thickness, do not bind HMM (see Fig. 3). Intermediate-sized, 100 A filaments (Ishikawa et al., 1968) are not well preserved in the HMM-treated myotubes. This is dramatically demonstrated in Colcemid-induced myosacs (Bischoff and Holtzer, 1968) in which massive aggregates of the 100-A filaments normally are found. Following the standard treatment with glycerol and HMM, the myosacs no longer exhibit 100-A filaments. If, however, after glycerination the myotubes are incubated with HMM for shorter periods of time (5–8 hr), then scattered 100-A filaments are preserved in the myotubes. These 100-A filaments do not bind HMM, although the thin myofilaments in the same cell do bind HMM.

These observations on HMM-treated myotubes in sectioned material have been confirmed by negative staining. Homogenates of muscle cultures of various ages were mounted on grids and treated with HMM. The thin filaments of 60–70 A diameter form the characteristic arrowhead structures (Fig. 7, inset). There is no detectable binding of HMM with any other component, such as myosin filaments, 100-A filaments, membrane fragments, ribosomes, and collagen fibrils (Fig. 7).

Interaction of HMM with Filaments in Nonmuscle Cells

Many mononucleated cells, especially in older cultures, are fibroblasts. Often, though not always, fibroblasts can be distinguished from presumptive myoblasts by the well-developed, rough-surfaced endoplasmic reticulum. After HMM treatment the arrowhead complexes are present both in

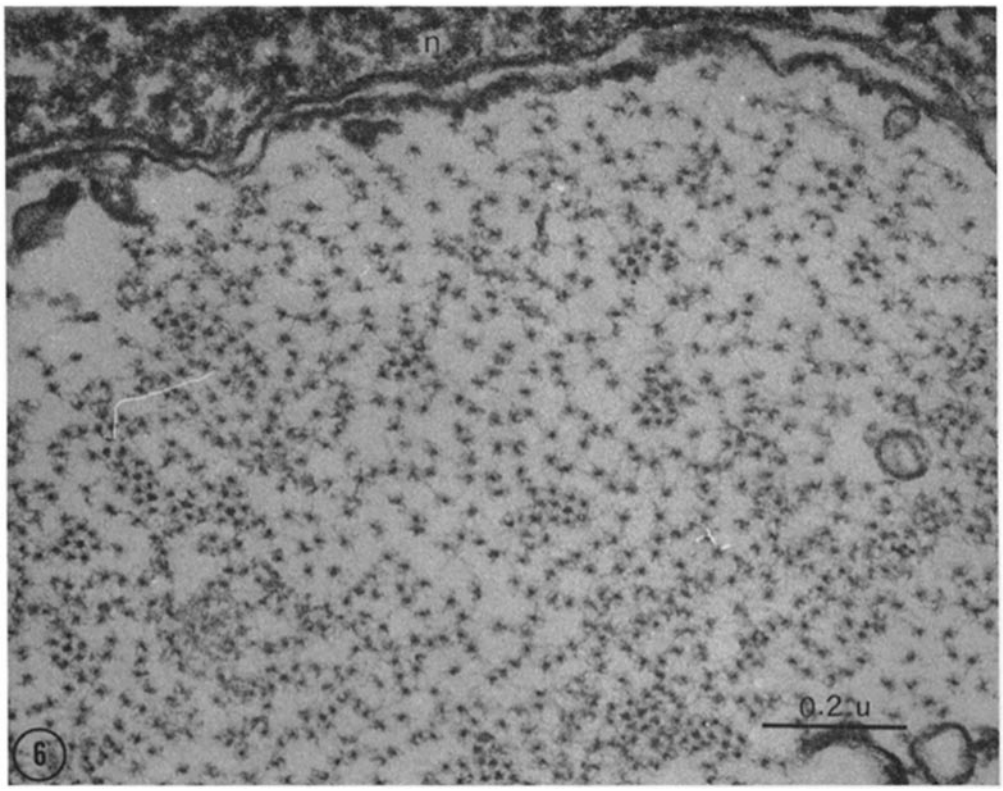

FIGURE 6 Cross-section of HMM-treated myotube from a 7-day culture. Filaments binding HMM show fuzzy coats surrounding the core filaments. Very often four to six filamentous projections can be seen extending radially from the core filaments. *n*, nucleus. × 92,000.

mononucleated presumptive myoblasts and in cells identified as fibroblasts (Fig. 8 *a*). HMM-filament complexes in these cells are also polarized, as judged by the direction of arrowheads (Fig. 8 *b*). Thus, virtually all the mononucleated cells in the muscle cultures, including cells in mitosis, exhibit HMM-reactive filaments.

To eliminate the possibility that fibroblasts in muscle cultures are unique with respect to forming HMM-filament complexes, cartilage cells, all derived from a single chondrocyte, were treated with HMM. Many chondrogenic cells grown in vitro dedifferentiate and transform into ameboid, flattened fibroblastic cells that cease to deposit chondroitin sulfate (Holtzer and Abbott, 1969). Under the phase microscope these cells exhibit phase-dense fibrils, particularly in the cortical regions and pseudopodial processes. These fibrils have been described in detail by Buckley and Porter (1967) and have been referred to as "stress fibers." Under the electron microscope the fibers have been resolved into aggregates of 50–75-A diameter filaments (Taylor, 1966; Buckley and Porter, 1967; Ishikawa et al., 1968). The interaction of these thin filaments with HMM is indistinguishable from the interaction of actin filaments and HMM in myofibrils (Fig. 9). The HMM-filament complexes in chondrogenic cells are polarized along the length of any given filament. The arrowheads have an average periodicity of 378 A (SD = 31 A, n = 180). There is no binding of HMM by the 100-A filaments which are also present in these cells in close proximity in some cases to HMM-binding, thin filaments (Ishikawa, et al., 1968). Since a detailed analysis of the filaments in these cells will be reported elsewhere, suffice it to say that cultured chondrogenic cells contain a minimum of two types of filaments that

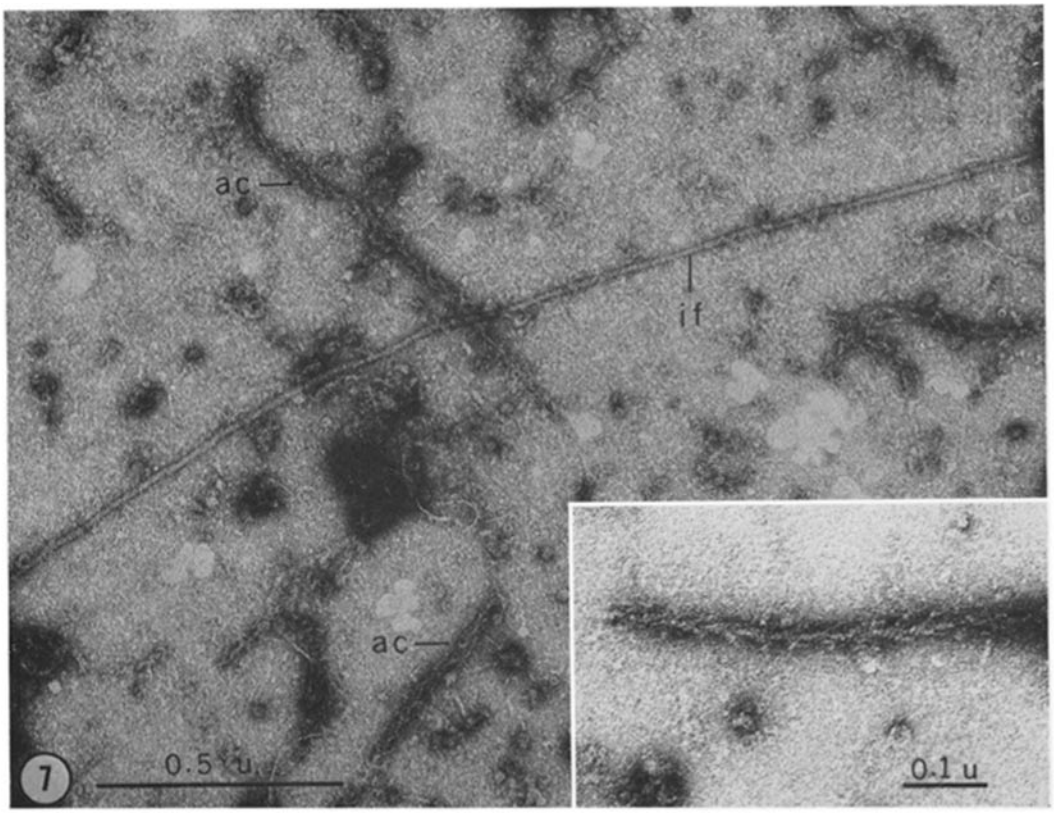

FIGURE 7 Negatively stained preparation of the HMM-treated homogenate of a 7-day muscle culture. Thin filaments (*ac*) are bound with HMM and show the characteristic arrowhead structures. Note that there is no binding of HMM with a filament of 100 A diameter (*if*). × 65,000. The inset represents a higher magnification of the arrowhead structures formed along the thin filament. × 110,000.

differ with respect to size and reactivity with HMM (see Fig. 9). As in the case of HMM-actin complexes, the HMM-filament complexes in chondrogenic cells also dissociate when rinsed with ATP or pyrophosphate solution for 30 min as described above.

The observations made on cultured chondrogenic cells have also been confirmed with negatively stained preparations. Negatively stained thin filaments isolated from chondrogenic cells display a beaded appearance (Fig. 10 *a*) and have a diameter of 60–70 A, similar to that of actin filaments (Hanson and Lowy, 1963; Huxley, 1963). These filaments complex with HMM to form arrowhead structures indistinguishable from those obtained from muscle homogenates (Fig. 10 *b*). Clearly, HMM-reactive filaments are not restricted to muscle cells. Again, in these preparations the 100-A filaments from chondrogenic cells do not bind HMM.

Prompted by these findings, we treated several different types of cells with HMM. Of particular interest was the possibility that ameboid mesenchymal cells might exhibit the HMM-filament complexes, whereas nonmotile epithelial cells might not. Accordingly, various epithelial cells from chick embryos, such as epidermal cells and intestinal and tracheal epithelial cells, were glycerinated and treated with HMM. HMM-filament complexes appeared in epidermal cells (Fig. 11) and in the epithelial cells of the small intestine and trachea (Fig. 12). Filaments associated with desmosomes of the epidermal cells do not complex with the HMM (Fig. 11). The thin filaments associated with microvilli in intestinal and tracheal cells form characteristic arrowhead structures

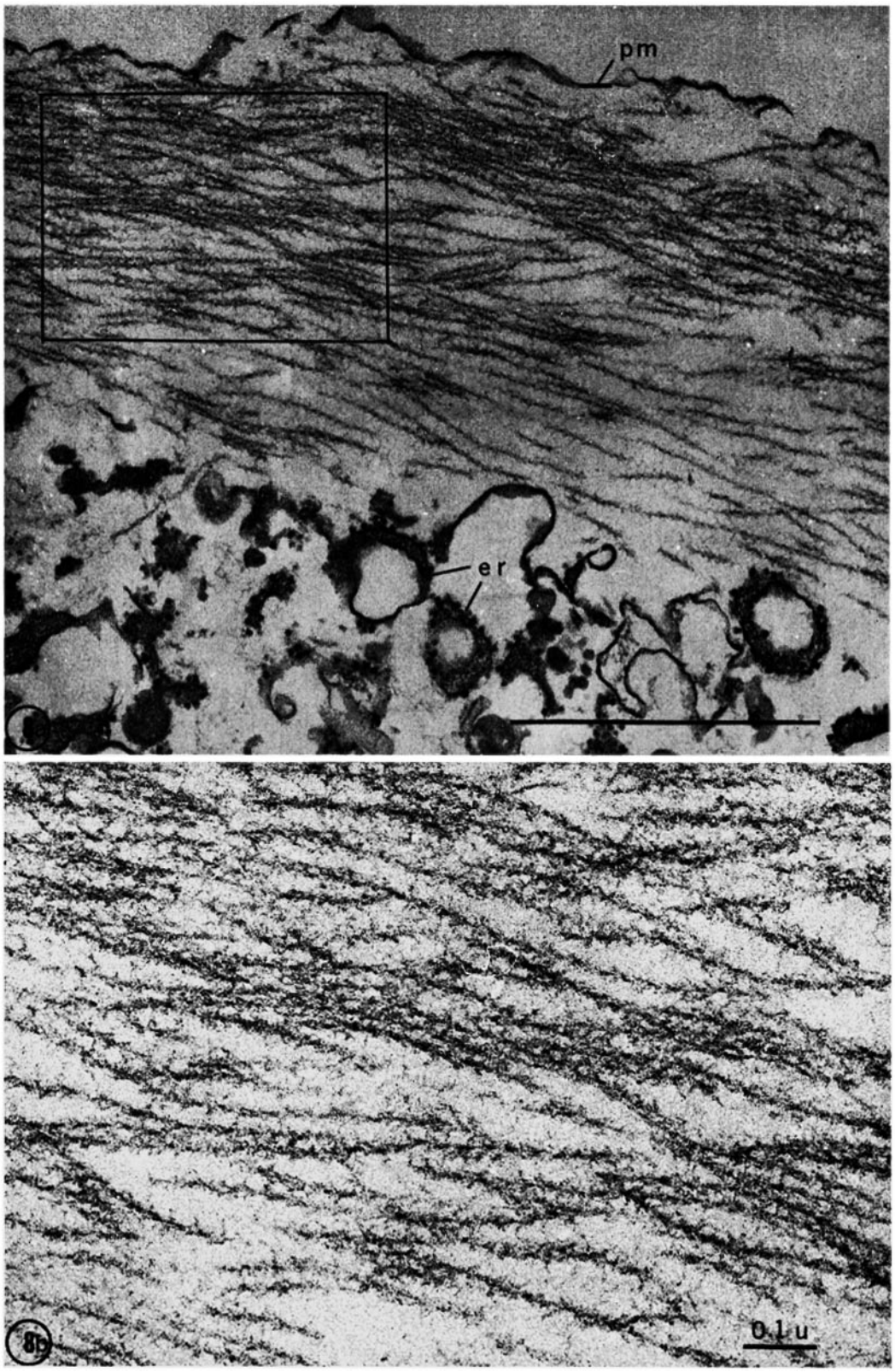

FIGURE 8 *a* and *b* HMM-treated mononucleated cell from a 12-day muscle culture. This cell is rich in rough surfaced endoplasmic reticulum and is probably a fibroblast. In Fig. 8 *a* all the thin filaments are seen forming the arrowhead structures. No interaction can be seen between HMM and other cellular components such as endoplasmic reticulum (*er*), and plasma membrane (*pm*). × 48,000. Fig. 8 *b* is an enlargement of the upper left portion of Fig. 8 *a*, as indicated by a square. The arrowhead structures along the thin filaments are indistinguishable from those formed in muscle cells. × 116,000.

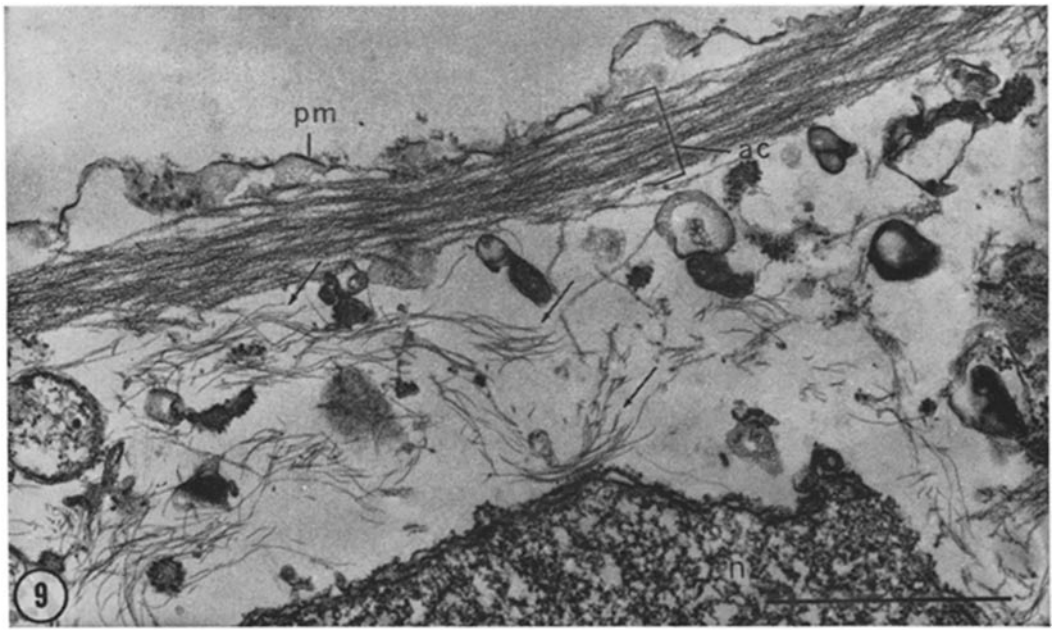

FIGURE 9 HMM-treated chondrogenic cell grown in vitro. The thin filaments (*ac*), which are prominent in the cortical regions and in the cell processes, are complexed with HMM. There is no binding of HMM by 100-A filaments in the same cell (arrows). *pm*, plasma membrane; *n*, nucleus. × 33,000.

(Figs. 12 and 13), showing a 346 A periodicity. These "core" filaments of the microvilli are polarized, as judged by the direction of the arrowheads; virtually all point away from the lumen and toward the interior of the cell (Figs. 12 and 13). In these cells there are many nonreacting filaments, 80–100 A in diameter, closely adjacent to the HMM-filament complexes. These filaments that do not react with HMM have the typical size and location of tonofilaments.

Nerve cells from the spinal cord of chick embryos were also glycerinated and treated with HMM. Arrowhead-bearing, polarized filaments, in addition to microtubules and 100-A filaments, appear in such treated nerve and glial cells. Owing, however, to the disruption of the fine cytology incidental to the glycerination, it is often difficult to determine the spatial relationships of the HMM complexes, the microtubules, and the 100-A filaments, all of which are present in the fixed and sectioned cells.

Cells in metaphase in myogenic cultures are of the following two types: fibroblasts and presumptive myoblasts. It is not yet possible to distinguish by electron microscopy between these two classes of cells during metaphase. Likewise, metaphase cells in chondrogenic cultures are of two types: functional chondrocytes depositing chondroitin sulfate, and dedifferentiated chondrocytes that do not deposit the mucopolysaccharide (Abbott and Holtzer, 1968). Nor can these two types, or states, of chondrogenic cells, as yet, be identified by electron microscopy during metaphase. When metaphase cells from myogenic or chondrogenic cultures are treated with HMM, filaments showing characteristic arrowhead structures appear just under the plasma membrane (Fig. 14). With ordinary fixation, the filamentous structures of the cortex are not evident. After extraction with glycerol and treatment with HMM, the cortex of virtually all the metaphase cells is characterized by prominent bundles of HMM-filament complexes parallel to the plasma membrane. Spindle tubules in the normal metaphase cells do not bind HMM.

Measurements of arrowhead periodicity in various types of cells are summarized in Table I.

DISCUSSION

Mild tryptic digestion cleaves myosin into two fragments: light (LMM) and heavy (HMM) mer-

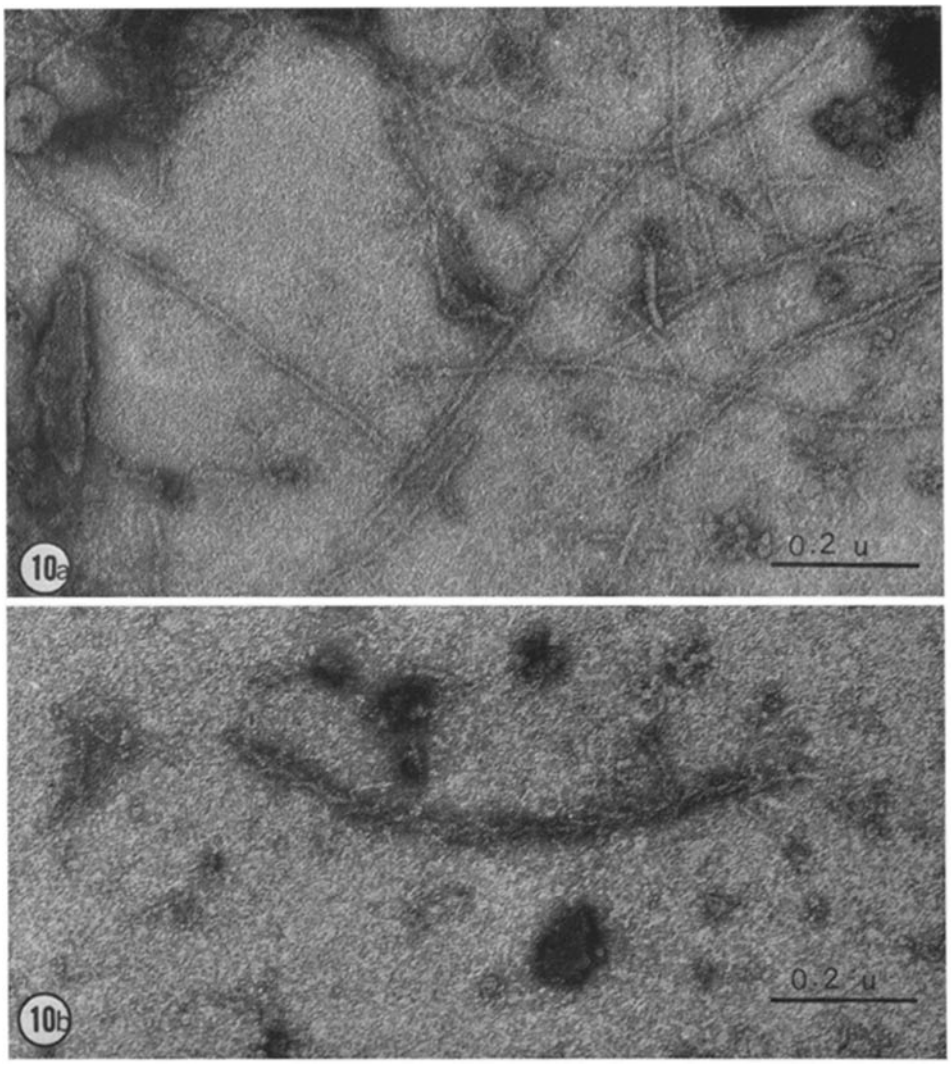

FIGURE 10 *a* and *b* Negatively stained preparations of the homogenate of a pure chondrogenic cell culture. × 99,000. In Fig. 10 *a*, the control preparation, the thin filaments isolated from chondrogenic cells show characteristic beaded appearance remniscent of actin filaments. When treated with HMM, as seen in Fig. 10 *b*, freshly isolated thin filaments are complexed with HMM to form the arrowhead structure similar to that obtained from muscle homogenates.

omyosin (Szent-Györgyi, 1953). The adenosine triphosphatase activity and the property of combining with actin are associated with the HMM fragment (see Perry, 1967). Huxley (1963) presented direct electron microscopic evidence for the interaction of HMM with thin filaments isolated from glycerol-extracted muscles. Negative staining of thin filaments following treatment with HMM yielded characteristic arrowhead complexes. This arrowhead complex was considered to be a highly specific and unique cytological method for identifying polymerized actin. As controls for the reaction are difficult to design, however, the specificity of the binding pattern has not as yet been unequivocally demonstrated. Whether HMM complexes with proteins other than actin (see Aronson, 1965), or if actin interacts with molecules other than HMM to form arrowhead complexes are still open issues.

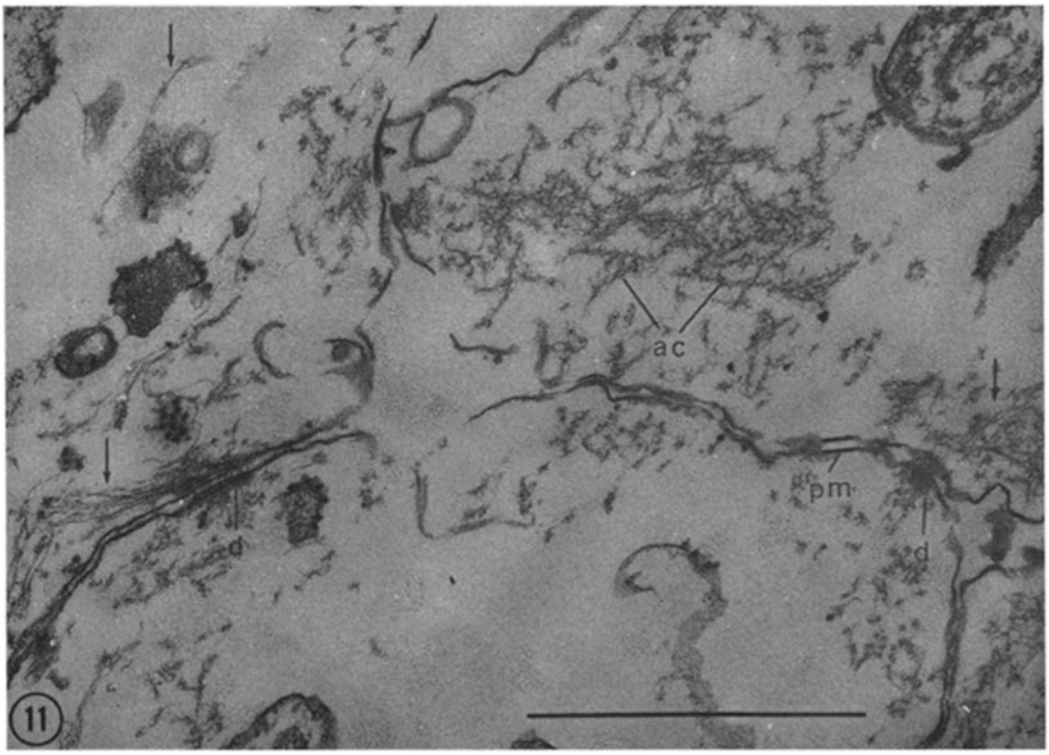

FIGURE 11 HMM-treated epidermal cells from a 10-day chick embryo. The thin filaments (*ac*) in the epidermal cells also bind HMM to form arrowhead complexes. However, the filaments associated with desmosomes (*d*) do not complex with HMM (arrows). *pm*, plasma membrane. × 45,000.

The experiments reported in this paper demonstrate the feasibility of using sectioned material to produce the typical arrowhead complexes formed by the interaction of definitive actin filaments with HMM. These procedures further demonstrate that HMM does not in fact interact indiscriminately with many other extra- or intracellular components. In a variety of glycerinated tissues no interaction could be detected between HMM and collagen or elastic fibrils, membranes of the endoplasmic reticulum, mitochondria, nuclei, tonofilaments, desmosomes, 100-A filaments, or microtubules. Clearly, the HMM-binding by thin filaments in myofibrils is not due to nonspecific protein–protein interactions. In this sense, HMM can be used as a molecular tracer for the identification of "thin" filaments. The formation of arrowhead complexes in a number of cell types, in addition to muscle, poses the question of whether the arrowhead complex is indeed a specific test for preexisting actin filaments, or at least for preexisting actin-like filaments.

There is a rapidly growing literature stressing the possibility that a number of labile intracellular filamentous structures such as microtubules, 100-A filaments, and actin filaments are related on the basis of amino acid composition, molecular weight, and nucleotide binding. According to this view these different structures consist of differing arrays of the same, or at least closely related, protein monomers (Gibbons, 1963; Sakai, 1966; Stephens, 1967; Inoué and Sato, 1967; Shelanski and Taylor, 1967; Renaud et al., 1968; Adelman et al., 1968; Schmitt, 1968). Recently Mazia and Ruby (1968) have included in this category two additional proteins, one isolated from mitochondria and one from erythrocyte membranes. These investigators have proposed that this class of structural proteins be termed "tektins." Antibody studies, however, suggest that actin is immunologically distinct from other filamentous structures. Fluorescein-labeled antibodies prepared against actin and/or tropomyosin were not bound in the areas capable of forming arrowhead complexes excepting the I

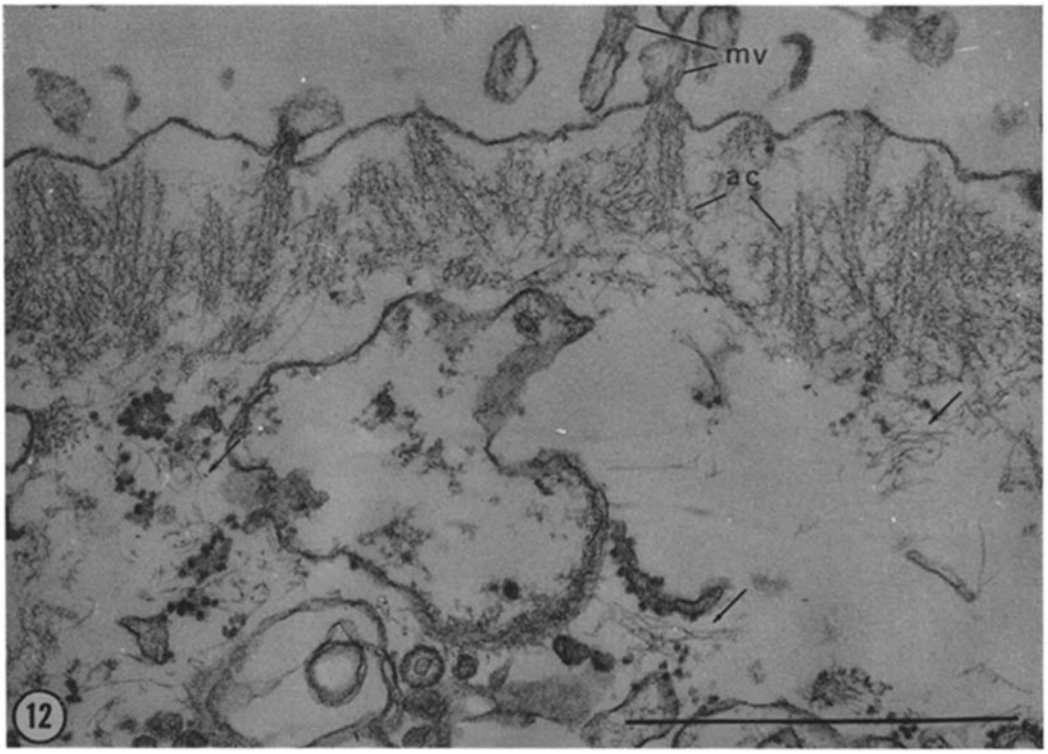

FIGURE 12 HMM-treated intestinal epithelial cells from a 15-day chick embryo. The thin filaments (*ac*) associated with microvilli (*mv*) form the arrowhead structures, whereas the tonofilaments of 100 A in average diameter can not bind HMM (arrows). × 52,000.

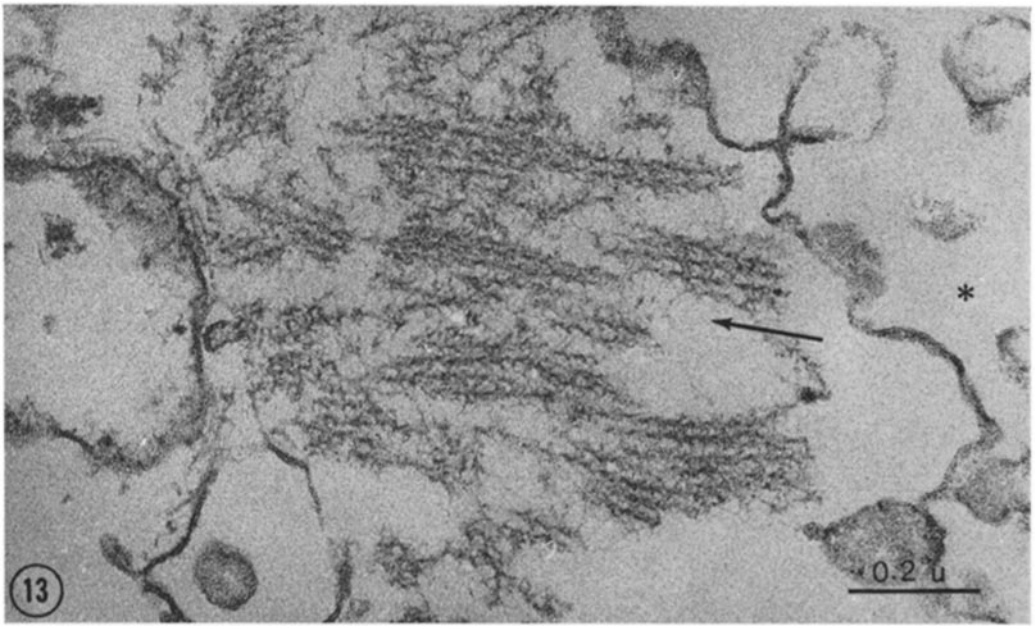

FIGURE 13 Higher magnification of the HMM-filament complexes formed in an intestinal epithelial cell from a 15-day chick embryo. The thin filaments of the microvilli are uniformly polarized as judged by the direction of the arrowheads, virtually all pointing away from the lumen (*) toward the interior of the cell (see direction of arrow). × 87,000.

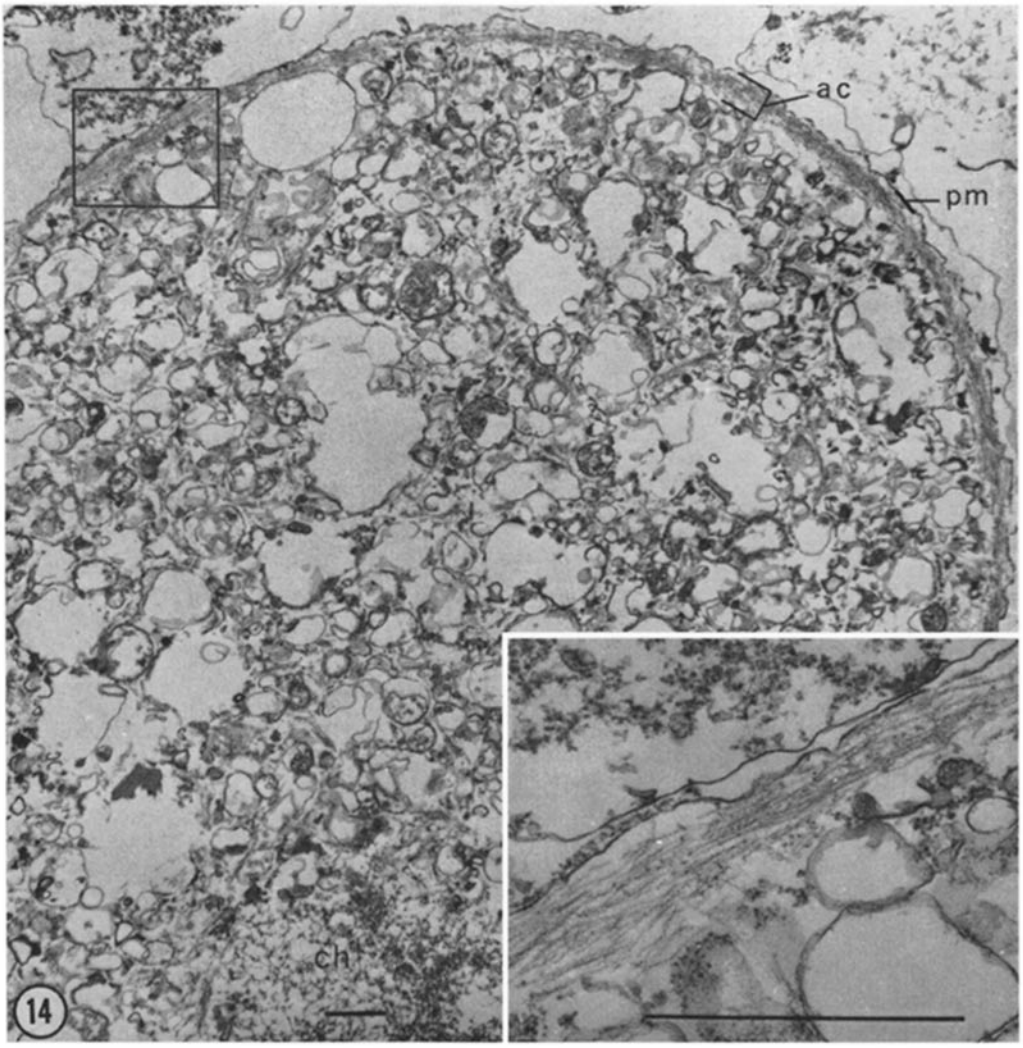

FIGURE 14 HMM-treated, metaphase-arrested cell from an early muscle culture exposed to 10^{-6} M Colcemid for 24 hr. When treated with HMM after glycerination, the cortex of the metaphase cell is characterized by prominent bundles of HMM-filament complexes (*ac*) parallel to the plasma membrane (*pm*). *ch*, chromatin mass. × 8,000. The inset shows an enlargement of the upper left portion of the figure as indicated by square. × 42,000.

TABLE I

Periodicity of Arrowheads

Cell type	Sectioned preparation				Homogenized with negative staining			
	M	±	SD*	(n‡)	M	±	SD	(n)
	A		*A*		*A*		*A*	
Adult skeletal muscle fiber	367	±	45	(214)				
Cultured skeletal muscle fiber	371	±	35	(100)	370	±	24	(70)
Embryonic smooth muscle cell	356	±	36	(150)				
Cultured chondrogenic cell	378	±	31	(180)	364	±	75	(50)
Embryonic intestinal epithelial cell	346	±	32	(100)				

* M ± SD = mean periodicity ± standard deviation

‡ n = number of arrowhead spaces measured

bands of myofibrils. Antiactin does not bind to the mitotic apparatus (see Holtzer, 1961; 1970 for discussion of the status of antiactin and antitropomyosin sera). Marchesi and Steers (1968) have reported that a protein from the cortex of red blood cells could be polymerized into filaments very similar in appearance to actin, but antibodies to this protein did not cross-react with actin. The observations in this report indicate that neither intact microtubules nor intact 100-A filaments interact with HMM, as do intact actin filaments in myofibrils. If actin filaments, microtubules, and 100 A-filaments do in fact consist of identical monomers packed in respectively different arrays, then failure of the latter two structures to complex with HMM must be attributed to conformational changes or steric effects.

If the formation of arrowheads following treatment with HMM is indeed a test for actin or actin-like proteins, then such a protein(s) is a constituent of a wide variety of cells. Actomyosin-like systems have been invoked many times to account for the contractile properties of different kinds of cells. Isolated cytoplasm of *Amoeba proteus* gelated with ATP has been found to contain 80–120-A filaments (Wolpert et al., 1964; Morgan et al., 1967). Fibrous aggregates of thin filaments 50–70 A in diameter are present in the cytoplasm of *Physarum* (Wolfarth-Bottermann, 1964; Rhea, 1966). Recently Hatano and Oosawa (1966 *a*, *b*) have isolated an actin-like protein, "plasmodium actin," from *Physarum*. The plasmodium actin combines with muscle myosin to form an actomyosin complex as muscle actin does. Clearly, actin-like proteins are not restricted to muscle cells. In most of the cells examined here, the HMM-bearing filaments are prominent in the cortical regions, a site well suited for their possible involvement in motile processes.

Whether the formation of the arrowhead complex indicates actin or only an actin-like protein has considerable bearing on the genetic controls regulating the synthesis of the contractile proteins of muscle. If the synthesis of the protein filament capable of binding HMM in nonmuscle cells is in fact controlled by the same gene that regulates actin synthesis in muscle cells, then actin could not be classified as a "luxury" molecule (Holtzer, 1967; Holtzer and Abbott, 1969); furthermore, its association with myosin in muscle would be facultative rather than obligatory. In this connection it will be interesting to assay the ability of 5-bromodeoxyuridine-suppressed myogenic cells, which do not form myofibrils (Okazaki and Holtzer, 1965; Bischoff and Holtzer, 1969; 1970), to form arrowhead complexes.

Further discussion of whether the arrowhead complexes formed in cells other than muscle are due to actin or only to actin-like molecules must await further detailed characterization of the involved filaments.

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