A FILAMENTOUS CYTOSKELETON IN VERTEBRATE SMOOTH MUSCLE FIBERS

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ABSTRACT

There are three classes of myofilaments in vertebrate smooth muscle fibers. The thin filaments correspond to actin and the thick filaments are identified with myosin. The third class of myofilaments (100 Å diam) is distinguished from both the actin and the myosin on the basis of fine structure, solubility, and pattern of localization in the muscle fibers. Direct structural evidence is presented to show that the 100-Å filaments constitute an integrated filamentous network with the dense bodies in the sarcoplasm, and that they are not connected to either the actin or myosin filaments. Examination of (*a*) isolated dense bodies, (*b*) series of consecutive sections through the dense bodies, and (*c*) redistributed dense bodies in stretched muscle fibers supports this conclusion. It follows that the 100-Å filament complexes constitute a structurally distinct filamentous network. Analysis of polyacrylamide gels after electrophoresis of cell fractions that are enriched with respect to the 100-Å filaments shows the presence of a new muscle protein with a molecular weight of 55,000. This protein can form filamentous segments that closely resemble in structure the native, isolated 100-Å filaments. The results indicate that the filamentous network has a structure and composition that distinguish it from the actin and myosin in vertebrate smooth muscle.

Despite the uniform optical density of the sarcoplasm in smooth muscle fibers, some classical histologists were able to demonstrate longitudinally oriented striations embedded in a homogeneous background material (36, 51). These striations were believed to reflect the presence of underlying fibrils in the sarcoplasm. This view was disputed (31), but later was proved in a study in which polarized light was used to show the fibrils in living muscle fibers (32). The structure of the fibrils was not further characterized until the advent of electron microscopy. Analysis then revealed that the sarcoplasm contained myofilaments described as ranging from 100 to 300 Å in diameter (1, 20, 35, 56). When methods of preparation affording high resolution were developed, the myofilaments were found to constitute a single class with a diameter of 60–80 Å that corresponds to the contractile protein F-actin (24, 48). There was no evidence that myosin was present in filamentous form (13, 40), although this protein could be isolated and induced to form filaments in vitro (25, 29, 48). Further work established that filaments resembling the myosin-containing filaments in striated muscles could be found in experimentally treated smooth muscle (5, 30, 39) and, more recently, when smooth muscles were fixed under "physiological" conditions, numerous thick myofilaments and

X-ray reflections characteristic of myosin filaments were found (8, 12, 21, 34, 44, 50).

In a number of studies over the last few decades, incidental mention has been made of another type of myofilament in smooth muscle fibers—the 100-Å filament (4, 16, 45). On the basis of their solubility properties and their unique pattern of distribution, the 100-Å filaments can be distinguished from the actin- and myosin-containing filaments (6, 54). The purpose of this report is to present further evidence that the 100-Å filaments have characteristics and properties that clearly distinguish them from actin and myosin filaments, and they form complex filamentous networks in the muscle fibers that are structurally independent of the actomyosin system.

MATERIALS AND METHODS

Muscles

Strips of *Taenia coli* dissected from the caeca of guinea pigs were used when it was necessary to have uniformly oriented muscle fibers in a physiologically or mechanically defined state for making observations on the distribution and organization of the myofilaments. Excised lateral muscles from chicken gizzards were used for the isolation and characterization of the myofilaments. Previous reports describe the specific conditions for the handling of *Taenia coli* (7, 8, 17) and gizzard smooth muscle (6).

High Voltage Eelectron Microscopy

Series of consecutive sections of *Taenia coli* muscle strips around 0.25 μm thick were stained with 2% uranyl acetate at 60°C and examined with a high voltage (1 MEV) electron microscope. Stereo-paired micrographs of series of consecutive sections were taken of each pertinent area at angles ±8–12° from the plane that is normal to the electron-optical axis at magnifications of ca. 10,000.

Isolation and Analysis of 100-Å Filaments

Coarsely minced, fresh lateral muscles from chicken gizzards were washed several times in 20 vol of cold (4°C) 50 mM sodium phosphate buffer (pH 7.5) with 1 mM hydroxyethyl mercaptan (SP buffer). The muscle fragments were washed again in several changes of distilled water and homogenized in a final mixture of 1 vol of muscle mince to 3 vol of distilled water in a Waring blender for 1–3 min. The resulting slurry was extracted to obtain actomyosin in 3 vol of 0.6 M KCl-0.05 M Tris(hydroxymethyl)-aminomethane (pH 8.6) for 48–72 h. A fresh extract was prepared every 24 h. The residue after each extraction step was recovered by centrifugation at 1,000 or 9,000 *g* and resuspended in fresh solution. The final "salt-insoluble" residue contained numerous 100-Å filaments. It was dispersed in 6–8 M urea in SP buffer and extracted for 12–24 h. The residue from this extraction does not contain 100-Å filaments. The extract was decanted and centrifuged at 100,000 *g* to remove suspended particles. The clear supernatant fluid was dialyzed against 20–40 vol of 10 mM SP buffer, and the flocculent precipitate consisting of filamentous segments which formed was collected by low-speed centrifugation. The remaining protein in solution was recovered by the addition of ammonium sulfate to 20% saturation at pH 7.5, or by the addition of cold ethanol to the solution of protein according to the procedure of Davison and Winslow (10).

Fractions of the muscle at each step in the isolation procedure were prepared for electron microscopy by conventional methods of embedding and negative staining, and for gel electrophoresis according to the method of Etlinger and Fischman (15). The relative molecular masses of the protein components were determined with a series of molecular weight standards as described by Weber and Osborne (55). The relative amounts of protein in the various bands were determined by densitometry, using the technique described by Lowy and Risby (33), with a Gilford spectrophotometer and linear transport accessory (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) at a wavelength of 5,400 Å and a slit width of 0.03 mm.

RESULTS

Filament Types

Recent ultranstructural studies have revealed the presence of three distinct classes of myofilaments (Fig. 1) in vertebrate smooth muscle fibers (6–8, 17, 43, 50, 52). The classes are defined on the basis of their diameters, fine structure, solubility, and pattern of localization in cross-sectional profiles of the fibers. The most abundant filaments are those around 75 Å in diameter. They are packed into bundles of 10–100 filaments showing a more or less regular center to center interfilament spacing that ranges from 75 to 150 Å. These filaments are homogeneously distributed throughout the sarcoplasm, and are identified with the actin-containing filaments of other muscle types. The class of filaments with the largest diameter (170 Å) is identified with the contractile protein myosin. These filaments can be consistently found in the fibers but their abosulte abundance and distribution are highly variable, owing to what is presumed to be an extraordinary lability of the aggregated form of this protein toward the methods of preparation for electron microscopy. The third class of filaments has a circular cross-sectional profile

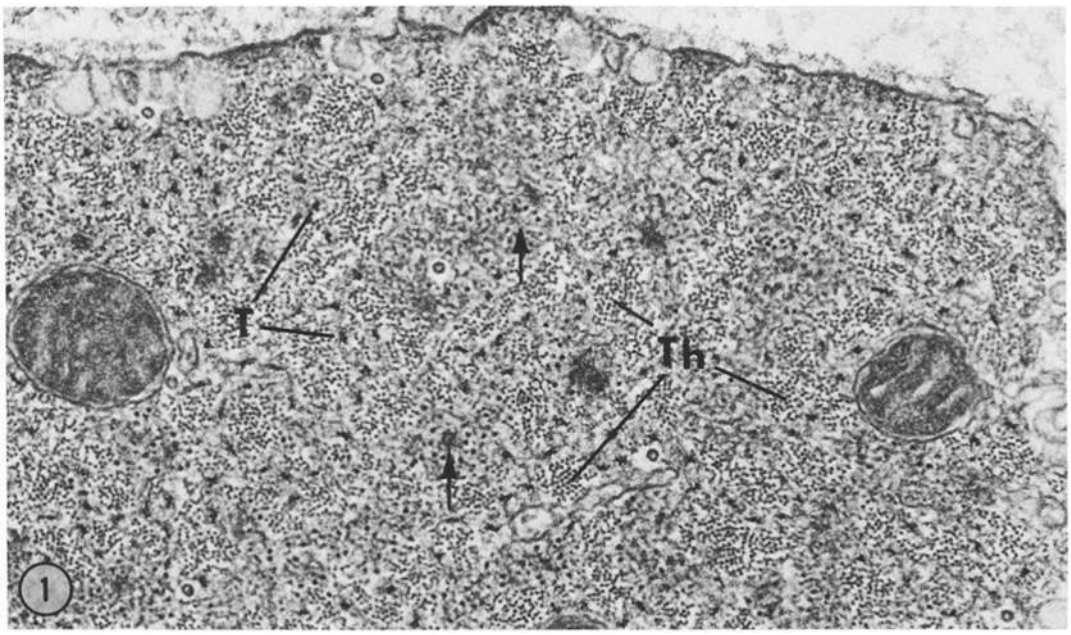


FIGURE 1 Portion of a cross section of a *Taenia coli* smooth muscle fiber showing the abundance, distribution, and diameter of the three types of myofilaments. The thin filaments (*Th*) are widely distributed and grouped into bundles. The thick filaments (*T*) are numerous and uniformly distributed. The 100-Å filaments are most clearly seen around the dense bodies (arrows) in the sarcoplasm. × 65,000.

around 100 Å in diameter. They are localized principally around the dense areas that are scattered in the sarcoplasm and subtend patches of the plasma membrane. The overall organization of these filaments in the muscle fibers has not been described in detail, and they have not been identified with any known muscle protein. Subsequent sections of this report will therefore describe the results of study designed to determine some of the properties and characteristics of this class of myofilaments.

The Filamentous Network

The 100-Å filaments have not been widely recognized as comprising a distinct system of myofilaments even though the ultrastructure of smooth muscle has been investigated in detail for over 20 yr. Failure to recognize this classs of filaments and their organization has probably been due to several factors. It is difficult to see the relatively small number of 100-Å filaments scattered among the extraordinarily large number of actin filaments which are characteristically found in vertebrate smooth muscle. The myosin filaments are labile, and in their absence there has been a misleading tendency to mistake 100-Å filaments for residual myosin filaments (4, 16). An effective way of enhancing the demonstration of the 100-Å filaments in smooth muscle is to decrease selectively the amount of actin- and myosin-containing filaments and at the same time to preserve the organization of the 100-Å filaments. This can be done by placing the tissue in solutions which cause the myosin and actin filaments to disaggregate or become labile during preparation for electron microscopy. Incubation of smooth muscle strips in physiological saline solution in which the Ca and Mg concentrations have been reduced to 1.6×10^{-8} and 1.6×10^{-6} M, respectively, by the addition of EDTA produces fibers in which the actin and myosin filaments are not detectable (17). This treatment allows clear demonstration of the 100-Å filaments and reveals that numerous connections are made between the dense bodies in the sarcoplasm and the bundles of 100-Å filaments (Fig. 2). The direct structural connections between the 100-Å filaments and dense areas suggests that these structures constitute a three-dimensional filamentous network within each muscle fiber. A test for this proposal can be made by altering the length of the muscle fibers and demonstrating how the networks are

FIGURE 2 Portion of a longitudinal section of a *Taenia coli* smooth muscle fiber treated with EDTA to remove both the thick and thin myofilaments and to reveal the 100-Å filaments connecting the dense bodies in the sarcoplasm and at the plasma membrane (arrows). × 26,000.

deformed. Earlier work (7) indicated that the distribution of 100-Å filaments and associated dense bodies was markedly changed when the muscle fibers were stretched to lengths at which the active tension developed by the entire muscle strip before fixation was equal to or less than the resting tension. Subsequently, further sampling of similar (stretched) preparations has shown what the effect of the length change is on the distribution of 100-Å filaments and dense bodies at various points along the length of muscle fibers. Fig. 3 illustrates the cross-sectional profile of several muscle fibers at three different points along the fiber length. At the central nuclear region the network is present as a concentric ring around the nucleus. In the juxtanuclear area the network is consolidated into a centrally located aggregate which is proportionally smaller in fiber profiles that are made near the end of the fibers. These observations show that the 100-Å filaments and dense bodies are integrated into a network that extends throughout the fiber length from end to end. It is necessary to have the network attached to the cell surface near the ends of the fibers in order to obtain the type of deformation found when the network and muscle fiber are stretched (7). The presence of the consolidated mass of 100-Å filaments and dense bodies extending into the ends of stretched fibers, as shown in Fig. 3, supports this idea. However, further work has also demonstrated that infrequent lateral connections between the network and the plasma membrane occur all along the length of the fibers. In order to show these features, high voltage electron microscopy was used in conjunction with the muscle strips that contained only the 100-Å filaments and dense bodies. Series of consecutive sections examined as stereo-pairs clearly show that there are infrequent lateral connections between the stretched 100-Å filament network and the plasma membrane dense bodies (Fig. 4).

Integrity of the Networks

The relationship of the 100-Å filament network to the arrays of actin and myosin filaments is not known. It is generally thought that the dense bodies in the sarcoplasm are analogous to the Z disks of striated muscle fibers. This would imply, therefore, that the actin filaments are integrated into a filamentous framework of what is described in this report as a cytoskeleton composed of 100-Å filaments and dense bodies. The possibility that actin filaments are connected to the dense bodies in

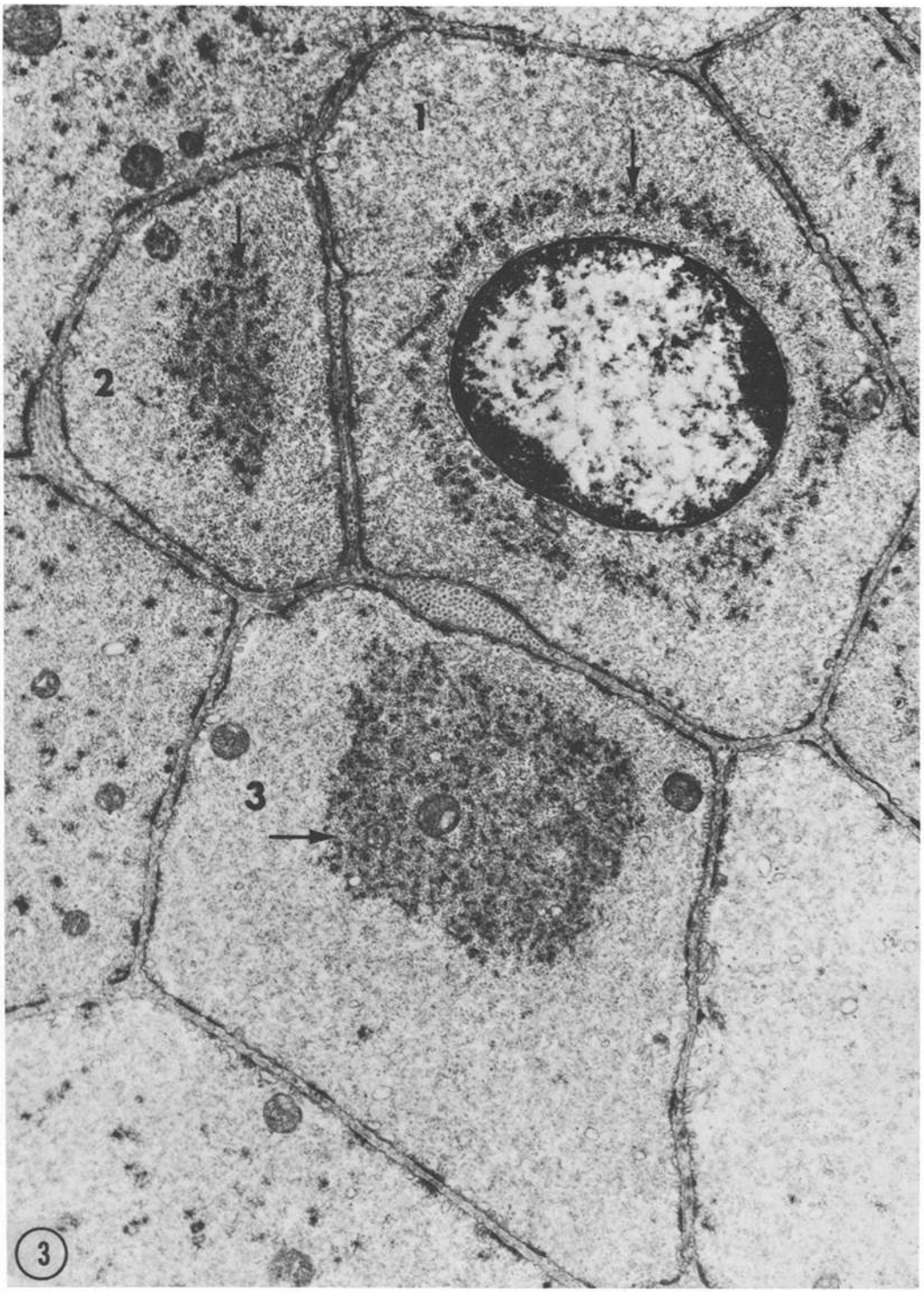


FIGURE 3 Cross-sectional profile of several *Taenia coli* smooth muscle fibers after treatment with EDTA to remove the thick and thin myofilaments and stretching in order to alter the distribution of the dense bodies and 100-Å filaments. Fibers labeled 1, 2, and 3 show what the distribution of the stretched 100-Å filament-dense body network is like at three different levels along the fiber length. Fiber profile 1 is at the midpoint along the cell length. Fiber profile 2 is near the end of the fiber, and profile 3 is between the center and the end of a fiber. This micrograph demonstrates that the 100-Å filament-dense body network extends throughout the length of each fiber. × 25,000.

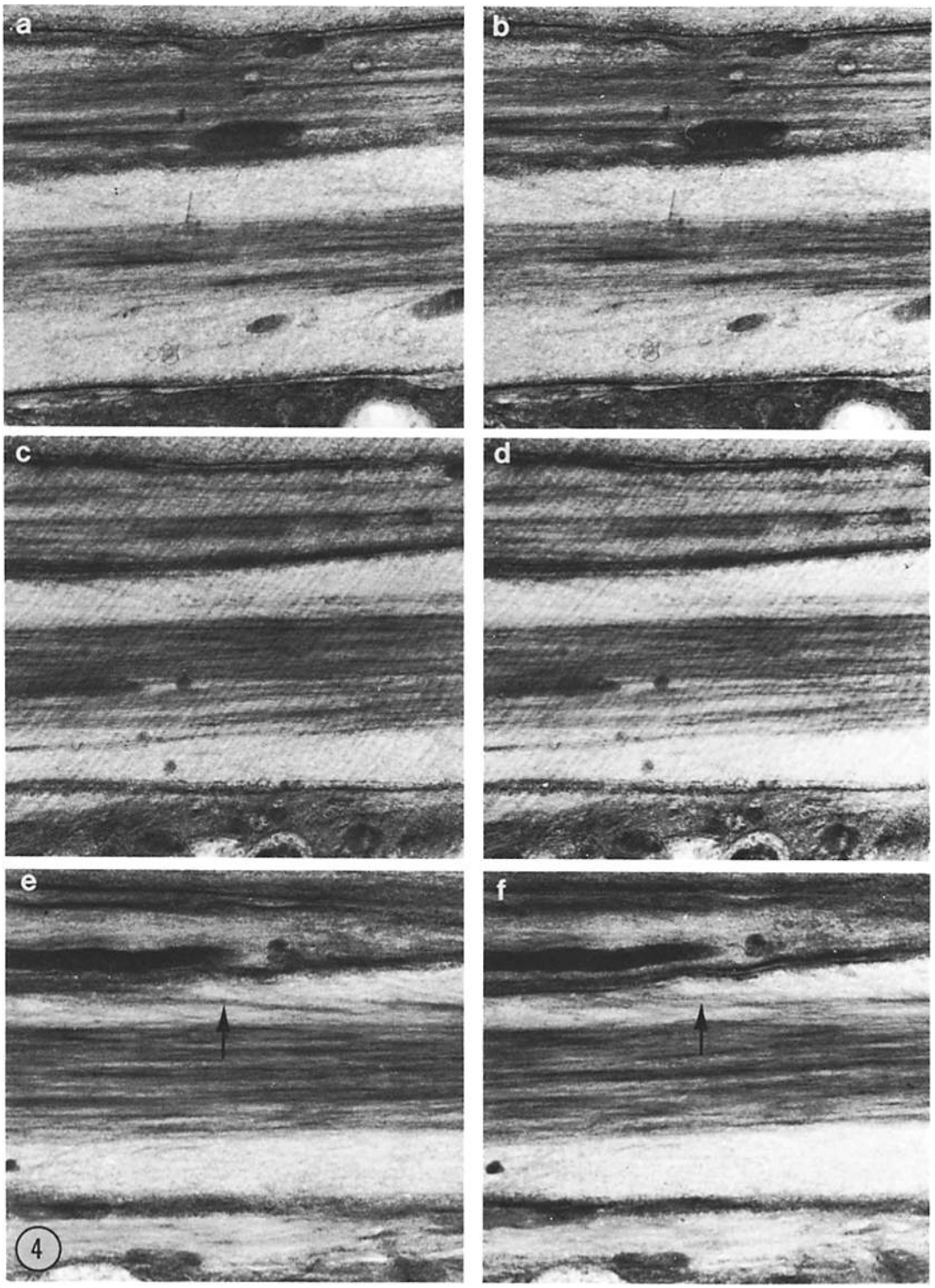


FIGURE 4 Consecutive, stereo-paired, high voltage electron micrographs that reveal a lateral connection between the consolidated 100-Å filament-dense body network and the plasma membrane of a stretched *Taenia coli* smooth muscle fiber (arrow). × 20,000.

the sarcoplasm has been examined in several different types of preparations.

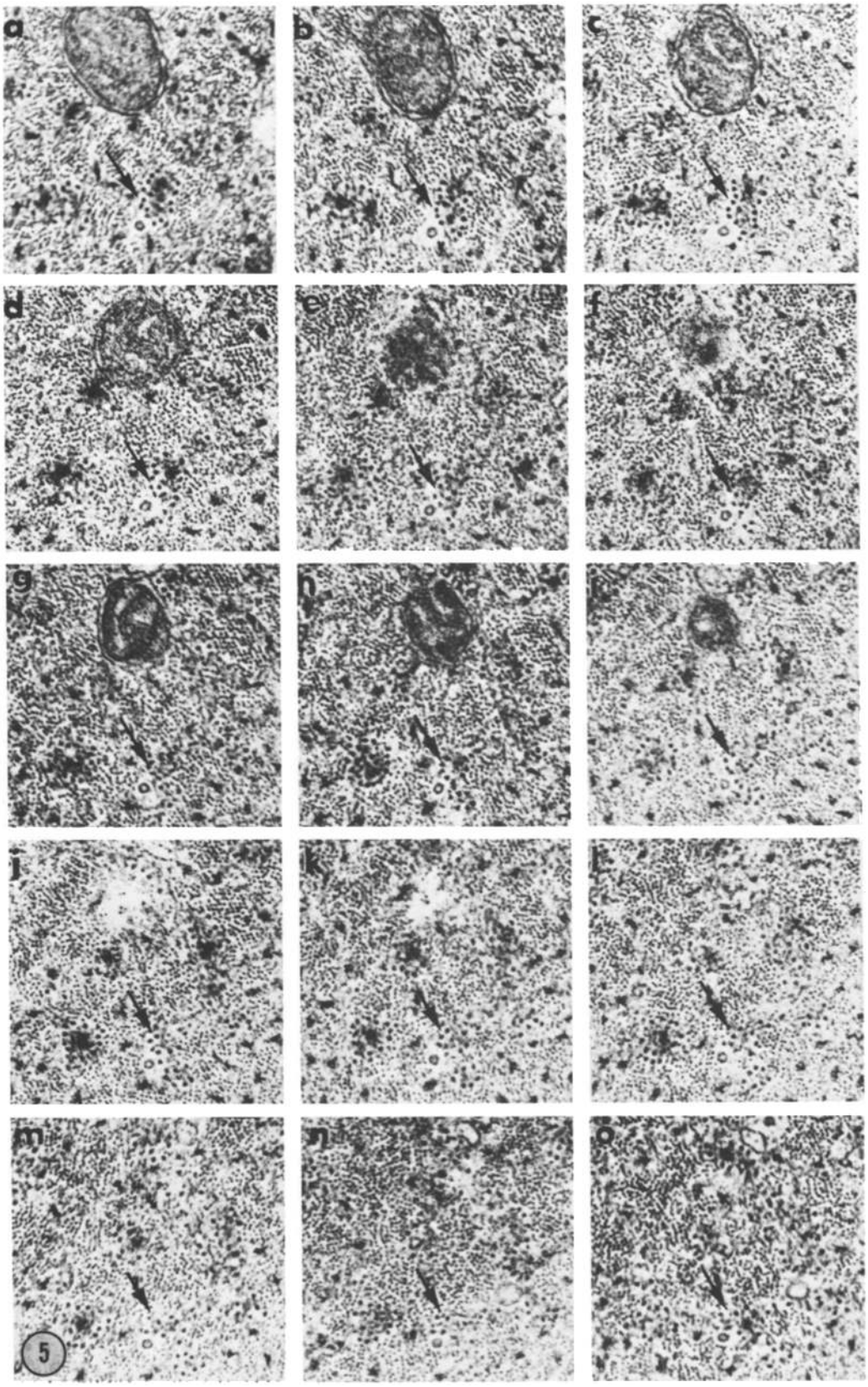
Certain longitudinal sections of muscle fibers indicate that the fusiform dense areas are not continuous with the bundles of actin filaments (52). Uncertainties arising over the relationship of the plane of the thin section to the orientation of the structures sampled in the section make the interpretation of structural continuity in these views very ambiguous. A more satisfactory approach to examining the structural relationship between the dense bodies and 100-Å filaments and the actin and myosin filaments can be made at high resolution with series of consecutive cross-sections. A representative series of sections which includes nearly 2 μm of sarcoplasm is shown in Fig. 5. The initial section shows the profile of a dense body through the electron-dense core region surrounded by a rosette of 100-Å filaments. As the series proceeds towards the end of the dense body, the size of the core region gradually decreases and the rosette of 100-Å filaments becomes less regular; at the end of the series the dense core disappears and the 100-Å filaments are present as a loosely organized bundle. Within this and similar series of sections, the bundles of contiguous actin filaments are never found to lie in the sampled "corridor" that extends from the core region of the dense body into the area at the end of the dense body containing the bundle of 100-Å filaments. This indicates that the actin filaments are not structurally continuous with the core region of the dense body or the attending array of 100-Å filaments.

Another, albeit less systematic appraoch to determine what filamentous connections may exist between the dense bodies and filaments is to utilize the fact that these structures are small enough to provide both adequate penetration of electrons and suitability for negative staining, so that they can be directly examined as isolated particles. When smooth muscle is mechanically disintegrated a number of fusiform subcellular particles can be recovered in the "nuclear" and "mitochondrial" fractions that closely resemble the structures identified as the dense bodies in sectioned preparations (6). Several of these bodies are shown in Fig. 6. They consist of discrete, closely packed bundles of filaments 40–100 Å in diameter and ranging from 0.3 to 1.5 μm in length. These regions are analogous to the electron-dense core region of the dense body seen in thin sections, and are usually attended by a tangled mass of 100-Å filaments. When the core region can be clearly seen, it appears to terminate abruptly. The only filaments that ever extend from the ends of the central core region are those characterized as 100-Å filaments. Filaments that could be identified with those containing actin or myosin are not directly associated with the isolated dense bodies, although they can be found in adjacent areas as separate filaments. These preparations, like the series of consecutive cross-sections, show that the actin filaments are not continuous with any structures that directly contribute to the dense body or its associated 100-Å filaments.

A further set of observations that has revealed the integrity of the cytoskeleton and the lack of connections between it and the actin and myosin filaments is derived from a study of the distribution of myofilaments in muscles that have been stretched to points on the length-tension curve where the active tension that can be generated is equal to or less than the resting tension. As described earlier, the 100-Å filaments and dense bodies in fibers of stretched muscle strips are grouped together in a mass that is centrally located in cross-sectional profiles of the fibers. In fibers from unstretched muscle strips, the dense bodies and 100-Å filaments are widely distributed throughout the fiber profiles. These differences in distribution are best seen in muscle strips that have been treated so as to remove both the actin and myosin filaments, but the effect is still noticeable in untreated muscle strips (i.e. in muscle strips that show the three types of myofilaments). When the three types of myofilaments are present, only those filaments that are structurally connected to the dense bodies should be coincidentally moved, as the dense bodies become grouped together during stretching. Fig. 7 shows a portion of a fiber in which the dense bodies and 100-Å filaments form a consolidated mass. Within this area bundles of actin filaments or myosin filaments are not present, but they are present in the peripheral areas. These observations indicate that movement of the actin and myosin filaments does not coincide with the relocalization of the dense bodies and the 100-Å filaments, and further imply that no structural continuity exists between the dense body-100-Å filament network and the actin and myosin filaments.

Isolation and Analysis of the 100-Å Filaments

The preparation of a cell fraction of smooth muscle that is enriched with respect to structures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comprising the filamentous networks has been described in an earlier report (6). Analysis of the protein composition of this fraction was undertaken by gel electrophoresis and correlated with observations made by electron microscopy.

The initial starting material for the isolation of the 100-Å filaments was minced lateral muscles from chicken gizzard, which consists of uniformly sized, flake-like groups of smooth muscle fibers (Fig. 8). After mechanical homogenization in a Waring blender and prolonged extraction (i.e. with salt solutions of high ionic strength to "solubilize" the actomyosin), the cell fractions corresponding to the nuclear and mitochondrial fractions were recovered by differential centrifugation. They contained large groups of 100-Å filaments and dense bodies together with mitochondria, a menagerie of vesicles, and membrane fragments (Fig. 9). Filaments corresponding to actin and myosin were not found in these fractions after prolonged extraction. Solubilization of the 100-Å filaments was effected with buffered solutions of urea. This treatment dissolved the 100-Å filaments and dense bodies, but it did not detectably alter the residual membranes and vesicles (Fig. 10). After high-speed centrifugation to remove suspended particles and dilution of the urea solution by dialysis, short segments of filaments that closely resembled the native 100-Å filaments formed a flocculent precipitate (Fig. 11). The remaining soluble protein was recovered by precipitation with the addition of ethanol to 60% by volume or by the addition of ammonium sulfate to 20% of saturation. These precipitates consisted of amorphous clumps of material when examined by negative staining and electron microscopy.

The polypeptide composition of the various cell fractions obtained during the isolation of the 100-Å filaments is shown in Fig. 12. The molecular weights of several major components in the original starting material (muscle flasks) range from around 200,000 to 20,000. The largest component and the two smallest components correspond to the polypeptide subunits of purified smooth muscle myosin (9). The component with a molecular weight of around 100,000 may correspond to α-actinin which is present in the dense bodies (46), and the component with a molecular weight of around 42,000 is isographic with actin. Hence, the only components that cannot be readily identified with a known muscle protein are a salt-insoluble component with a molecular weight of around 55,000 and two salt-soluble components with molecular weights of ca. 38,000 and 30,000. After prolonged extraction of the muscle homogenate with salt solutions of high ionic strength, the material recovered in the nuclear and mitochondrial fractions had only three major components. The largest component corresponds to the heavy chain of myosin, and the smallest is actin. There was a large increase in the relative amount of the intermediate component which has a molecular weight of around 55,000. This result is correlated with the relative abundance of 100-Å filaments in these cell fractions, and it suggests that this polypeptide is the subunit of the 100-Å filaments. Treatment of these salt-insoluble cell fractions with solutions of urea resulted in the preferential dissolution of the 55,000 mol wt component; this component was recovered as a principal constituent of the flocculent precipitate that consists of short segments of 100-Å diam filaments which formed upon removal of the urea by dialysis, although some high molecular components were also present. The remaining protein in solution was precipitated with ethanol or ammonium sulfate; it consists of the 55,000 mol wt component (Fig. 12), but suitable conditions for forming filaments from this protein have not yet been found.

From the preceding section of Results, it is deduced that the polypeptide component with a molecular weight of around 55,000 is the subunit of the 100-Å filaments. The mobility of this component was compared with that of several other proteins of known molecular weight in order precisely to establish its relative molecular mass (Fig. 13). This comparison indicates that the 100-Å filament subunit has a molecular weight of approximately 55,000 and that it does not comigrate in electrophoresis gels with any known muscle

FIGURE 5 Consecutive sections (*a–o*) of a region of a *Taenia coli* smoth muscle fiber showing a microtubule marker (arrow) adjacent to a dense body and the attending array of 100-Å filaments. The core region of the dense body gradually diminishes from (*a*) to (*d*), and the rosette of 100-Å filaments gradually becomes less regular. Bundles of thin myofilaments are present in the contiguous areas but they do not emerge from the core region of the dense body as is shown in the sections from (*e*) to (*o*) or from the 100-Å filaments that attend the dense body. × 65,000.

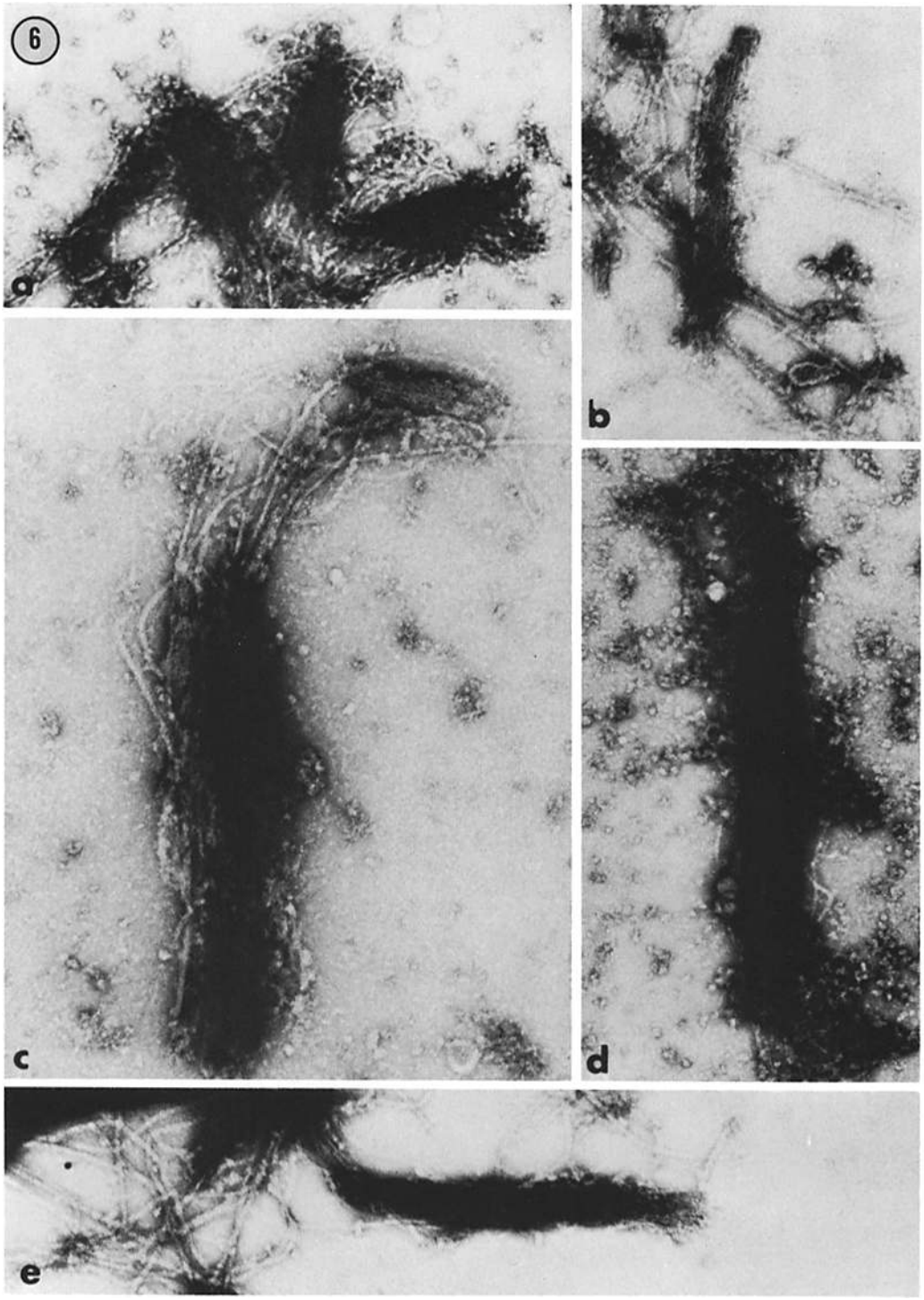

FIGURE 6 Micrographs of negatively stained, isolated dense bodies with attending 100-Å filaments from chicken gizzard lateral muscles. At *a* several dense bodies are grouped together with a tangled mass of 100-Å filaments. At *b* and *e* the filamentous substructure of the core region of the dense body terminates abruptly, and at *c* and *d* the dense bodies are only associated with more or less 100-Å filaments. Thin actin-like filaments are never found directly connected to the dense bodies. (*a*) × 50,000. (*b–e*) × 80,000.

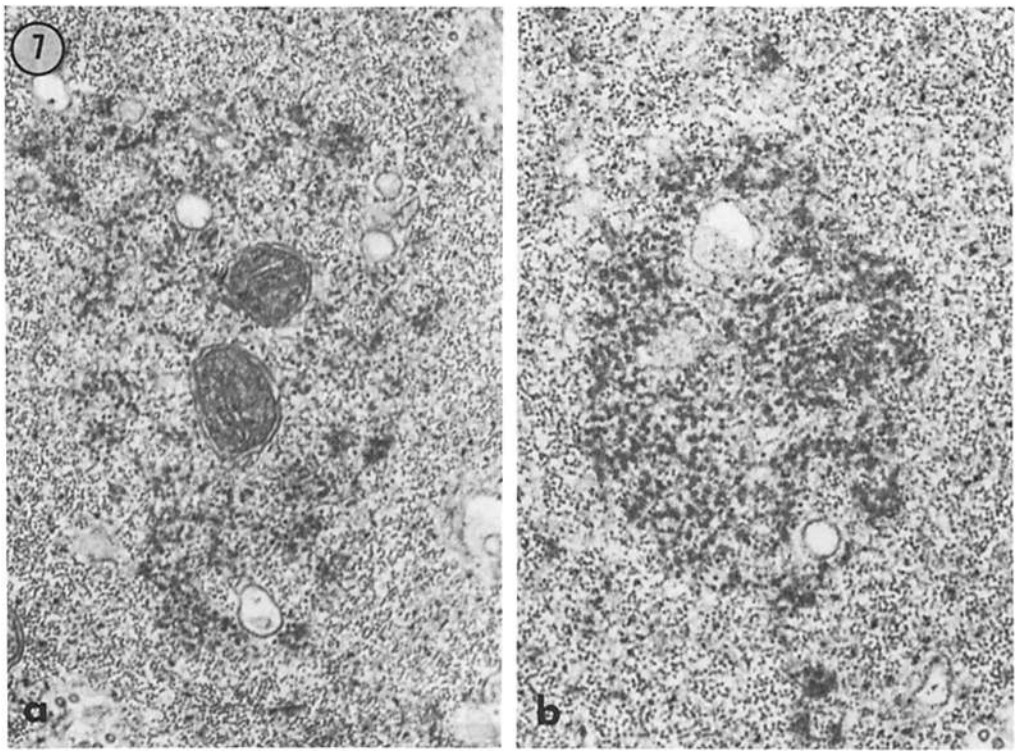

FIGURE 7 Region of sarcoplasm from highly stretched fibers from *Taenia coli*. The consolidated mass of 100-Å filaments and dense bodies is segregated from the surrounding thick and thin myofilaments. × 65,000.

FIGURE 8 Uniform fragments of minced gizzard muscle used to correlate the analyses of structure by electron microscopy and protein composition by gel electrophoresis. × 40.

protein or with the microtubule subunit, tubulin. Several different samples of purified brain tubulin consistently had a greater mobility than the 55,000 mol wt component in smooth muscle and had a calculated molecular weight of around 53,000. Densitometric scanning of the acrylamide gels stained with Coomassie brilliant blue was used to determine the relative amounts of the 100-Å filament subunit in the various cell fractions. Integration of the areas under the curves in Fig. 14 shows that it constitutes around 5% of the total protein in the original muscle mince, 20% of the salt-insoluble nuclear and mitochondrial fractions, and 40% of the urea-soluble fraction from which the short filaments are formed.

DISCUSSION

Relatively little is known of the three-dimensional arrangement of the elements comprising the contractile apparatus of vertebrate smooth muscle. The class of filaments with the smallest cross-sectional diameter is identified with the actin-containing filaments of striated muscles, because they compare closely with respect to protein composi-

FIGURE 9 Micrograph of the residue of gizzard muscle after prolonged extraction with salt solutions of high ionic strength. The residue contains large numbers of 100-Å filaments. × 10,000.

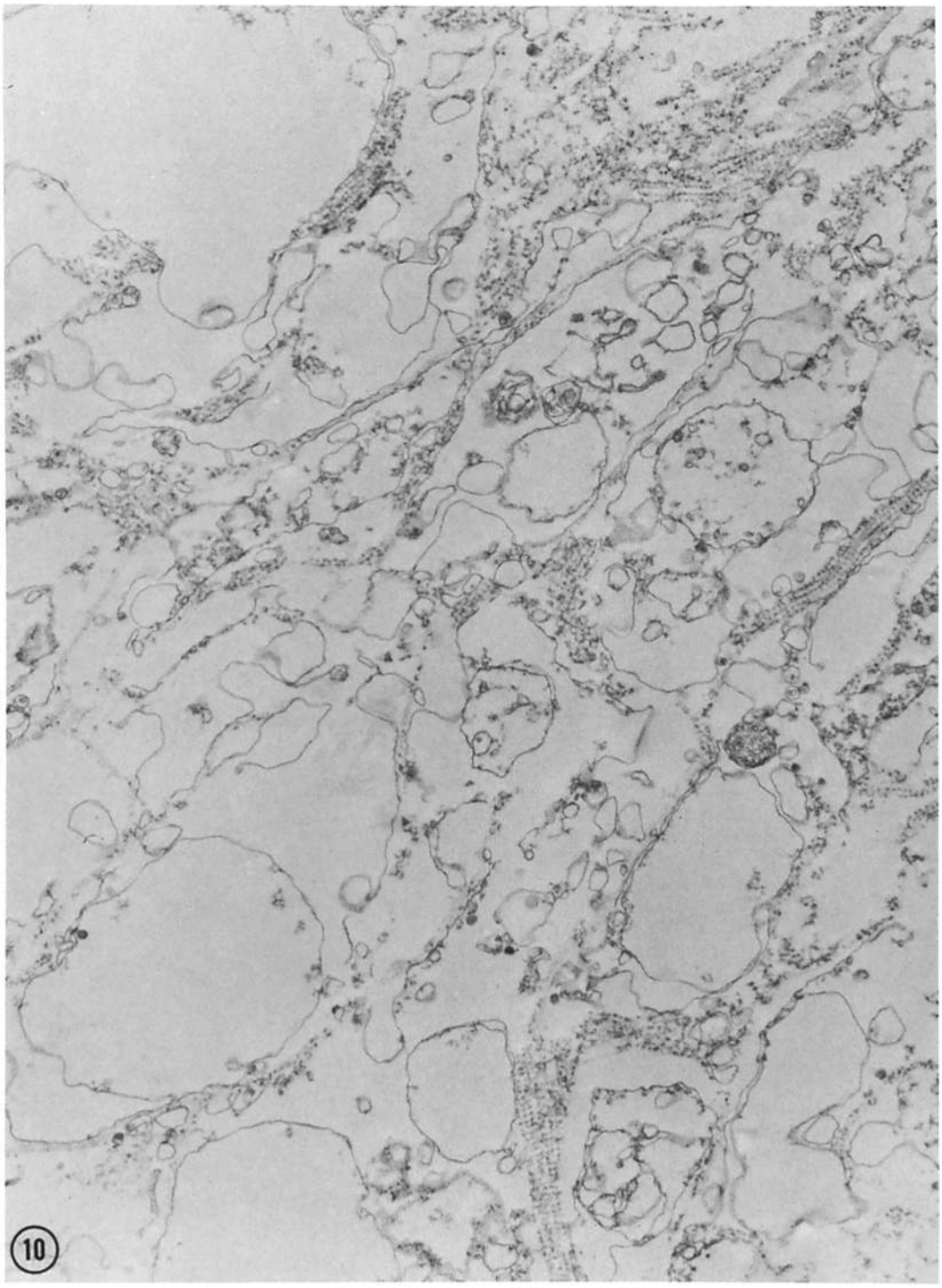

FIGURE 10 Micrograph of the residue of the salt-insoluble fraction of gizzard muscle after extraction with 6 M urea. Membrane fragments and vesicles remain together with insoluble collagen. The 100-Å filaments are not found in the muscle residue after extraction with urea. × 7,000.

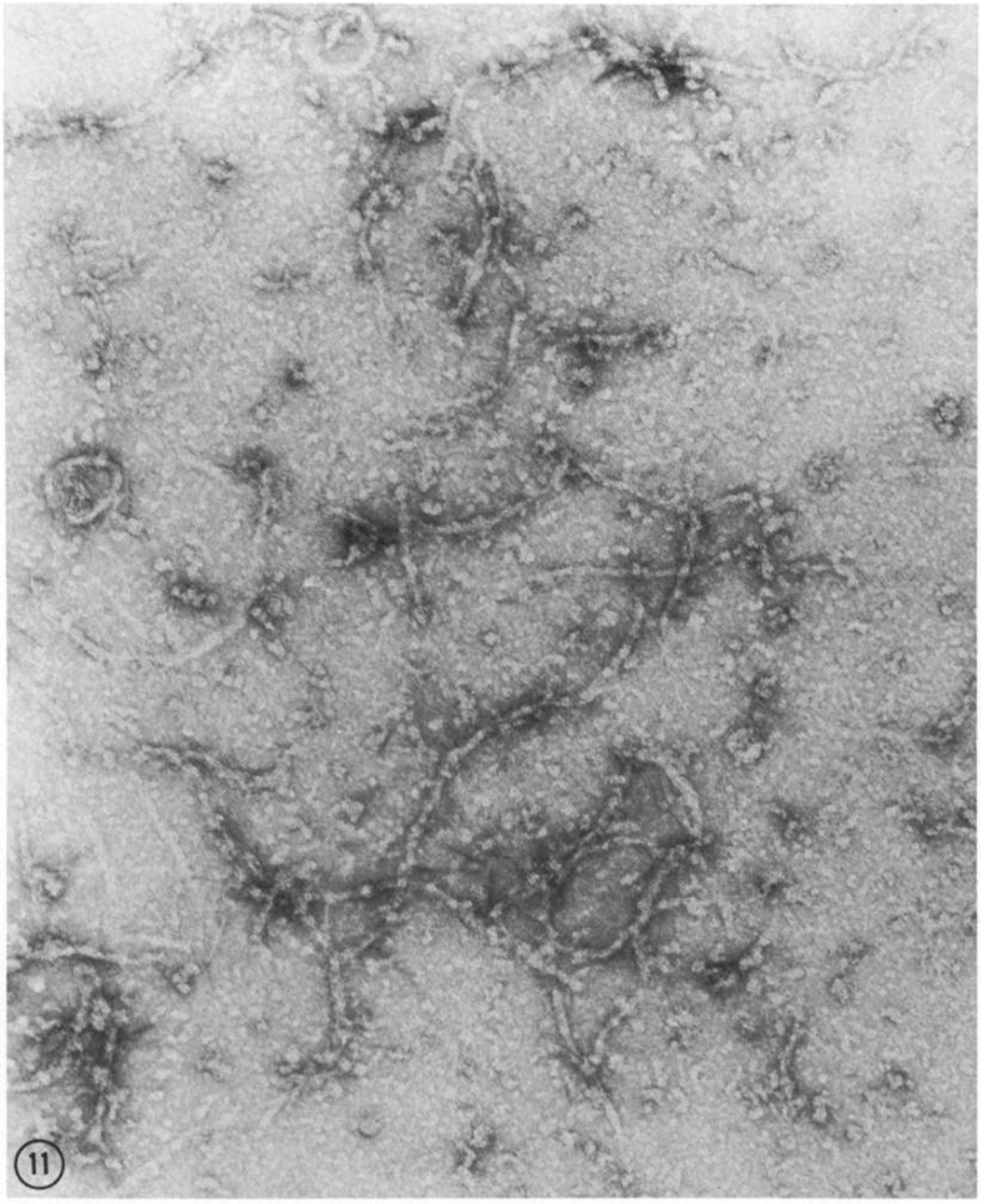

FIGURE 11 Negatively stained filament segments that form upon dialysis of the urea-soluble fraction of the residue. The filamentous segments resemble native 100-Å filaments. × 100,000.

tion and substructure (3, 23, 24). They differ markedly in their organization and relative abundance. The actin filaments of smooth muscle are grouped together into bundles with approximately a 120-Å center to center spacing showing a hexagonal packing arrangement (14, 26, 34). The ordering within and among these bundles is limited to small areas. This accounts for the presence of only a single observable maximum in diffraction patterns that corresponds to the spacing of the

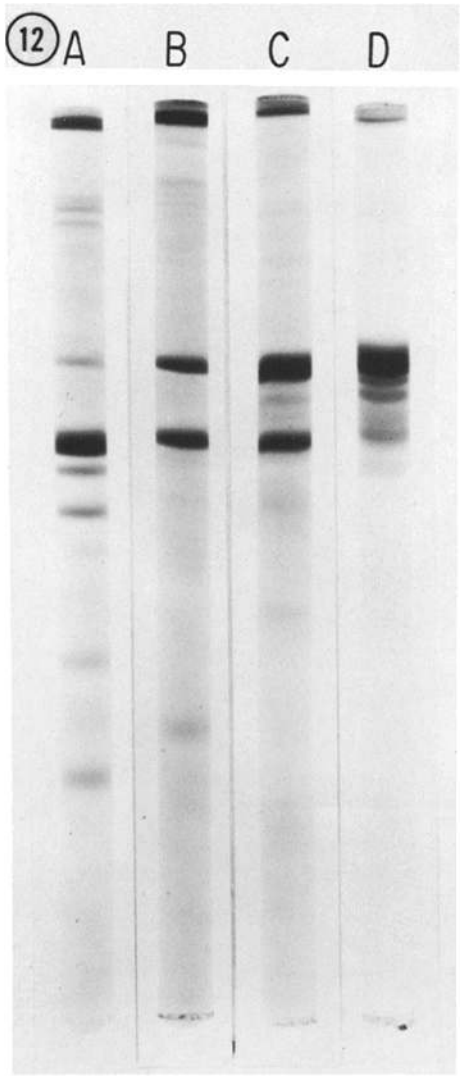

FIGURE 12 Acrylamide gels (8%) showing the protein composition of several steps in the isolation procedure. The washed muscle mince is shown at *A*; *B* is the salt-insoluble material, *C* is the urea-soluble material, and *D* is the soluble protein that is recovered at 20% ammonium sulfate saturation. The original gels are approximately 93 mm long.

filaments in a limited lattice as explained by Rice et al. (43), and by Small and Squire (51). The actin is much more abundant than in striated muscles. Molar ratios of around 30:1, actin to myosin, are reported (38, 53) in contrast to 3:1 for skeletal muscle. Direct counts of actin filaments reveal also that they are more abundant than filaments containing myosin: values of 15–60:1 are reported (43, 50), although this particular issue is complicated by the difficulty in preserving the myosin-containing filaments in smooth muscle. Determining the abundance and distribution of the myosin filaments has been a subject of considerable difficulty in recent studies (28, 40, 44, 50). The class of filaments with the largest diameter were earlier assumed to contain myosin only because of their overall resemblance to the thick myosin-containing filaments in striated muscles and because of the similarity in the form of "synthetic" filaments made from the myosin from both smooth and striated muscle (5, 12, 30, 39). This entire viewpoint was substantially strengthened by X-ray diffraction studies that revealed a diffraction maximum that is characteristic of myosin filaments (34). The intensity distribution in the X-ray diffraction patterns relating to myosin filaments correlates well with the form and relative paucity of thick filaments in smooth muscle and their lack of lateral registration into repeating lattice-like arrays. Therefore, there is some assurance that the class of thick myofilaments that is observed by electron microscopy corresponds to myosin.

The class of myofilaments around 100 Å in diameter has been observed in several types of smooth muscle (2, 6, 43, 50), and they appear to constitute a distinct class of filament because they differ from both the actin and the myosin filaments in their fine structure, solubility, and pattern of distribution in the muscle fibers (6). These filaments form structural attachments with the dense bodies in the sarcoplasm and at the plasma membrane. These points are most clearly demonstrated in muscle preparations in which the actin and myosin filaments are either selectivity extracted or removed (6, 17). The muscle residue after the extraction or selective removal of the actomyosin contains large numbers of 100-Å filaments and structures that closely resemble the dense bodies within untreated, intact smooth muscle fibers. Mechanical disintegration of the muscle residue after extractions of actomyosin produces small groups of 100-Å filaments and dense bodies suggesting that homogenization fragmented the large networks. This view is supported by the relationship between the size of the 100-Å filament networks and the extent of mechanical disintegration (6). This idea and its functional consequence were examined in another type of muscle preparation. Strips of muscle were incubated in vitro and

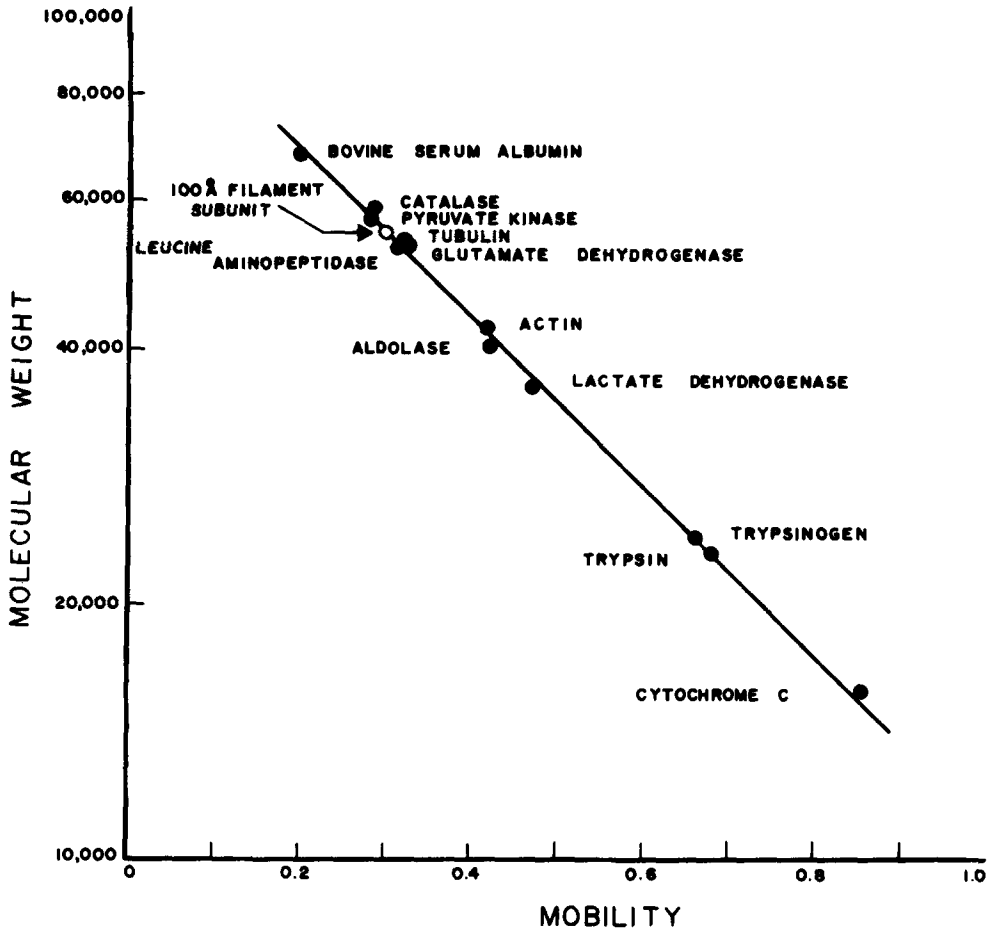

FIGURE 13 Standard curve used to determine that the molecular weight of the 100-Å filament subunit is 55,000.

changes in the length and tension of the whole muscle strip were correlated with the length distribution of the component muscle fibers. Stretching the muscle strips led to an increase in the average length of the fibers, and to consolidation of the 100-Å filaments and dense bodies into a centrally located mass. It was clearly shown that there is a strong correlation between (*a*) the degree of stretch, (*b*) the average length of the component fibers, and (*c*) the ultrastructural distribution of the 100-Å filaments and dense bodies (7). The changes in the distribution of the filaments and dense bodies indicated that these components formed networks whose shape is length dependent. A fundamental feature of the model developed to explain how the change in length affected the distribution of the proposed network was that the 100-Å filaments must attach to the cell surface principally at or near the ends of the muscle fibers. Extensive sampling now indicates that this does occur, but there are also a number of lateral attachments made between the network and the plasma membrane all along the length of the fibers. Hence the basic model must be revised to include these infrequent lateral connections.

Are the actin filaments structurally related to the 100-Å filament networks? The sets of observations reported in this study suggest that actin filaments do not attach to the sarcoplasmic dense bodies. Analysis of series of consecutive thin sections that include dense bodies, and observations on both isolated dense bodies and *in situ* relocalized dense bodies indicate that actin filaments are not connected to the network through

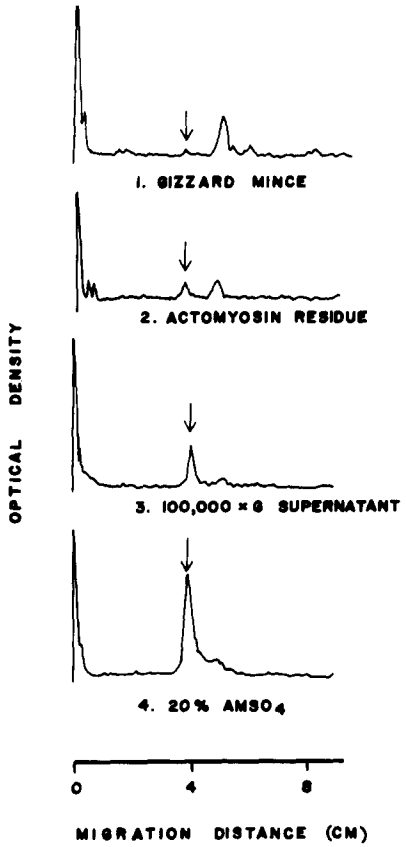

FIGURE 14 Densitometric scans of the gels shown in Fig. 12 to determine the relative amount of 100-Å filament subunit (arrows) in the various cell fractions.

these dense bodies. Other studies indicate that the actin filaments do attach to the plasma membrane dense bodies (18, 19, 41), and this view is consistent with the observations made here.

The principal purpose of this report is to present evidence that the 100-Å filaments constitute a distinct class of myofilaments. The foregoing discussion serves to suggest that the 100-Å filaments consitute a structurally distinct system of filaments. The results of the analysis of their composition also indicate that the 100-Å filaments are qualitatively distinct from the actin and myosin filaments. Because of a close correspondence between the number of 100-Å filaments in various smooth muscle cell fractions and the relative abundance of a polypeptide with a molecular weight of around 55,000, it is very likely that this polypeptide represents the protein subunit of the 100-Å filaments, but this point cannot yet be made with absolute assurance since there are high and some low molecular weight components that also appear in these cell fractions. These minor components may possibly attend the 100-Å filaments or have a role in the aggregation of the putative 55,000 mol wt subunits into filaments. Identical results were found for the protein composition with SDS gels of neurofilaments which closely resemble the 100-Å filaments (47), so there is a possibility that the 100-Å filaments that are found in a variety of cell types, such as cardiac muscle cells (37, 42), fibroblasts (22, 27), and macrophages (11), may have a similar protein composition and substructure. Unpublished observations indicate that there is a minor salt-insoluble component (2–5% of the total protein) in cardiac and skeletal muscle that is isographic with the proposed 100-Å filament subunit from smooth muscle. Further work on the chemical and physical properties of this protein must now be done to provide a basis for clearly demonstrating a role for these filaments. All the results accumulated thus far indicate that the 100-Å filaments are distinct from the actin and myosin filaments and therefore that they constitute an integrated and probably independent system of filaments—a cytoskeleton.

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