LOCUS AND STATE OF AGGREGATION OF MYOSIN IN TISSUE SECTIONS OF VERTEBRATE SMOOTH MUSCLE

BERNARD J. PANNER and CARL R. HONIG

From the Departments of Pathology and Physiology, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14620

ABSTRACT

Structures with the characteristics of molecular myosin were identified by electron microscopy in tissue sections of vertebrate smooth muscle. No thick filaments of myosin were found regardless of preparative procedures, which included fixation at rest and in contraction, glycerine extraction, and storage at low pH prior to fixation. Absence of thick myosin filaments and presence of what appear to be myosin molecules is in accord with conclusions based on X-ray diffraction (3, 12) and birefringence data (4) from living smooth muscles at rest and in contraction. Explanations are provided for appearances thought by others (6, 20, 21) to represent thick myosin filaments. Our present observations are in accord with the model for smooth muscle contraction which we have previously proposed (1).

INTRODUCTION

We have previously suggested a contraction hypothesis for vertebrate smooth muscle (1) based on the following electron microscopic observations: (*a*) All longitudinal filaments in sections of gizzard smooth muscle are composed of actin. (*b*) No thick myosin filaments can be found in tissue sections. (*c*) Monomers and dimers of myosin exist, both free and attached to actin, in fresh gizzard homogenates prepared at very low ionic strength, even in the presence of added Ca^{++} and Mg^{++}. (*d*) Actin filaments are collated by the elliptical, dense bodies into sets which are the equivalent of the sarcomere of striated muscle. It was suggested that contraction occurs through sliding of actin filaments of opposite sense, driven by interaction with numerous myosin dimers or tetramers.

The foregoing is consistent with observations based on filament widths (*a*), filament counts (*b*), X-ray crystallography (*c*), birefringence (*d*), and smooth muscle mechanics (*e*), all of which indicate that a sliding mechanism is involved. Our hypothesis reconciles these evidences in favor of a sliding mechanism with the presence of only one type of longitudinal filament in fresh homogenates and tissue sections (1). Failure to find thick myosin filaments cannot prove their absence. The principal observation we now report is a positive one, namely, that structures with the morphologic characteristics of myosin molecules and/or dimers exist in sections of gizzard fixed both at rest and in contraction.

METHODS

Sections of turkey gizzard were prepared for electron microscopy in both methacrylate and Epon as described previously (1). In a recent report by Kelly and Rice (6), prolonged storage and low pH were thought to favor aggregation of myosin into thick filaments comparable to those of striated muscle. We have attempted to reproduce their results by using the experimental conditions they described. These conditions include preparation at pH 6.2 and 7.4, prolonged storage in glycerin before fixation,

use of high concentration of Ca-ATP, and removal of Ca^{++} by EGTA. Our techniques (1) and those of Kelly and Rice (6) have been previously described. Most electron microscopic images were recorded at primary magnifications of 50,000. Primary magnifications were checked by photographing a diffraction grating replica. Final photographic magnifications were calibrated by using plates of the grating replica to obtain final print magnifications of 100,000–500,000.

RESULTS

Identification of Myosin

In sectioned material, structures having the appearance of molecular myosin are found (Figs. 1–3). A typical molecule is shown at high magnification in Fig. 1, *inset*. It consists of a head which appears to be composed of two globular subunits arranged linearly. Similar head substructure has been described for isolated, shadow-cast myosin by Carney and Brown (7) and by Lowey et al. (8). Dimensions of this particular head are 60 × 120 A. The tail is almost aligned in long axis of the head and can be followed for about 600 A. It is 15–20 A wide. Between head and tail a small break in continuity can be seen. The position of this break corresponds to the position of the trypsin-sensitive "hinge" region of the myosin molecule. Several other structures with similar morphology are seen in Fig. 1 and in other sections prepared under different conditions.

The four principal morphologic features of myosin are again evident in Fig. 2. These are: dimensions, globular head, linear tail, and apparent bend and/or break at head-tail junction. Fig. 2 was photographically enlarged to 500,000 ×, the highest magnification we have employed. Images which we interpret as myosin molecules stand out as structures of uniformly decreased electron opacity against a darker globular background. The qualitative difference in electron opacity of the myosin molecules shown in Figs. 1 and 2 is attributable to uncontrolled variations in focus, phase contrast, section thickness, intensity of heavy metal staining, and possibly other physical factors. It has been our experience that primary magnifications of 50,000 or more are necessary for resolution of the myosin molecule in thin tissue sections. Neither in Figs. 1 and 2 nor in any other electron micrograph of sectioned gizzard have we been able to identify thick filaments of myosin.

Effect of Preparative Conditions on Appearance of Myosin

FIXATION, EMBEDDING AND STAINING: Use of glutaraldehyde without osmium tetroxide

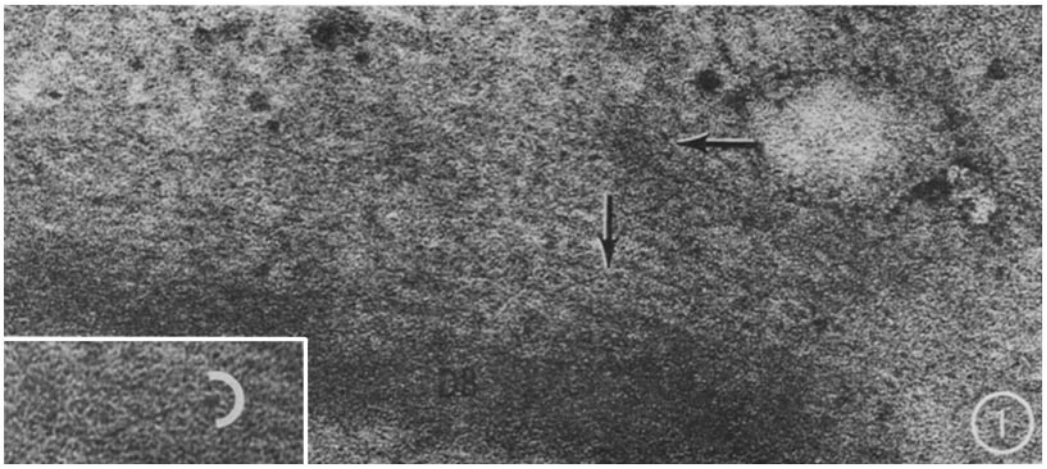
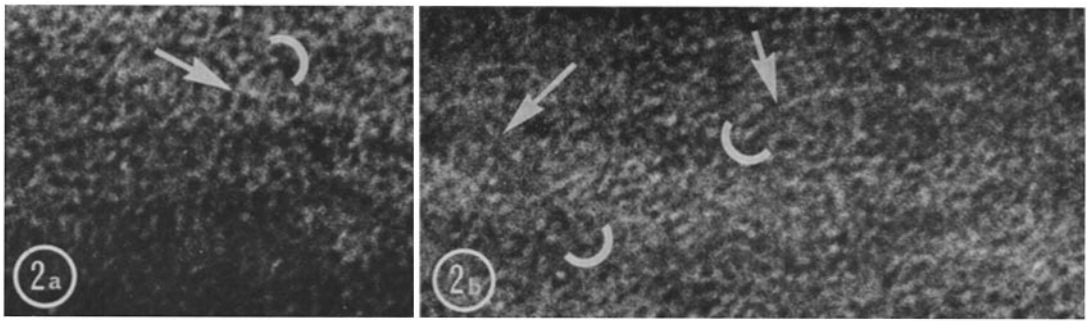


FIGURE 1 Longitudinal section of gizzard smooth muscle fixed in glutaraldehyde buffered to pH 6.2 immediately after removal and embedded in Epon. Sections stained with lead. An elliptical dense body (*DB*) is present. Arrows point to individual myosin molecules with ovoid heads and linear tails. Parts of other myosin molecules are also apparent. × 150,000. The myosin molecule indicated by the vertical arrow is shown in the *inset*. The head is about 60 A wide and 120 A long (semicircle). It appears to contain two globular subunits. The tail is 15–20 A wide and can be followed about 600 A. × 250 000



FIGURES 2 *a* and 2 *b* Longitudinal sections of smooth muscle from gizzard soaked for 1 1/2 hr in buffered salt solution pH 6.2 with added 1 mM EGTA, fixed in glutaraldehyde, pH 6.2, postfixed in OsO_4, and section stained with lead. Semicircles indicate myosin heads while arrows point to myosin tails. Linear arrays of dark globular actin subunits can be seen. Myosin heads are about 50–60 A wide and about 120 A long. Myosin tails can be identified with lengths up to about 600 A and widths of about 15–20 A. Break in continuity appears to exist between head and tail. × 500,000.

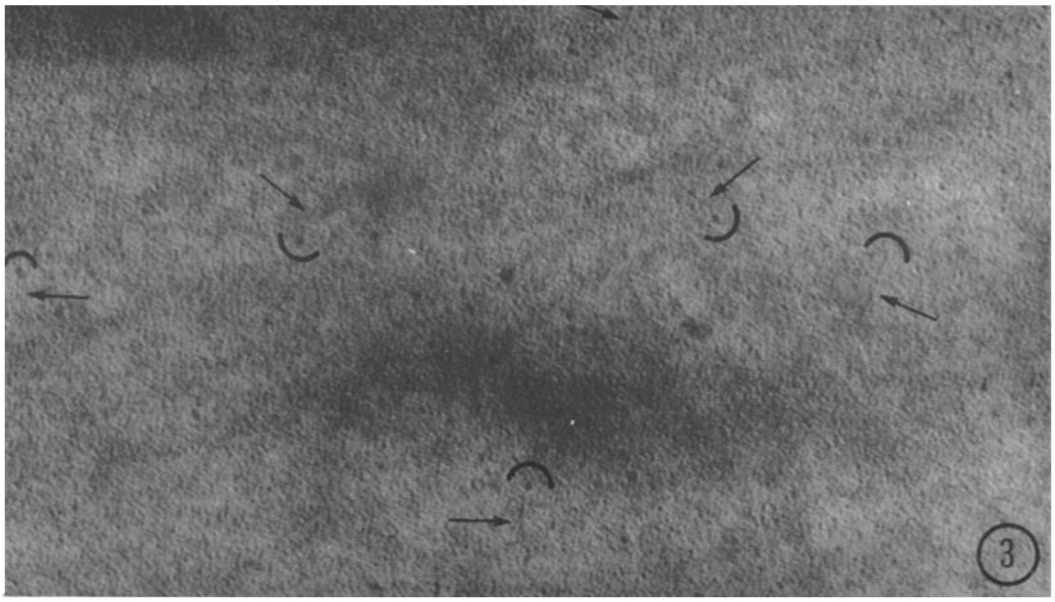


FIGURE 3 Section of longitudinally-oriented gizzard smooth muscle fixed in glutaraldehyde, pH 7.4, immediately after removal and embedded in Epon. Section was lightly stained with uranyl acetate. Reduced staining of actin aids in identifying myosin molecules (semicircles and arrows). × 150,000.

postfixation minimizes electron opacity of the predominant longitudinal actin filaments. Under these conditions structures which lie between actin filaments and thought to be myosin molecules can be more readily identified (Fig. 3). It is our impression that absence of osmium improves preservation of myosin structure, as might be expected from the known effects of osmium on muscle proteins (9). Brief staining of the section with uranyl acetate, as in Fig. 3, improves visibility of myosin, especially the globular portion of the molecule, without substantially increasing actin electron opacity. It also permits easier recognition of the large number and wide distribution of these myosin appearances.

Sections embedded in methacrylate exhibit

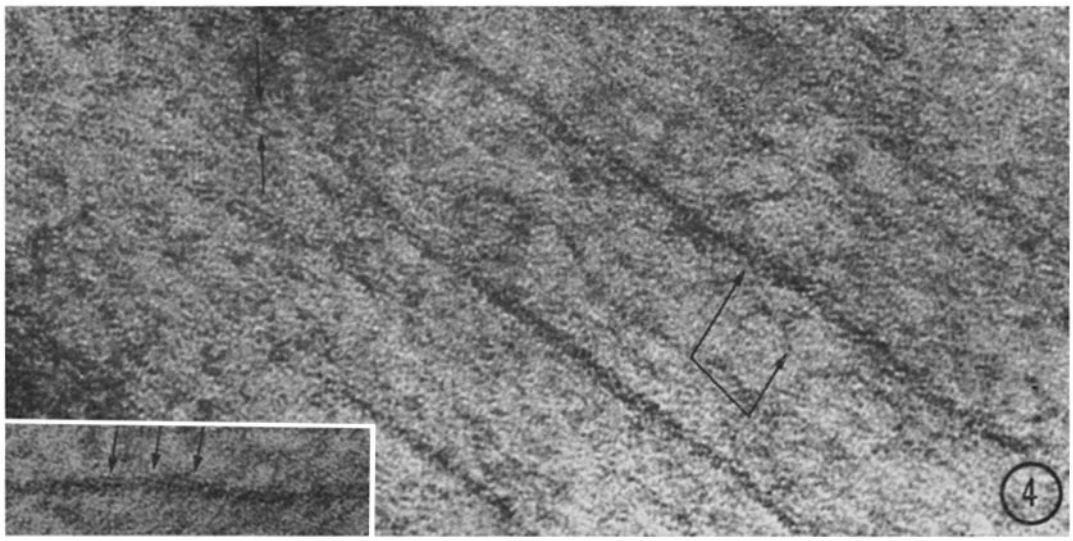


FIGURE 4 Gizzard smooth muscle fixed in glutaraldehyde at pH 7.4, postfixed in OsO_4, and embedded in methacrylate. Actin filaments composed of globular subunits have an average width of 60 A. Dense areas along individual actin filaments are also composed of globular subunits and are also about 60 A wide. Arrows point to myosin molecules. The linked arrows indicate a myosin molecule which appears attached or in proximity to an actin filament. × 250,000. *Inset.* Muscle prepared as in Fig. 4. Short arrows point to myosin molecules which may be attached to actin to form a compound actomyosin filament. × 250,000.

specialized regions of increased electron opacity along actin filaments (1). In some of these dense regions structures thought to be myosin are aligned in closer longitudinal approximation to actin than in others (compare Fig. 4 and Fig. 4, *inset*). Where myosin lies close to actin it accentuates the apparent density and width of the longitudinal filaments (Fig. 4, *inset*). It is probable, however, that not only myosin, but tropomyosin, troponin, and possibly other proteins (10) also contribute to variations in electron opacity along the smooth muscle actin filament, and contribute to its apparent width.

EFFECTS OF LENGTH AND CONTRACTION: No thick filaments of myosin are observed in sections from muscles fixed in contraction or from muscles at rest before application of fixative. Longitudinal filament widths are the same in muscles allowed to shorten and in those held at rest length during fixation. This width is consistent with the dimensions of actin. Under all the above conditions structures with the morphological characteristics of myosin molecules could be identified.

Effect of Storage, Glycerination, and pH

Muscles trimmed to 1–2 mm in width and 1 cm in length were stored at rest length at −20°C for up to 4 months in 50% glycerol buffered at pH 7.4. These specimens showed no areas of empty cytoplasm and only slight disruption of cytomembranes. Filament ultrastructure in sections from such tissue blocks is in no way different from that illustrated in Figs. 1, 3, and 4, which were obtained from muscles fixed immediately upon removal from the animal. In contrast, comparable muscle samples stored for 4 months at −20°C in glycerol buffered at pH 6.2 showed extensive tissue alteration even to the naked eye; the muscle blocks were decolorized and translucent. There is complete absence of recognizable ultrastructure in sections prepared from these blocks, except for a few nuclear fragments and cytoplasmic debris. Muscles fixed after 1 wk of storage at −20°C in glycerol buffered at pH 6.2 were not decolorized, but sections taken from the superficial layers of the specimen blocks reveal loss of filament ultrastructure and partial disruption of cytomembranes. No thick filaments of myosin are found in these

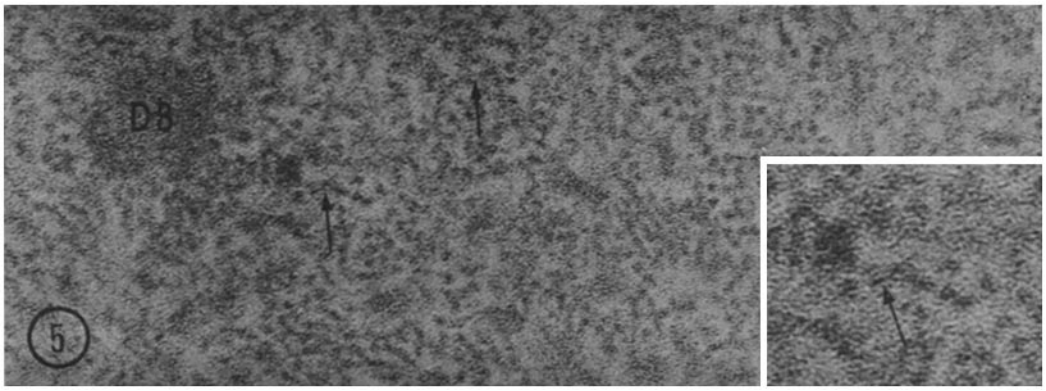


FIGURE 5 Cross-section of gizzard smooth muscle soaked 1½ hr in salt solution, pH 6.2, with 5 mM ATP added. Fixed in glutaraldehyde, pH 6.2, postfixed in OsO_4, and embedded in Epon. Two dense bodies (*DB*) are shown in cross section. No thick filaments are seen. Actin filaments are not all cut in true cross section. Dimensions are similar for both dark- and light-staining filaments (about 60 A). Arrows point to structures regarded as myosin molecules. × 150,000. *Inset* shows myosin molecule with two linear structures joining at the neck. × 250,000.

damaged regions. Sections obtained from central portions of blocks stored at pH 6.2 are not significantly different from sections from muscles conventionally prepared, and they contain numerous appearances like those identified as myosin in Figs. 1–3.

Effect of Ca^{++} and ATP

Structures typical of myosin molecules can be found in specimens from muscles soaked 1½ hr at 4°C in 1 mM $MgCl_2$, 50 mM KCl, and either 20 mM Tris HCl, pH 7.4 or 20 mM imidazole, pH 6.2. The results are the same in presence of 5 mM ATP and in absence of added ATP. No Ca^{++} was added to the extraction medium, but the concentration of ionized contaminant Ca^{++} was between 5×10^{-6} and 1×10^{-5} M. These concentrations fully activate the actomyosin ATPase, promote superprecipitation of gizzard myosin B, and maximally activate the ATPase of glycerinated gizzard myofibrils (unpublished observations). No thick myosin filaments are observed in sections of gizzard soaked either at pH 7.4 or 6.2. Instead, individual myosin molecules are found in longitudinal and in cross sections (Fig. 5). Fig. 5, *inset*, at higher magnification, shows what appears to be a myosin molecule with two tails, similar to Fig. 6 of Carney and Brown (7). It is not possible to measure the individual tails with sufficient accuracy to determine whether these may represent separation of a two-stranded structure or superimposition.

Myosin in Fresh Homogenates

Gizzards were homogenized in 50 mM KCl, 20 mM imidazole, pH 7.0, and the homogenates were examined within 15 min by negative staining (1). No myosin aggregates were observed, even if 5×10^{-4} M Ca^{++} was added and up to 10 mM Mg was present. On one occasion spindle-shaped structures devoid of projections and up to 2300 A long and 200 A wide were found (Fig. 6). These exhibit longitudinal substructure but no axial periodicity, as in the "needles" of light meromyosin shown in Plate XV (*b*) of Huxley (11). Thus we can obtain aggregates from freshly homogenized gizzard, but only if catheptic activity is present, as indicated by loss of myosin heads.

Actin

Smooth muscle fixed in glutaraldehyde exhibits uniform longitudinal filaments with regular interfilament spacing (Fig. 7; reference 1, Fig. 2). The regular interfilament distance of 110–120 A is in remarkably good agreement with values obtained by Elliott and Lowy (12) for living taenia coli from low angle X-ray diffraction diagrams. This regularity of packing appears responsible for the high form birefringence of vertebrate smooth muscles (4). Primary fixation in osmium tetroxide rather than glutaraldehyde leads to variability in electron opacity of individual actin filaments (1) and destroys the regularity of filament spacing which exists in vivo (12).

FIGURE 6 Specimen from gizzard homogenized in 20 mM imidazole, pH 7.0, 50 mM KCl, without added Mg^{++} or ATP; contaminant Ca^{++} was about 10^{-5} M. Negatively stained about 15 min after homogenization. Sharply tapering needlelike aggregates with longitudinal substructure and devoid of lateral projections are shown, consistent with "crystals" of light meromyosin. Actin filaments are also present. × 150,000.

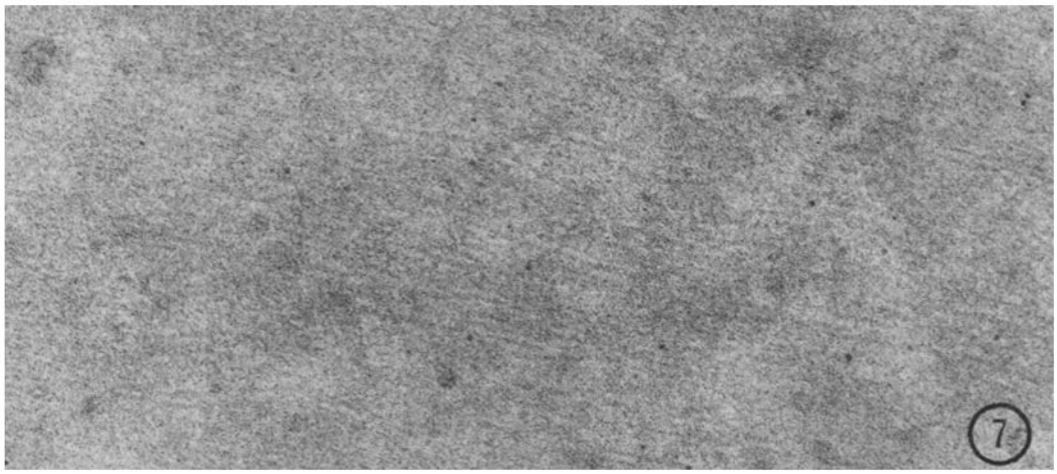


FIGURE 7 Longitudinal section of glutaraldehyde-fixed smooth muscle postfixed in OsO_4 and embedded in Epon. Regular spacing between actin filaments is well maintained at 100–125 A. × 100,000.

Phosphotungstic acid (PTA) greatly increases the electron opacity of actin and, if applied excessively in the block, nonspecific staining of background material and of proteins associated with actin can increase filament width as an artifact. Regions of increased width also can be produced by disjunction of the two actin strands. Both artifacts can give rise to appearances which might be mistaken for a thick filament. Material embedded in methacrylate (1) or in Araldite (13) exhibits electron-opaque regions along actin filaments (Fig. 4). These are not observed in Epon-embedded material similarly fixed and stained. Despite the dark appearance which, especially at low magnification, gives an erroneous impression of a thick filament, the dimensions and globular

substructure seen at high magnification identify the filament as actin (Fig. 4 and Fig. 4, *inset*).

DISCUSSION

Experimental

Our principal result is that tissue sections of gizzard contain structures identical in morphology to individual molecules of myosin isolated by chemical methods and visualized after shadow-casting (7, 8, 11, 14) or negative staining (1). The dimensions of these structures are also consistent with dimensions of smooth muscle myosin inferred from hydrodynamic data (15). It is considerably easier to obtain well-resolved images of molecular myosin by use of shadow-casting than it is to demonstrate myosin in sectioned material. The best resolution obtainable in conventional electron microscopy of stained, sectioned tissue is about 20 A. Only in exceptional cases can this performance be significantly improved (16). Since myosin heads are about 20–30 A wide, and the tail is about 15 A wide, it is clear that we are working at about the practical limits of the method in the present study. Though the pictures reflect this fact, it is nevertheless possible to identify the characteristic morphological features of myosin.

We have tried to improve definition of myosin in the final prints by use of optical filtration (17). Unfortunately, enhancement of high frequencies characteristic of myosin tails obscures ovoid myosin heads, and enhancement of low frequencies to improve resolution of heads eliminates the tails. Spatial filtering of Fig. 7, which was designed to enhance structures oriented at an angle to the longitudinal actin filaments, made possible visualization of images thought to be myosin which were not observable in conventional prints. Fig. 3 also indicates that the predominant actin filaments tend to obscure myosin. It is therefore unlikely that the amount of myosin could ever be judged from electron micrographs. The amount which could be accommodated by the arrangement we propose (1) seems sufficient, however, to account for the myosin found by chemical analysis (18).

Problems of resolution are compounded by possible disruption of molecular structure during specimen preparation. Our inability to observe lengths greater than about 1200 A in the plane of section, in addition to the foregoing technical factors, render it impossible to determine whether the long myosin tails seen in sectioned smooth muscle existed in vivo as individual molecules or as side-side or end-end dimers as required by the contraction mechanism we have proposed (1).

Results of Others

THICK FILAMENTS FROM HOMOGENATES: Several investigators have reported that thick filaments can be produced in vitro by lowering the ionic strength of solutions of smooth muscle myosin (6, 19, 20, 21). These filaments tend to be somewhat shorter than those obtainable from striated muscle and exhibit widths ranging from 110–250 A. In our experience, fresh, negatively stained gizzard homogenates do not contain thick myosin filaments. Occurrence of light meromyosin "crystals" in some homogenates (Fig. 6) indicates how rapidly smooth muscle myosin can be degraded in vitro. It is therefore not surprising that in aged preparations (6) or in those deliberately treated with a proteolytic enzyme (20) thick filaments may be found. Even skeletal muscle myosin, which normally remains in molecular form at high ionic strength, will aggregate in 0.6 M KCl upon aging.

Further evidence that thick myosin filaments do not exist in living smooth muscle arises from comparison of width of thick filaments with the 110–125 A interactin distances observed by X-ray diffraction (12) and electron microscopy (Fig. 7). The maximum interactin distance in such a lattice would be about 180 A. A functional myosin aggregate must therefore be less than 180 A in diameter, but some structures thought by others to be thick filaments of myosin are of considerably greater width (6, 21). Filaments between 110 A and 180 A could be accommodated in the regular interactin spaces of living smooth muscle, but only if the arrangement of thick filaments is also regular. Such regularly spaced large myosin filaments might be expected to yield reflections in X-ray diffraction diagrams, and in electron micrographs the cross sections should resemble A bands. No myosin reflections have ever been observed by X-ray diffraction, nor has regularity of spacing been demonstrated among thick filaments purported to be myosin.

If thick myosin filaments do not exist in vivo, how can one account for the thick filaments which have been reported in sectioned smooth muscle (6, 21, 22)? Upon careful examination of these structures we conclude that they cannot be identified as myosin on the basis of their substructure.

Since thick filaments can readily be produced in smooth muscle sections as preparative artifacts, we suggest that the following criteria be met before concluding that a thick filamentous structure is indeed composed of myosin:

(*a*) The spacing of thin actin filaments should be regular.

(*b*) Order should be demonstrable in the arrangement of thick filaments, with respect both to each other and to actin.

(*c*) Magnification and resolution should be adequate to identify the substructure of the myosin aggregate, namely projections characteristic of myosin heads, bare central region, and distal taper. In most previous studies of sectioned vertebrate smooth muscle, magnifications have been used which are too low to visualize molecular dimensions and, indeed, often too low for accurate measurement of filament width.

(*d*) Technique should be adequate to resolve the globular substructure of actin filaments, in order to further exclude the possibility that thick filaments about 140 A in diameter are due to side-to-side association of two actin filaments, or to formation of a compound actomyosin filament.

(*e*) The diameter of presumed myosin filaments in sectioned muscle should not exceed the maximum diameter of aggregates of smooth muscle myosin produced synthetically in vitro.

Review of all published electron micrographs that are thought to demonstrate thick filaments of myosin have failed to reveal any filaments which meet the above criteria. The thick filaments recently described by Kelly and Rice (6) are randomly distributed. Large areas of their sections contain no thick filaments, and some areas are devoid of all filaments (Figs. 8 and 12 of reference 6). Figs. 1, 2, and 4 of Nonomura (21) also demonstrate randomly arranged thick filaments of variable diameter, with minimum distances between thick filaments of about 700 A. In cross-section, structures identified by Nonomura as thick filaments are resolvable into contiguous ovoid densities each with dimensions characteristic of actin. The mean filament diameter for the primary peak in Nonomura's bimodal frequency distribution of filament diameters is consistent with actin dimensions, and the mean diameter for the small secondary peak is about twice the primary one, again suggesting side-to-side apposition of actin. Actin shows strong electrical birefringence (23) and may be held in its regular array in vivo by mutual repulsion provided by fixed charges along the filaments (12). Charge neutralization by preparative techniques could account for loss of regular actin filament spacing and apposition of filaments.

Kelly and Rice attribute the presence of thick filaments in their sections to aggregation of myosin during prolonged storage of the specimens in glycerin at pH 5.8–6.6 (6). In our experience, however, glycerination at pH 6.2 for even 1 wk resulted in disruption of normal ultrastructure in the superficial portions of the tissue blocks, and material so stored for 4 months showed loss of ultrastructure throughout the specimen. We can readily find molecules of myosin in superficial portions of specimens stored briefly at pH 6.2 and in the relatively intact central portions of blocks stored at pH 6.2 for up to 1 wk. Perhaps the blocks which Kelly and Rice stored for 6 months were sufficiently large to provide less damaged tissue in the depths of the specimen, but is is difficult to know what the pH might have been in these usable portions.

Shoenberg (20) has recently proposed that myosin exists in molecular form in resting smooth muscle and reversibly aggregates into thick filaments when activation raises intracellular Ca^{++}. Though one cannot exclude the possibility of contraction-coupled aggegation of myosin, it seems extremely unlikely. The belief that smooth muscle cells contract slowly enough to permit reversible aggregation of myosin is based on older data for tetanic contractions in large cell populations. When a single action potential in an individual smooth muscle cell is correlated with the tension developed by very small populations of cells, the time course of the twitch is comparable to that of a slow skeletal muscle. For example, peak twitch tension in a 5 mm strip of taenia coli (24) and in a strip of fowl anterior mesenteric artery (25) is attained in about 500 msec. Time until onset of contraction should be even shorter, especially if it were possible to eliminate conduction time in the strips by correlating electrical and mechanical events for a single cell. Minimum time for aggregation of smooth muscle myosin in vitro is about 1 min (20). We conclude that smooth muscle contraction is at least 100 times too fast to be explained by reversible, contraction-coupled aggregation of myosin. Direct evidence against the idea of contraction-coupled aggregation is the fact that living vertebrate smooth muscle exhibits no change in birefringence during isometric contraction (4).

The birefringence data are consistent with evidence from X-ray diffraction in living and fixed smooth muscles (3, 11) and with the electron microscopic findings described in this paper. Whether apparent failure of smooth muscle myosin to aggregate in vivo is a property of the individual myosin molecules or of a controlling reactant, such as a recently described myosin aggregation factor (26), remains to be determined.

Ultrastructure and Contraction Mechanism

The existence of myosin in relatively unaggregated form (dimers or tetramers) is consistent with the contraction mechanism we have previously suggested (1). In our view, shortening is attributable to interdigitation and sliding of actin filaments of opposite sense. Motion is imparted by numerous myosin dimers which form linkages between actin filaments. Asynchronous making and breaking of links along the entire free length of actin outside the dense body allows for sustained tension even though individual dimers become disconnected during shortening. A similar mechanism may underlie movement in slime molds (27) and in other primitive motile systems in which actomyosin-like proteins have been described (28, 29, 30).

Two pieces of evidence crucial to proof of this hypothesis are still missing. Myosin dimers have not been visualized in tissue sections of smooth muscle, for reasons cited above, though they have been found in fresh homogenates (1). Secondly, evidence for a difference in the directionality of actin at either end of the dense body (the homologue of the Z disc) has not yet been obtained. The hypothesis is nevertheless consistent with all present biochemical and ultrastructural observations, and it accounts for the broad length optimum for tension development so characteristic of smooth muscle (5). In striated muscles maximum tension is developed only over the small range of sarcomere lengths within which actin and myosin sites overlap to a maximum extent. This range is determined by the length of the bare central zone of the A band filament (31). Smooth muscle, in contrast, subserves functions which require much larger per cent changes in length. In the molecular arrangement we propose, actin and myosin can interact over at least 2 μ, or more than 10 times the length of the bare zone of the A band filament. We therefore regard maintenance of myosin in unaggregated form as an adaptation which facilitates shortening by increasing the range of lengths over which tension-generating actomyosin linkages can be formed.

We thank Dr. Clarence N. Nelson, physicist, Eastman Kodak Co., Rochester, N. Y., for applying optical filtering to our electron photomicrographs.

This research was supported by grants #AM-11720 and HE 03290 and 5K3 HE 15519 from the United States Public Health Service.

Received for publication 19 May 1969, and in revised form 19 August 1969.

REFERENCES

1. 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.
2. SHOENBERG, C. F. 1962. Some electron microscopic observations on the contraction mechanism in vertebrate smooth muscle. *In* Electron Microscopy: Fifth International Congress on Electron Microscopy. S. S. Breese, Jr., editor. Academic Press Inc., New York. **2**:M8.
3. ELLIOTT, G. F. 1964. X-ray diffraction studies on striated and smooth muscles. *Proc. Roy. Soc. (London) Ser. B.* **160**:467.
4. FISCHER, E. 1944. The birefringence of striated and smooth mammalian muscles. *J. Cell Comp. Physiol.* **23**:113.
5. CSAPO, A. 1960. Molecular structure and function of smooth muscle. *In:* The Structure and Function of Muscle. G. Bourne, editor. Academic Press Inc., New York. **1**:246.
6. KELLY, R. E., and R. V. RICE. 1968. Localization of myosin filaments in smooth muscle. *J. Cell Biol.* **37**:105.
7. CARNEY, C. A., and A. L. BROWN, JR. 1966. An electron microscopic study of canine cardiac myosin and some of its aggregates. *J. Cell Biol.* **28**:375.
8. LOWEY, S., H. S. SLAYTER, A. G. WEEDS, and H. BAKER. 1969. Substructure of the myosin molecule I. Subfragments of myosin by enzymic degradation. *J. Mol. Biol.* **42**:1.
9. PAGE, S. 1964. Filament lengths in resting and excited muscle. *Proc. Roy. Soc. (London) Ser. B.* **160**:460.
10. 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.

11. HUXLEY, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7**:281.
12. ELLIOTT, G. F., and J. LOWY. 1968. Organization of actin in a mammalian smooth muscle. *Nature (London).* **219**:156.
13. 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. (London) Ser. B.* **160**:517.
14. ZOBEL, C. R., and F. D. CARLSON. 1963. An electron microscopic investigation of myosin and some of its aggregates. *J. Mol. Biol.* **7**:78.
15. BÁRÁNY, M., K. BÁRÁNY, E. GAETJENS, and G. BAILIN. 1966. Chicken gizzard myosin. *Arch. Biochem. Biophys.* **113**:205.
16. HAYDON, G. B. 1968. On the interpretation of high resolution electron micrographs of macromolecules. *J. Ultrastruct. Res.* **25**:349.
17. CUTRONA, L. J., E. N. LEITH, and L. J. PORCELLO. 1959. Filtering operations using coherent optics. *Proc. Nat'l. Electron. Conf.* **15**: 262.
18. NEEDHAM, D. M., and J. M. WILLIAMS. 1963. Proteins of the uterine contractile mechanism. *Biochem. J.* **89**:552.
19. HANSON, J. and J. LOWY. 1964. The problem of the location of myosin in vertebrate smooth muscle. *Proc. Roy. Soc. (London) Ser. B.* **160**: 523.
20. SHOENBERG, C. F. 1969. An electron microscopy study of the influence of divalent ions on myosin filament formation in chicken gizzard extracts and homogenates. *Tissue and Cell* **1**:83.
21. NONOMURA, Y. 1968. Myofilaments in smooth muscle of guinea pig's taenia coli. *J. Cell Biol.* **39**:741.
22. LANE, B. P. 1965. Alterations in the cytologic detail of intestinal smooth muscle cells in various stages of contraction. *J. Cell Biol.* **27**:199.
23. KOBAYASI, S., H. ASAI, and F. OOSAWA. 1964. Electric birefringence of actin. *Biochim. Biophys. Acta.* **88**:528.
24. BÜLBRING, E. 1962. Electrical activity in intestinal smooth muscle. *Physiol. Rev.* **42**(suppl. 5): 160.
25. BOLTON, T. B. 1968. Electrical and mechanical activity in the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. *J. Physiol.* **196**:283.
26. KOZUKI, Y., and Y. TONOMURA. 1967. A protein factor regulating the state of aggregation of myosin. *J. Biochem. (Tokyo).* **62**:726.
27. HATANO, S. and M. TAZAWA. 1968. Isolation, purification, and characterization of myosin B from myxomycete plasmodium. *Biochim. Biophys. Acta.* **154**:507.
28. KINOSHITA, S., B. ANDOH, and H. HOFFMANN-BERLING. 1964. Das Erschlaffungssystem vom Fibroblastenzellen. *Biochim. Biophys. Acta.* **79**:88.
29. OHNISHI, T., H. KAWAMURA, K. TAKEO, and S. WATANABE. 1964. Propriété des Protéines Contractiles Ressemblantes à l'Actine et à la Myosine Extraites des Mitochondries du Foià. *J. Biochem. (Tokyo).* **56**:273.
30. GRETTE, K. 1963. Relaxing factor in extracts of blood platelets and its function in the cells. *Nature (London).* **198**:488.
31. GORDON, A. M., F. J. JULIAN, and A. F. HUXLEY. 1964. The length tension diagram of single vertebrate striated muscle fibers. *J. Physiol.* **171**:28.