MYOSIN-LIKE AGGREGATES IN TRYPSIN-TREATED SMOOTH MUSCLE CELLS

JACK ROSENBLUTH

From the Departments of Physiology and Rehabilitation Medicine, New York University Medical Center, New York 10016

ABSTRACT

Segments of the lower small intestine of the toad *Bufo marinus* were excised and soaked for approximately 2 hr in Ringer's solution (pH 7.4 or 7.8) containing crystalline trypsin and then fixed for electron microscopy at approximately the same pH. Thin sections of the tunica muscularis of these specimens show smooth muscle cells ranging in appearance from severely damaged at one extreme to apparently unaffected at the other. Among these are cells at intermediate stages, including some which exhibit large and conspicuous populations of thick filaments closely resembling artificially prepared aggregates of smooth muscle myosin. The thick filaments have the form of tactoids $\sim$ 250–300 A in diameter in their middle regions and are $\sim$ 0.5–1.0 μ in length. In some preparations they also display an axial periodicity approximating 143 A. They are usually randomly oriented and segregated from the thin filaments, which tend to form closely packed, virtually crystalline bundles at the periphery of these cells. "Dense bodies" are absent from cells showing these changes. The simplest interpretation of these data is that smooth muscle myosin normally exists among the actin filaments in a relatively disaggregated state and that trypsin induces aggregation by altering the conformation of the myosin molecule. Alternatively, trypsin may act indirectly through an effect on some other smooth muscle protein which normally forms a stable complex with relatively disaggregated myosin.

INTRODUCTION

Although vertebrate smooth muscle has been shown by biochemical methods to contain substantial amounts of actin, myosin, and tropomyosin (1, 3, 18, 29), the mechanism by which contraction is accomplished in this type of muscle is by no means clear. In order to postulate a realistic model of contraction, it is essential that the state of aggregation of the contractile proteins be determined. So far, X-ray diffraction studies indicate (4) that the actin of smooth muscle is aggregated into strands (F-actin) as it is in striated muscle; however, the X-ray evidence bearing on myosin aggregation is still equivocal (4, 17). Similarly, ultrastructural studies of vertebrate smooth muscle have demonstrated thin filaments of about the same dimensions as the I-band filaments of striated muscle (2, 15, 18, 20), but the existence of myosin aggregates comparable to the thick filaments that occur in vertebrate striated muscles (9) and in various invertebrate muscles (7, 25) is a matter of debate (14, 21, 23, 29).

Recently several reports of thick filaments in thin sections of vertebrate smooth muscles have appeared (13, 14, 19, 22). However, it was not established that these filaments are composed of myosin; nor was it clear whether such filaments exist under normal conditions but are not usually preserved by conventional fixation methods, or, alternatively, whether they form only under abnormal conditions and do not occur as such in

any normal physiological state. If the latter is the case and if myosin is normally disaggregated in vertebrate smooth muscle, then another question arises as to what prevents its aggregation *in situ* in view of the fact that extracted smooth muscle has been shown to be capable of forming thick filaments in vitro (8, 11).

The present paper reports observations made on one example of a vertebrate smooth muscle in which thick filaments are ordinarily not seen in thin sections by electron microscopy. When this muscle is subjected to trypsin treatment, however, large populations of thick filaments having the distinctive characteristics of myosin aggregates, including their periodicity, appear in some of the cells. The implication that myosin is normally relatively disaggregated in this muscle and the possible means by which trypsin induces aggregation are considered.

METHODS

Adult toads (*Bufo marinus*) weighing approximately 250 g were anesthetized with 5 ml of a 10% solution of ethyl carbamate injected into the dorsal lymph sac, or were pithed. The abdominal wall was opened; the lower small intestine was located, and segments about a half inch long were tied off and dissected out. Each segment was then "inflated" with one of the solutions listed below and immersed in the same solution at room temperature. In most instances specimens were immersed for 2 hr (range: 45 min–5 hr).

Immersion solutions:

(*a*) ¾ strength Ringer's injection (Baxter) adjusted to pH 7.4 (unbuffered), plus trypsin[1] (0.5–3.0 mg/ml).

(*b*) ¾ strength Ringer's injection (Baxter) plus 0.02 M or 0.1 M Tris-HCl buffer, pH 7.4 or 7.8.

(*c*) ¾ strength Ringer's injection (Baxter) plus 0.02 M or 0.1 M Tris-HCl buffer, pH 7.4 or 7.8 plus trypsin[1] (2 mg/ml).

(*d*) ¾ strength Ringer's injection (Baxter) plus 0.02 M Tris-HCl buffer, pH 7.4, plus 3.45 mg/ml of diisopropyl fluorophosphate-inactivated salt-free trypsin.[2]

(*e*) ¾ strength Ringer's injection (Baxter) plus 0.02 M Tris-HCl buffer, pH 7.4, plus 2 mg/ml of trypsin[1] and 2 mg/ml of 3 × crystalline soybean trypsin inhibitor.[3]

At the end of this time, some of the solution was withdrawn from the lumen of the segment and replaced by ~2% glutaraldehyde (biological grade) in 0.1 M phosphate buffer (pH 7.5) and the segment was immersed in this fixative also at room temperature for approximately 1–2 hr. Rings and strips were then cut from the fixed intestine, rinsed in 0.65% NaCl, postfixed in 1% OsO_4 in phosphate buffer (pH 7.5) for approximately 1–2 hr, dehydrated in a graded series of methanol solutions, and embedded in Araldite 502. In some cases the specimens were soaked overnight in 0.5% uranyl acetate in acetate-Veronal buffer (pH 5.0) before the dehydration step. In one experiment a piece of cardiac ventricle was treated as in *c* and then fixed in glutaraldehyde and processed as described.

Thin sections cut from the blocks were stained with uranyl acetate followed by lead hydroxide (12). Alternatively, some grids were stained with 1% phosphotungstic acid in acetone for 1 hr, in some instances following immersion in 5% hydrogen peroxide for 15 min, and then rinsed briefly in acetone followed by water. These grids were either examined without further treatment or were stained also with lead hydroxide. Staining with 1% potassium permanganate alone or followed by lead hydroxide was also carried out on some specimens but was much less successful. Segments of intestine, isolated as described above, were in some instances fixed immediately at lengths from extreme contraction to extreme extension. In one instance, trypsinized and control segments of intestine were fixed by the acrolein-dichromate method of Robison and Lipton (24). After fixation, the tissue was rinsed in 20% acrolein, then dehydrated in 100% acrolein and embedded. Sections of these blocks were double stained with uranyl acetate and lead hydroxide. Details in these specimens were best seen when electron micrographs were printed negatively by way of an intermediate reversal negative. All micrographs were taken with a Philips EM 300 electron microscope.

RESULTS

Trypsin-treated intestine retains its basic organization but exhibits several changes from normal. Epithelial cells show a loss of the basement membrane covering their basal surfaces, and at a deeper level bundles of C fibers also lack their usual enveloping basement membrane and display an unraveled appearance resulting from the apparent withdrawal of Schwann cell processes from around the axons (Fig. 4). In the tunica muscularis, muscle cells are normally surrounded intimately by a fine reticulum of extracellular filaments (26) which are thinner (~100 A) than collagen fibrils and have no apparent periodicity. These "microfibrils," which may serve to transmit tension developed by the muscle cells, are reduced in number after trypsin treatment (Figs. 1–3, 8).

[1] 2 x or 3 x crystalline salt-free trypsin (Mann Research Labs Inc., New York or Worthington Biochemical Corp., Freehold, N.J.).

[2] Worthington Biochemical Corp.

[3] Calbiochem, Los Angeles, Calif.

In addition, the muscle cells display a range of alterations involving intracellular constituents. These effects are always most pronounced in the most superficial muscle cells, i.e., in those cells in most direct contact with the trypsin solution. Even among the superficial muscle cells, however, the changes do not occur uniformly, and consequently any one section may show apparently unaffected smooth muscle cells at one extreme and severely damaged "ghosts" at the other, as well as small numbers of intermediate stages. Presumably the cells are resistant to trypsin up to a point, after which it brings about a sequence of changes culminating in cytolysis.

Fig. 1 illustrates a typical trypsin-treated specimen. It shows portions of several muscle cells, one

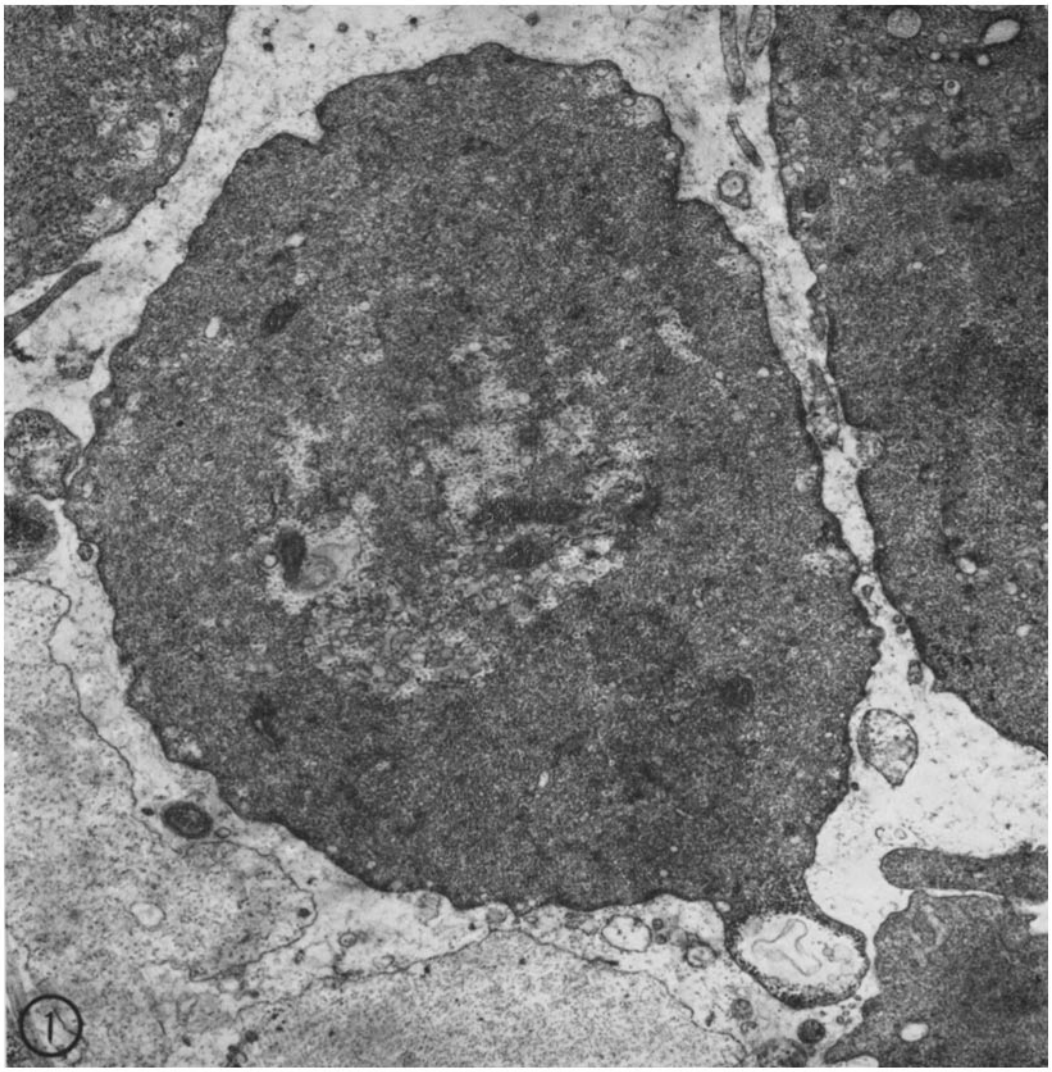

FIGURE 1 Cells of the tunica muscularis after trypsin treatment. Except for one bleb, the muscle cell in the center is normal in appearance. The cell at the upper right contains a number of densely rimmed vacuoles presumably derived from invaginations of plasma membrane. The cell at the upper left exhibits a population of thick filament profiles among the thin ones, and the cells at the bottom center and bottom left are ghosts containing no recognizable organelles. × 20,000.

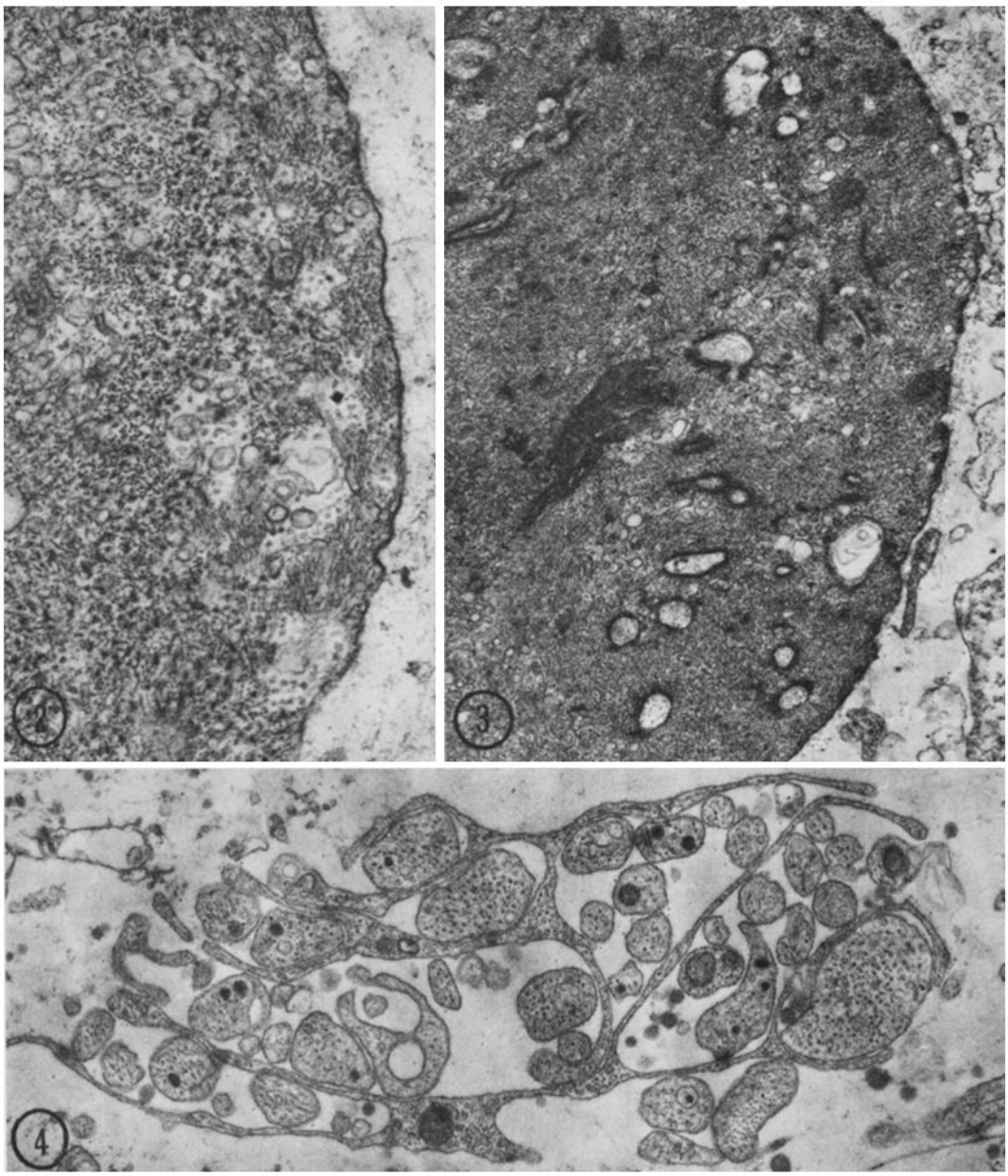

FIGURE 2 Detail of Fig. 1. Both thick and thin filaments are visible. Dense bodies and membrane-associated dense patches are absent. × 46,000.

FIGUER 3 Adjacent portion of the cell shown at the upper right of Fig. 1. Several densely rimmed vacuoles are conspicious in the cytoplasm. × 20,000.

FIGURE 4 Nerve fiber bundle of the myenteric plexus. Both the Schwann cell and the axons appear normal but the basement membrane that normally surrounds such a bundle is absent and the Schwann cell sheets appear unraveled. × 19,000.

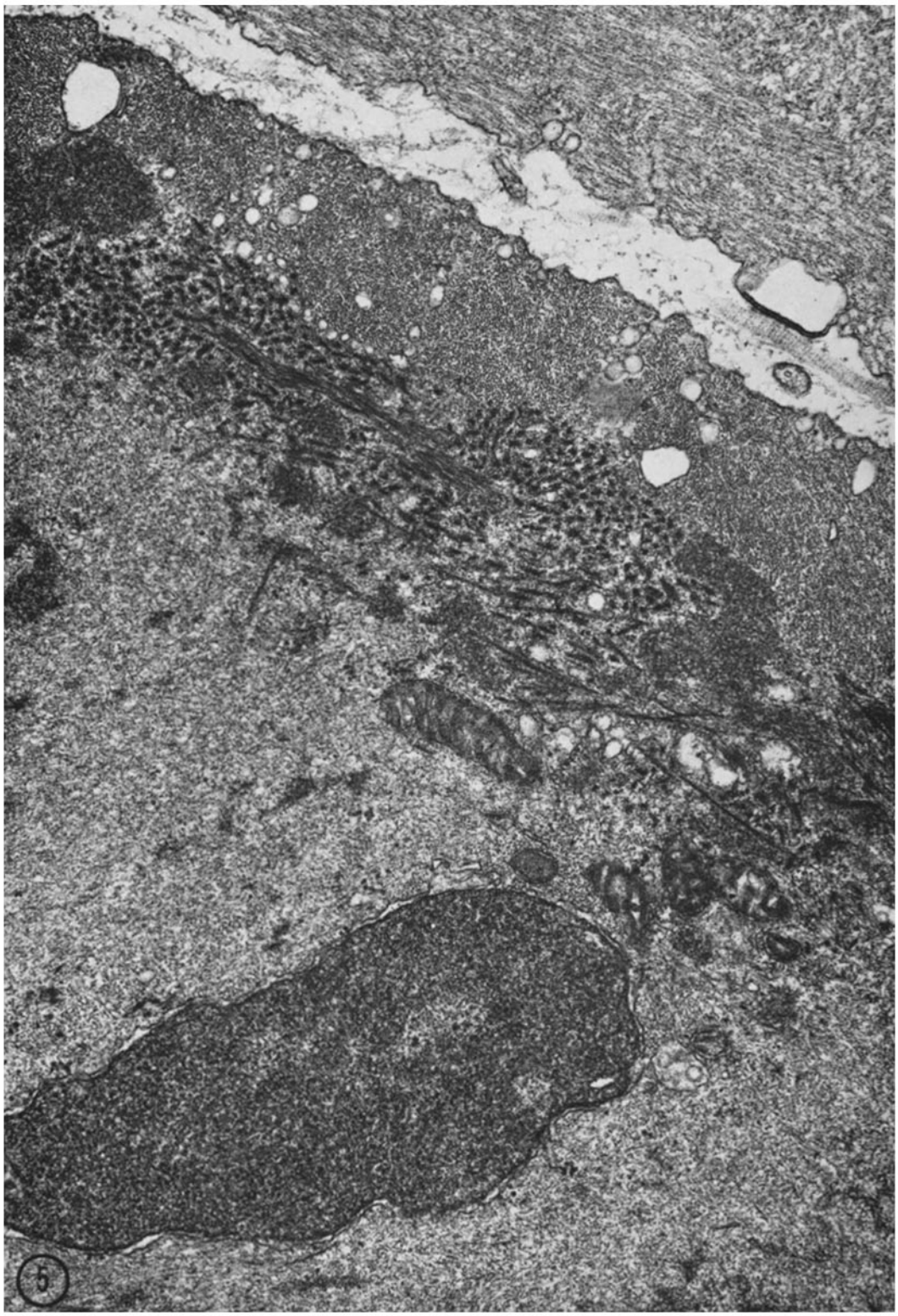

FIGURE 5 Trypsin-treated smooth muscle cell showing pronounced segregation of intracellular constituents. The nucleus is seen at the bottom left. A layer of tightly packed thin filaments underlies the plasma membrane. Dense patches are absent from the plasma membrane but large dense aggregates occur at a deeper level. Thick filaments cut both longitudinally and transversely are prominent. × 37,000.

of which (center) does not differ from untreated controls except for a vacuolar protrusion at the lower right. This cell has a central sarcoplasmic region containing glycogen, mitochondria, and vesicular profiles. Surrounding this core, and virtually filling the rest of the cell, are thin myofilaments. Dense bodies are scattered among the myofilaments and dense patches are applied to the cytoplasmic surface of the plasma membrane. The cell at the upper right is similar but contains several densely rimmed vacuoles presumably derived from invaginations of the plasma membrane. These vacuoles are even more numerous in an adjacent portion of the same cell (Fig. 3). The cell at the upper left of Fig. 1 exhibits a population of thick filament profiles among the thin ones (seen at higher magnification in Fig. 2), and the cell at the bottom center is a ghost lacking any internal organization or recognizable organelles. This report will concentrate on those cells that exhibit prominent arrays of thick filaments.

The plasma membrane surrounding cells of this type may display interruptions, and the dense intracellular material which normally coats a large portion of the cytoplasmic surface of this membrane is either completely or virtually absent (Figs. 2, 5, 6, 8). Any large vacuoles that appear deeper in the cell are also devoid of such a coating (Figs. 5, 9). The small inpocketings normally so common along the surface of smooth muscle cells are reduced and in some cases absent. Mitochondria may display an increase in the density of their matrix (Fig. 5) but this is not invariably true and normal-looking or swollen mitochondria may also be seen in such cells (Fig. 12). "Dense bodies" or "fusiform densities," which presumably correspond to the Z lines of striated muscle, are absent (cf., reference 6 and Figs. 2, 6, 8).

The most striking feature of the cytoplasm of these cells is the presence of two distinct classes of filaments. Those of one group (Fig. 6) look like the ~70 A filaments present in untreated cells but have a greater tendency to form tightly packed bundles in which the filaments are virtually in crystalline array (center-to-center distance ~100–125 A; cf., reference 5). Filaments of the second

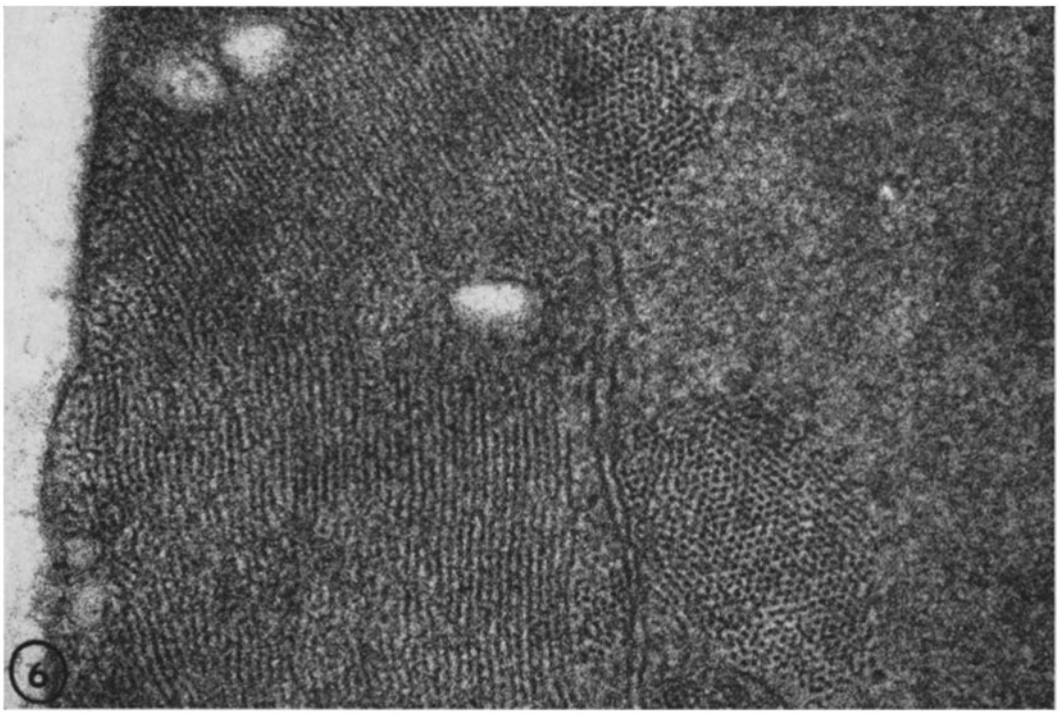

FIGURE 6 Tightly packed thin filaments at higher magnification. Filaments are cut both transversely and longitudinally. The center-to-center distance of the filaments is ~100–125 A. × 118,000.

class are much thicker. They appear as a relatively uniform population of tactoids, ~250–300 A in diameter at the widest point, which taper towards both ends (Fig. 7). Lengths up to about 1 μ have been followed in thin sections (Fig. 8) but are more usually about half this amount. When cut transversely (Fig. 7) their profiles are variable in area, presumably because of the taper along their length.

In addition to the components already described, such cells may also exhibit regions of granulo-filamentous material of low or high density (Figs. 5, 9). The low-density areas resemble the sarcoplasmic core of normal cells and the high-density areas look like aggregates of the material composing dense bodies. Thus trypsin treatment seems to bring about a dissociation and segregation of intracellular consituents into discrete territories.

The location of thick filaments within the cells is not constant. They may virtually fill a cell, in some instances extending to the very margin of the cell (Fig. 8); in most cases, however, the thin filaments are situated peripherally in the cell and the thick filaments centrally (Fig. 12). Occasionally a cell is seen in which the cytoplasm is strikingly layered; i.e., its various components occupy concentric rings or the interfaces between rings (Fig. 9). Although the populations of thin and thick filaments are usually segregated from each other, in some places they adjoin, and in such regions configurations can be found resembling the interdigitating thick and thin filaments of cross-striated muscles (Fig. 10). The average spacing of the thick filaments is ~300–500 A (center-to-center) where they are parallel to each other.

The orientation of the thin filaments in the trypsin-treated cells is usually roughly longitudinal, but groups perpendicular to each other may be seen in the same section (Fig. 6). Thick filaments, in contrast, usually display an entirely random orientation (Figs. 7, 8), although in some cases they too exhibit a degree of alignment with one another, especially where they are mingled with thin filaments (Fig. 12).

The detailed structure that can be visualized in the thick filaments depends on the preparative methods used. In specimens fixed in glutaraldehyde followed by osmium tetroxide and stained, after sectioning, with uranyl and lead salts (Fig. 10), thick filaments rarely exhibit an axial periodicity but occasionally display fine bridges extending from them to adjacent thin filaments; images reminiscent of the arrowhead pattern created by adding heavy meromyosin (HMM) to F actin (9)

FIGURE 7 Thick filaments at higher magnification. In longitudinal sections these filaments are straight and taper to a point at either end. They are ~0.5 μ long and ~250–300 A in maximum width. The transverse sections through them vary in diameter depending on the level of the cut. × 81,000.

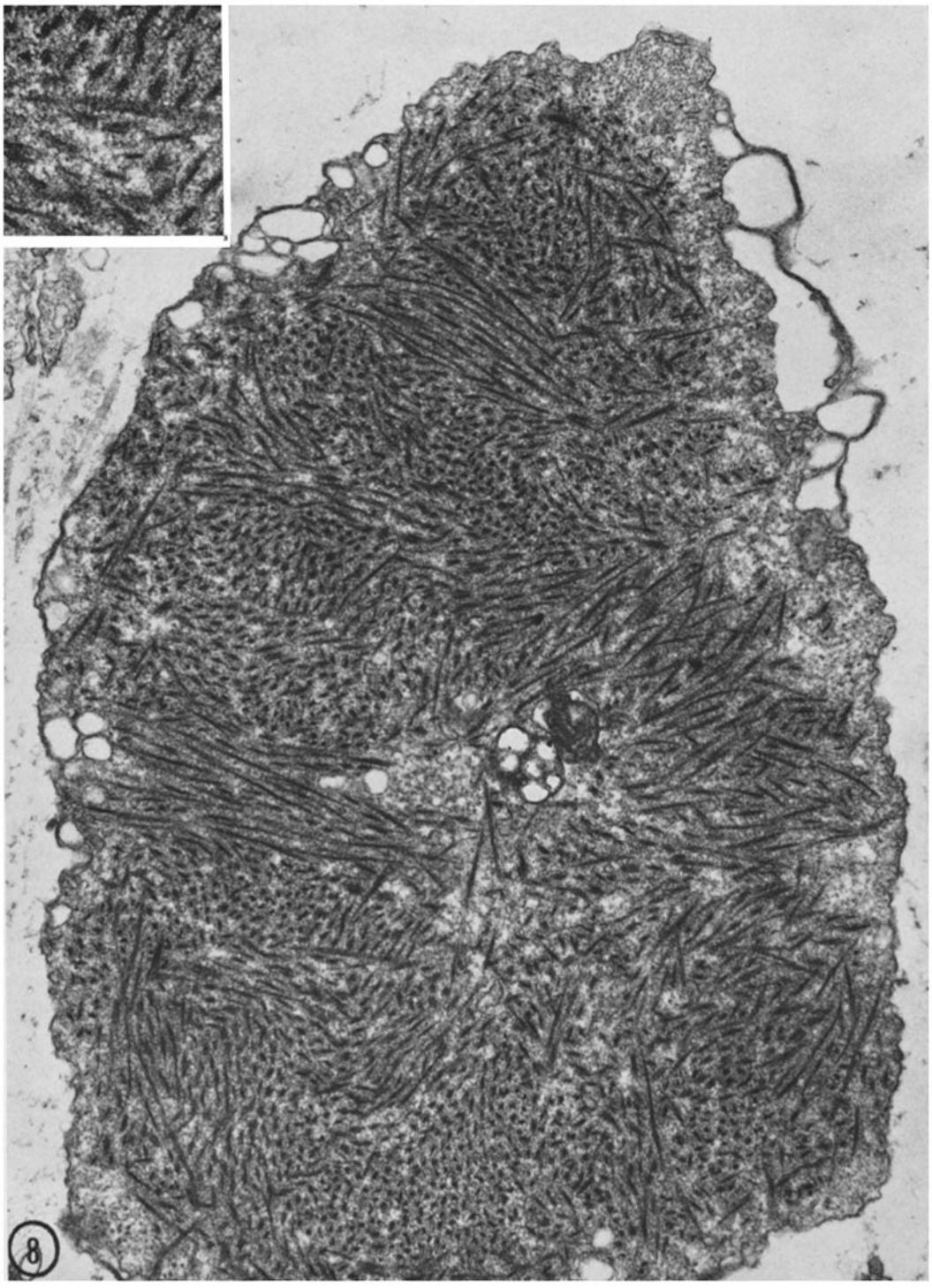

FIGURE 8 Trypsin-treated cell which is virtually filled with thick filaments. The filaments are strikingly uniform in size, shape, and spacing with respect to one another. *Inset.* Detail at higher magnification showing transverse striations in a thick filament. × 29,500; *inset*, × 68,000.

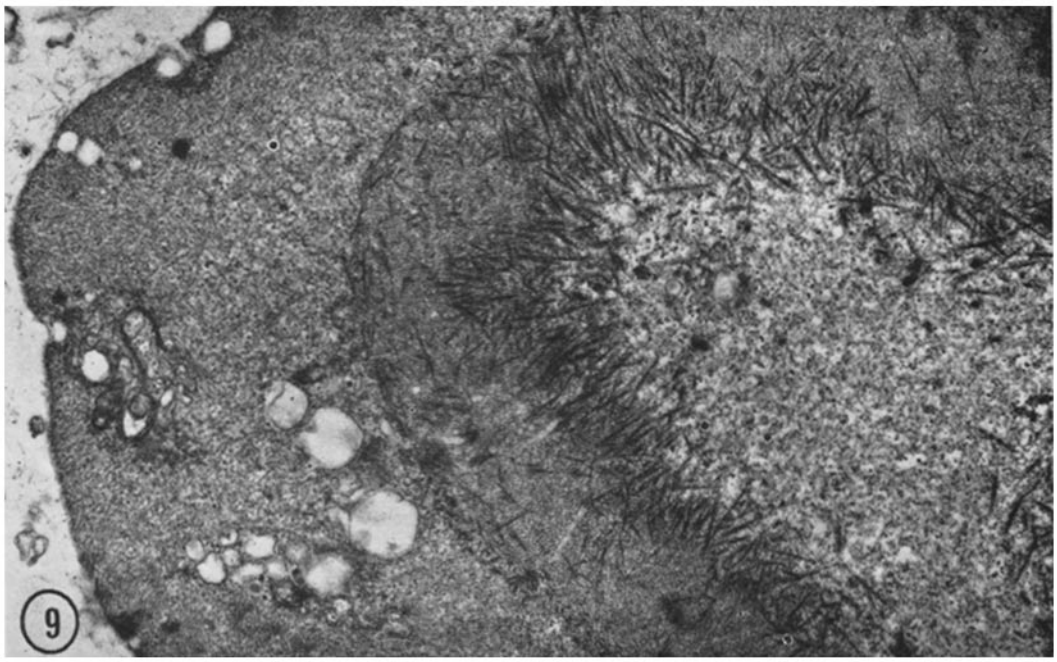

FIGURE 9 Trypsin-treated cell showing pronounced layering. Thick filaments are concentrated at two different interfaces. The cell looks almost as if it had been centrifuged. × 18,500.

also appear in such preparations, and, in addition, individual thick filaments are sometimes surrounded by a weblike halo which appears to be composed of very fine "whiskers" (cf., reference 10) oriented at a small angle to the thick filaments themselves. Substitution of the uranyl stain with either phosphotungstic acid or permanganate enhances the contrast of the thick filaments more and also brings out a transverse band pattern as well as a roughening or notching of the contour of some of the thick filaments (not shown). An even greater enhancement of thick filament contrast but without roughening, is achieved by en bloc staining of the tissue with uranyl acetate at pH 5.0, prior to dehydration, plus double staining of the sections with uranyl and lead salts (Figs. 5, 7, 8). Indeed, for surveying sections to find cells containing thick filaments, this last preparation is most convenient. An axial periodicity approximating 143 A is occasionally visible in such preparations (Fig. 8, inset). In specimens prepared by acrolein-dichromate fixation, large concentrations of thick filaments ~250–300 A in diameter can also be found, and in these preparations crossbanding at a period approximating 143 A is even more distinct (Fig. 11).

In control preparations which are not exposed to any artificial solutions before fixation and which are fixed in glutaraldehyde immediately upon removal from the living animal, followed by post-fixation in osmium tetroxide, images that might be interpreted as thick filaments are encountered infrequently in both longitudinal and transverse sections, regardless of the degree of contraction at the time of fixation. Specimens soaked in either buffered saline without trypsin or in diisopropyl fluorophosphate–inactivated trypsin or in a mixture of trypsin and soybean trypsin inhibitor in buffered saline display occasional thick filaments ~100–180 A in diameter, but in most instances, the cell containing them also has obvious interruptions in its plasma membrane, clumped nucleoplasm, absence of thin filaments, or swelling of mitochondria. Such thick filaments are encountered more commonly when the concentration of Tris buffer is 0.1 M rather than 0.02 M, and they are found frequently in cells prepared by the acrolein-dichromate method, which, like "inert dehydration" (22), does not preserve thin filaments or dense bodies. In general, thick filaments are encountered in those control prepara-

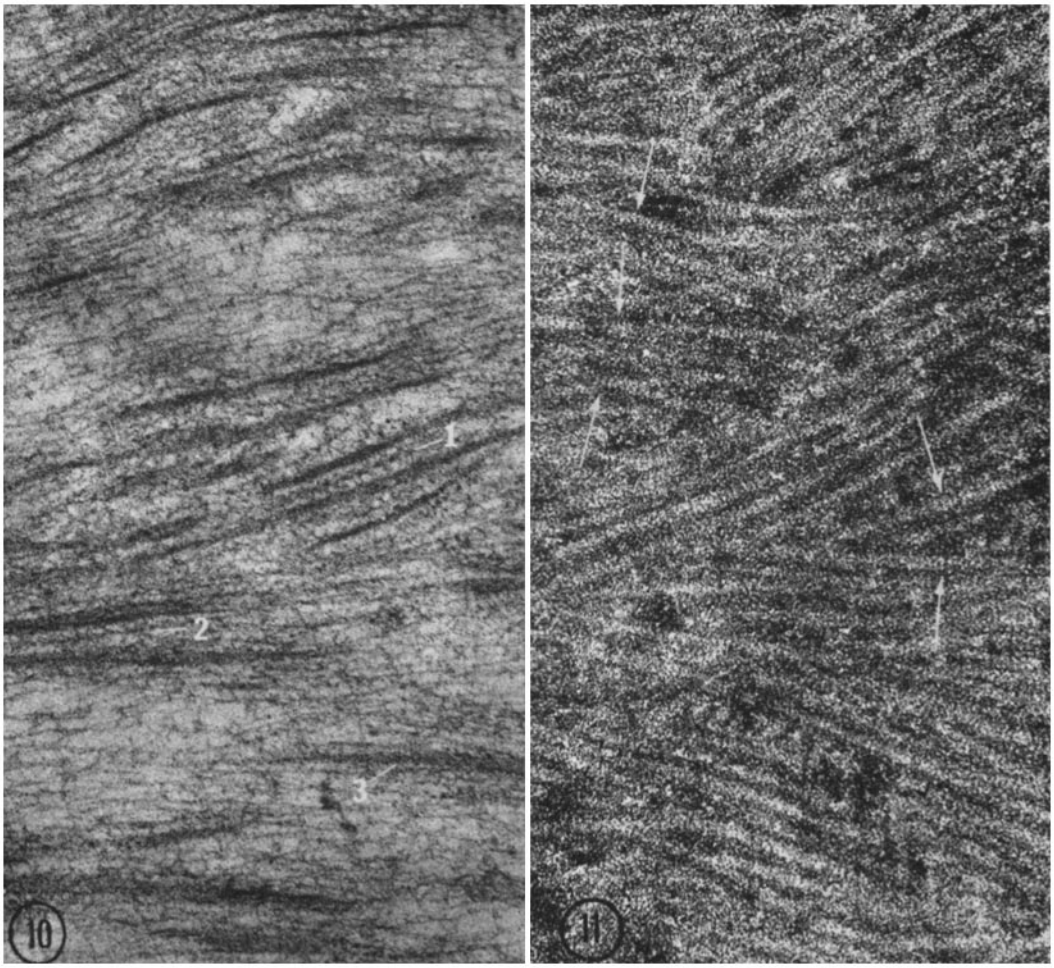

FIGURE 10 Thick and thin filaments intermingled in a trypsin-treated smooth muscle cell. One thin filament (*1*) situated between thick filaments appears beaded (270 A period) and resembles an "arrow-headed filament." A second thin filament (*2*) appears to be connected to thick filaments by fine bridges. A thick filament (*3*) appears to have fine filaments running obliquely into it. × 82,500.

FIGURE 11 Acrolein-dichromate fixed, trypsin-treated muscle (contrast reversed). The thick filaments, which appear light, exhibit cross striations repeating at a period approximating 143 A (arrows). × 63,000.

tions in which the preservation of other cell components is defective.

Cardiac muscle cells soaked in trypsin in the same way as the intestinal smooth muscle exhibit several changes. The effects are again nonuniform, some cells appearing quite like untreated cells, others as ghosts, and still others with intermediate changes. Fig. 13 shows two adjacent ventricular muscle cells, one of which exhibits the typical band pattern of striated muscle. The other cell, in contrast, has lost its internal organization: Z lines are absent, and thick and thin myofilaments are grossly disordered. As in the smooth muscle cells, thin filaments have become segregated to the periphery of the cell where they form tightly packed bundles. The altered cardiac muscle cell is not readily distinguishable from the trypsin-treated smooth muscle cell in Fig. 12.

DISCUSSION

This study shows that some smooth muscle cells exposed to trypsin and fixed by standard methods

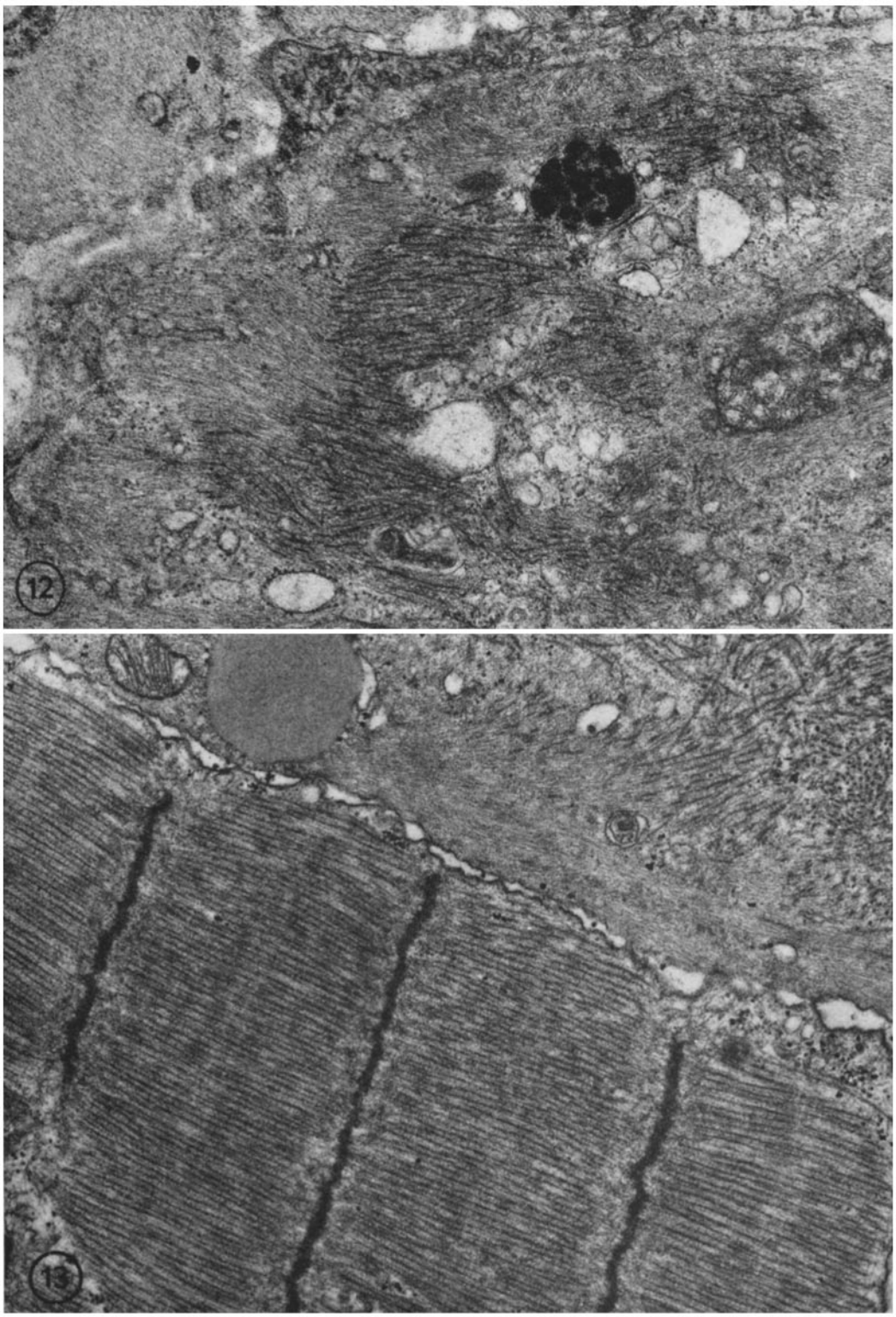

FIGURE 12 Trypsin-treated smooth muscle. Oriented thin filaments occupy the periphery of the cell. Thick filaments in this case are in approximate alignment with the thin filaments (cf., Fig. 13). × 27,500.

FIGURE 13 Trypsin-treated cardiac muscle. The lower cell is normal in appearance. The upper cell, in contrast, lacks Z lines, contains tightly packed thin filaments peripherally, and disorganized thick filaments centrally (cf., Fig. 12). × 27,500.

exhibit large numbers of thick filaments which resemble those that can be produced in vitro from extracted smooth muscle myosin (8, 11). In addition, these presumptive myosin aggregates are shown to have an axial periodicity approximating the 143 A period of myosin evident in X-ray diffraction diagrams of skeletal muscle. The thick filaments are usually segregated from the thin filaments, but where the two populations adjoin, configurations reminiscent of the A bands of striated muscle may be seen. Dense bodies are absent from the cells containing thick filaments, and the thin filaments have a tendency to form highly regular arrays at the cell periphery, perhaps reflecting the removal of myosin from among them. Such large numbers of thick filaments were not encountered in the cells of control samples fixed in the same way.

The first question raised by these findings is: What are the thick filaments composed of? Their resemblance in both size and shape to the thick filaments that can be produced in vitro from extracted smooth muscle myosin has already been pointed out. Aggregates of light meromyosin (LM M) assume the shape of tactoids as well but tend to be larger and more irregular in size. They are free of projections and may form a square lattice under some conditions (9). Such lattices were never seen in the present study. Ikemoto et al. (10) have shown what are probably composite aggregates of myosin and LMM in trypsin-treated preparations of actomyosin. Such aggregates tend to be larger than filaments composed of myosin alone, but unlike LMM aggregates they display projections from their surfaces. Thin filaments "decorated" with myosin (9) are only about half the width of the tactoids seen in the present study and are not tapered. Thus, the arrays of thick filaments observed here might represent aggregates of myosin or light meromyosin or a mixture of the two. The uniformity of their size suggests that they are predominantly myosin and that *in situ* as in vitro the aggregation of smooth muscle myosin ceases after reaching a maximum predetermined size.

The thick filaments described in this report have appeared under circumstances that are unquestionably abnormal. The obvious question raised is whether or not such structures also exist in smooth muscle under physiological conditions. Several reports of elongated, 120–300 A diameter filaments in "normal" smooth muscle cells have been published; however, in none of these was it established that the thick filaments seen in tissue sections did in fact have the characteristics of smooth muscle myosin aggregates. Moreover, certain associated morphological findings, such as clumped nucleoplasm (14) and the absence of thin filaments (22), suggest that the cells in which these thick filaments were found may have undergone changes before or during the preparative procedures and therefore cannot be considered to represent normal smooth muscle faithfully. The possible deleterious effects of extracellular ATP on living cells (14), of ischemia, particularly in excised muscle strips undergoing contraction, of ionic shifts due to the use of fixatives of high osmolarity (13, 14) or during inert dehydration (22), and of slow or incomplete penetration of either glutaraldehyde or osmium tetroxide into the depths of contracted muscle coils are all potential sources of artifactual change in these studies. Comparable filaments were also found in scattered cells of some control preparations in the present study; however, as noted in the Observations, these were usually associated with signs of cell damage or deficient preservation.

In an X-ray diffraction study of guinea pig taenia coli, Lowy et al. (17) report a meridional reflection at 144 A. In contrast to Kelly and Rice (14) who found thick filaments only in *contracted* smooth muscle, Lowy et al. found that this reflection, presumed to have arisen from myosin, was most prominent in *relaxed* muscle (at 12°–15°C) and was markedly diminished during contraction (at 37°C). In view of the possibility of cell damage or of conformational changes in myosin or other muscle proteins at temperatures below 16°C (16), which might predispose to the aggregation of myosin, the significance of this result remains to be determined.[4]

[4] In another paper, Rice et al. (*J. Cell Biol.* **47**:183) again report both thick and thin filaments (ratio 1:50) in contracted but not relaxed taenia coli muscle. The primary fixative used for the former was, however, 6.25% glutaraldehyde, which is strongly hyperosmotic, and for the latter 3% glutaraldehyde. Lowy and Small (*Nature, London.* **227**:46) have also published a new study in which the contractile response of guinea pig taenia coli muscle was eliminated by suspending it for 3–4 hr in Ringer's solution at 0°C. The muscle was then fixed at pH 7.1 at the same temperature for electron microscopy. The authors state that ribbon-shaped filaments, presumed to be myosin, and ordered arrays of thin filaments appear only under these "normal" conditions or after re-

Although vertebrate smooth muscle myosin can apparently form large aggregates *in situ* under certain extraordinary conditions, there is no clear evidence that such aggregates ever occur under physiological conditions. Contraction hypotheses based on the existence of myosin in the form of thick filaments in smooth muscle or based on reversible aggregation of myosin should therefore await studies in which extraneous causes of aggregation have been eliminated.

If it is assumed that smooth muscle myosin is normally not in the form of thick filaments, then a question arises as to what might prevent such aggregates from forming in the cells in view of the fact that extracted smooth muscle myosin is capable of aggregating in vitro. Several possible explanations are suggested by the data:

(*a*) The conformation of the smooth muscle myosin molecule may not allow aggregation to occur except after mild denaturation such as from "aging," glycerination, acidification, dehydration, repeated extraction (27), or, in the present case, trypsin treatment. Shoenberg (28) has suggested that proteolytic activity associated with impure collagenase may also induce the aggregation of smooth muscle myosin.

(*b*) Smooth muscle myosin may normally form a complex with some other protein which prevents aggregation, but which is sensitive to tryptic digestion. The absence of dense bodies and dense patches along the surface membrane of trypsin-treated cells exhibiting thick filaments suggests that some proteolysis has occurred in these cells.

(*c*) Smooth muscle myosin may normally be bound, in relatively disaggregated form, to actin filaments. Just as trypsin brings about a segregation of thick and thin filaments in cardiac muscle, it may serve to dissociate smooth muscle myosin from actin to a much greater degree than ever occurs normally. Once dissociated, the myosin may become segregated and then aggregate *in situ* in the same way that extracted smooth muscle myosin aggregates in vitro.

(*d*) The thick filaments that form after trypsin treatment may contain LMM or some other myosin fragment which presumably does not exist in the muscle normally. Even if LMM constituted only a small proportion of each filament, that small quantity might act as a nidus for aggregation *in situ*.

(*e*) Ionic or other conditions in the cytoplasm of these cells may not normally be conducive to the aggregation of myosin, but trypsin may damage the cell membrane in a selective way resulting in a change in the composition of the cytoplasm towards one which favors the aggregation of myosin. Shoenberg has suggested that an influx of calcium ions, for example, could promote myosin aggregation (28). Similarly, Ikemoto et al. showed that aggregation of myosin tails in an actomyosin gel can be induced not only by trypsin treatment but also by alterations in the ionic environment (10). The fact that occasional thick filaments, which may represent myosin aggregates, sometimes appear in soaked control specimens, especially those showing signs of damage, is consistent with this possibility; however, the effect could also be ascribed to the action of endogenous proteases in cells undergoing autolysis, or to the leaching out of relatively soluble muscle proteins, e.g. tropomyosin, during prolonged soaking.

Of these various possibilities, the simplest one is the first, i.e., that trypsin brings about a change in the conformation of smooth muscle myosin by a direct action on the myosin molecule itself. Conformational changes have also been assumed to account for the increase in calcium-activated ATPase activity that can be produced in smooth muscle myosin, but not in skeletal muscle myosin, by a variety of agents including trypsin (1). On the other hand, it has been possible to induce the formation of thick filaments from smooth muscle myosin in vitro, suggesting that denaturation of the myosin may not be necessary for its aggregation. Although such procedures as glycerination, or dialysis at acid pH used in extracting and isolating smooth muscle myosin may themselves introduce some conformational changes in the molecule, the alternative possibility remains that *in situ* smooth muscle myosin is normally maintained in a relatively disaggregated state as a part of a stable complex with other muscle proteins and that it is one of these other proteins or its association with myosin that is somehow disturbed by trypsin as well as by other agents that promote the aggregation of smooth muscle myosin.

Regardless of how trypsin acts to produce two distinguishable populations of filaments in these cells, the phenomenon shows that large numbers of filaments having the characteristic shape, size, and periodicity of smooth muscle myosin aggregates

---

laxation has been achieved by exposing the muscle to solutions made hyperosmolar with sucrose. The ratio of ribbons to thin filaments was 1:70.

can be seen easily in tissue sections prepared by standard methods when such filaments are present. The fact that they have appeared so prominently under completely abnormal circumstances and are not seen ordinarily in this muscle is consistent with the view that normally smooth muscle myosin exists, intermixed with actin, in some relatively disaggregated state by virtue of its conformation or its association with other muscle proteins, but that under certain abnormal conditions it is capable of forming thick filaments *in situ*.

A report of this study was presented at the annual meeting of the American Association of Anatomists held in April, 1970 (26).

This investigation was supported by Grants NS 07197 and NS 07495 from the National Institutes of Health, United States Department of Health, Education, and Welfare.

Received for publication 15 April 1970, and in revised form 28 July 1970.

REFERENCES

1. BÁRÁNY, M., K. BÁRÁNY, E. GAETJENS, and G. BAILIN. 1966. Chicken gizzard myosin. *Arch. Biochem. Biophys.* **113**:205.
2. CHOI, J. K. 1962. Fine structure of the smooth muscle of the chicken's gizzard. *In* Electron Microscopy. S. Breese, Jr., editor. Academic Press Inc., New York. **2**:M9.
3. EBASHI, S., H. IWAKURA, H. NAKAJIMA, R. NAKAMURA, and Y. OOI. 1966. New structural proteins from dog heart and chicken gizzard. *Biochem. Z.* **345**:201.
4. ELLIOTT, G. F. 1967. Variations of the contractile apparatus in smooth and striated muscles. *J. Gen. Physiol.* **50**(Suppl.):171.
5. ELLIOTT, G. F., and J. LOWY. 1968. Organization of actin in a mammalian smooth muscle. *Nature (London).* **219**:156.
6. FISCHMAN, D. A., and A. A. MOSCONA. 1969. An electron microscope study of in vitro dissociation and reaggregation of embryonic chick and mouse heart cells. *J. Cell Biol.* **43**:37 a.
7. HANSON, J., and J. LOWY. 1960. Structure and function of the contractile apparatus in the muscles of invertebrate animals. *In* Structure and Function of Muscle. G. H. Bourne, editor. Academic Press Inc., New York. **1**:265.
8. HANSON, J., and J. LOWY. 1964. The problem of the location of myosin in vertebrate smooth muscle. *Proc. Roy. Soc. Ser. B.* **160**:523.
9. HUXLEY, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7**:281.
10. IKEMOTO, N., S. KITAGAWA, A. NAKAMURA, and J. GERGELY. 1968. Electron microscopic investigations of actomyosin as a function of ionic strength. *J. Cell Biol.* **39**:620.
11. KAMINER, B. 1969. Synthetic myosin filaments from vertebrate smooth muscle. *J. Mol. Biol.* **39**:257.
12. KARNOVSKY, M. J. 1961. Simple methods for "staining with lead" at high pH in electron microscopy. *J. Biophys. Biochem. Cytol.* **11**:729.
13. KELLY, R. E., and R. V. RICE. 1968. Localization of myosin filaments in smooth muscle. *J. Cell Biol.* **37**:105.
14. KELLY, R. E., and R. V. RICE. 1969. Ultrastructural studies on the contractile mechanism of smooth muscle. *J. Cell Biol.* **42**:683.
15. LANE, B. P. 1965. Alterations in the cytological detail of intestinal smooth muscle cells in various stages of contraction. *J. Cell Biol.* **27**: 199.
16. LEVY, H. M., N. SHARON, E. M. RYAN, and D. E. KOSHLAND, JR. 1962. Effect of temperature on the rate of hydrolysis of adenosine triphosphate and inosine triphosphate by myosin with and without modifiers. Evidence for a change in protein conformation. *Biochim. Biophys. Acta.* **56**:118.
17. LOWY, J., F. R. PAULSON, and P. J. VIBERT. 1970. Myosin filaments in vertebrate smooth muscle. *Nature (London).* **225**:1053.
18. NEEDHAM, D. M., and C. F. SHOENBERG. 1964. Proteins of the contractile mechanism of mammalian smooth muscle and their possible location in the cell. *Proc. Roy. Soc. Ser. B.* **160**: 517.
19. NONOMURA, Y. 1968. Myofilaments in smooth muscle of guinea pig's taenia coli. *J. Cell Biol.* **39**:741.
20. PANNER, B. J., and C. R. HONIG. 1967. Filament ultrastructure and organization in vertebrate smooth muscle. Contraction hypothesis based on localization of actin and myosin. *J. Cell Biol.* **35**:303.
21. PANNER, B. J., and C. R. HONIG. 1970. Locus and state of aggregation of myosin in tissue sections of vertebrate smooth muscle. *J. Cell Biol.* **44**:52.
22. PEASE, D. C. 1968. Structural features of unfixed mammalian smooth and striated muscle prepared by glycol dehydration. *J. Ultrastruct. Res.* **23**:280.
23. RICE, R. V., A. C. BRADY, R. H. DEPUE, and R. E. KELLY. 1966. Morphology of individual macromolecules and their ordered aggregates by electron microscopy. *Biochem. Z.* **345**:370.

24. ROBISON, JR., W. G., and B. H. LIPTON. 1969. Advantages of dichromate-acrolein fixation for preservation of ultrastructural detail. *J. Cell Biol.* **43**:117 a.
25. ROSENBLUTH, J. 1968. Obliquely striated muscle. IV. Sarcoplasmic reticulum, contractile apparatus, and endomysium of the body muscle of a polychaete, *Glycera*, in relation to its speed. *J. Cell Biol.* **36**:245.
26. ROSENBLUTH, J. 1970. Thick myofilaments in trypsin treated smooth muscle cells. *Anat. Rec.* **166**:369.
27. SCHIRMER, R. H. 1965. Die Besonderheiten des contractilen Proteins der Arterien. *Biochem. Z.* **343**:269.
28. SHOENBERG, C. F. 1969. An EM study of the influence of divalent ions on myosin filament formation in chicken gizzard extracts and homogenates. *Tissue and Cell.* **1**:83.
29. SHOENBERG, C. F., J. C. RÜEGG, D. M. NEEDHAM, R. H. SCHIRMER, and H. NEMTCHEK-GANSLER. 1966. A biochemical and EM study of the contractile proteins in vertebrate smooth muscle. *Biochem. Z.* **345**:255.