THE ORGANIZATION OF CONTRACTILE FILAMENTS IN A MAMMALIAN SMOOTH MUSCLE

ROBERT V. RICE, JOAN A. MOSES, G. M. McMANUS, ARLENE C. BRADY, and LORRAINE M. BLASIK

From the Department of Chemistry and the Mellon Institute of Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213

ABSTRACT

Ordered arrays of thin filaments (65 A diameter) along with other apparently random arrangements of thin and thick filaments (100–200 A diameter) are observed in contracted guinea pig taenia coli rapidly fixed in glutaraldehyde. The thin-filament arrays vary from a few to more than 100 filaments in each array. The arrays are scattered among isolated thin and thick filaments. Some arrays are regular such as hexagonal; other arrays tend to be circular. However, few examples of rosettes with regular arrangements of thin filaments surrounding thick filaments are seen. Optical transforms of electron micrographs of thin-filament arrays give a nearest-neighbor spacing of the thin filaments in agreement with the "actin" filament spacing from x-ray diffraction experiments. Many thick filaments are closely associated with thin-filament arrays. Some thick filaments are hollow circles, although triangular shapes are also found. Thin-filament arrays and thick filaments extend into the cell for distances of at least a micron. Partially relaxed taenia coli shows thin-filament arrays but few thick filaments. The suggestion that thick filaments aggregate prior to contraction and disaggregate during relaxation is promoted by these observations. The results suggest that a sliding filament mechanism operates in smooth muscle as well as in striated muscle.

INTRODUCTION

The literature on smooth muscle was reviewed recently (1). In the past, much confusion was generated by conflicting reports of thick filaments, although most laboratories have consistently found thin filaments. Several recent reports from three different groups (2–5), who found both thick and thin filaments in sections of smooth muscle by electron microscopy, strongly indicated that a mechanism of sliding between thick and thin filaments may operate in smooth muscle as well as striated muscle. One team failed to find thick filaments but instead claimed to have located myosin molecules in sections, and proposed a sliding mechanism involving myosin dimers (6, 7). X-ray diffraction studies (8, 40) have generally failed to detect myosin thick-filament reflections, although one study on guinea pig taenia coli did find evidence for ordered arrays of thin filaments (9). In this study, the degree of order was not discussed, but the diffuse single reflection reported indicated only limited arrays of actin filaments. Ordered arrays of thin (actin) filaments from mammalian smooth muscle have never been reported, despite numerous electron microscopic examinations (2, 3, 10–21). A recent report on gizzard smooth muscle claimed to have visualized "regular spacing between actin filaments" (6). However, the only evidence presented

was a small area of a longitudinal section. The interpretation given to this electron micrograph implied that regularly spaced thin filaments fill the myofibrillar area of the smooth muscle cell. A hexagonal lattice of thin filaments from molluscan smooth muscle was mentioned in the X-ray report (9) as a personal communication from H. E. Huxley.

We have now found, by electron microscopy, limited ordered arrays of thin filaments in guinea pig taenia coli *together with thick filaments*. Optical diffraction patterns of these electron micrographs are closely related to the X-ray diffraction patterns of "living unstimulated" guinea pig taenia coli.

MATERIALS AND METHODS

Mature male guinea pigs usually were sacrificed with ether. More recently, animals were stunned and bled. Strips of taenia coli were carefully dissected while kept moist with modified Krebs-Ringer solution (22). Most preparations of muscle in this study were "contracted" by placing strips of tissue in room temperature Krebs-Ringer solution which contained 5 mM ATP at pH 7.2 (2). Almost all dissected smooth muscle showed coiled shortening even before immersing in ATP solution. The tissue was placed in 6.25% glutaraldehyde at pH 7.2 and cut into very small strips (0.2 mm by 2 mm) with razor blades; fixation was continued for 1 hr at room temperature (22–23°C). Postfixation was done in 1% OsO_4 at pH 7.4 for 1 hr. The tissue was then block-stained for 1½ hr in 2% uranyl acetate at pH 7.0. An Epon-Araldite mixture was used for embedding (26). Sections were cut on a Porter-Blum MT-2 ultramicrotome with DuPont diamond knives. The sections were stained with both lead citrate and uranyl acetate (27).

Attempts at providing completely relaxed taenia coli were carried out by using adrenaline (24, 25). Tissue was prepared by flushing out the intestine with a modified Krebs solution (23) and then carefully dissecting the muscle strips away from the intestinal wall. The strips were placed in a thermostated chamber of 37°C. The mechanical activity was monitored with a transducer connected to a lever. In this manner, contraction-relaxation cycles could be continuously monitored. Glutaraldehyde (3%) in the Krebs solution at 37°C was added after relaxation was obtained with adrenaline (23–25). After 5–10 min, the strips were removed from the apparatus, placed in the usual 6.25% glutaraldehyde at room temperature, and the outer regions of the strip were separated from the central region while in the fixative. Thereafter, fixation and embedding were carried out as usual. Electron micrographs were taken with either a Philips 300 or a JEM-7 electron microscope, using a 30 μ objective aperture and operated at 60 kv. Electron micrographs were taken at magnifications ranging from 25,000 to 100,000, with surveying carried out at 50,000 after initial surveys at lower magnifications.

Optical transforms (light-diffraction patterns) of electron micrographs were taken on a Pullin optical diffractometer, using masks made with a Pullin pantograph punch (R. B. Pullin and Co., London, England) (28). The only changes made were to use a Spectra Physics 130 gas laser as a light source and a Polaroid camera back for recording diffraction patterns. Enlargements to about 1,000,000 diameters were used on the pantograph to punch masks from exposed X-ray film. Approximately 500 holes corresponding to filament positions were punched for each mask. High-contrast reversals of similar electron micrographs have more recently been used in place of masks, with comparable results.

RESULTS

During the past several years of examining vertebrate smooth muscle in this laboratory, we have continually seen very limited ordered arrays of thin filaments. These usually consisted of widely scattered groups of thin filaments composed of two rows of three or four filaments in each row. The first observations of large, well-ordered arrays of thin filaments were found from an embedding of about 25 blocks. Approximately 50% of these blocks showed arrays when sectioned and examined in the electron microscope. However, when the tissue was carefully cut into very small strips in glutaraldehyde (as described under Materials and Methods), all blocks were found to contain ordered arrays along with numerous thick filaments.

Fig. 1 is an example of electron micrographs of guinea pig taenia coli rapidly fixed. Arrays of closely packed thin filaments are present throughout this area of the smooth muscle cell. Numerous thick filaments are also seen; some thick filaments (arrows) are partially surrounded by one or more circles of thin filaments. Careful inspection of the arrangement of thin filaments near thick filaments indicates that most of the latter are associated with partial or distorted circles of thin filaments. A count of the total number of thin and thick filaments in micrographs similar to Fig. 1 gave a total of approximately 7500 thin filaments and 150 thick filaments, if only thick filaments with diameters larger than 140 A were considered. Dense bodies are seen, and several microtubules are usually present. In

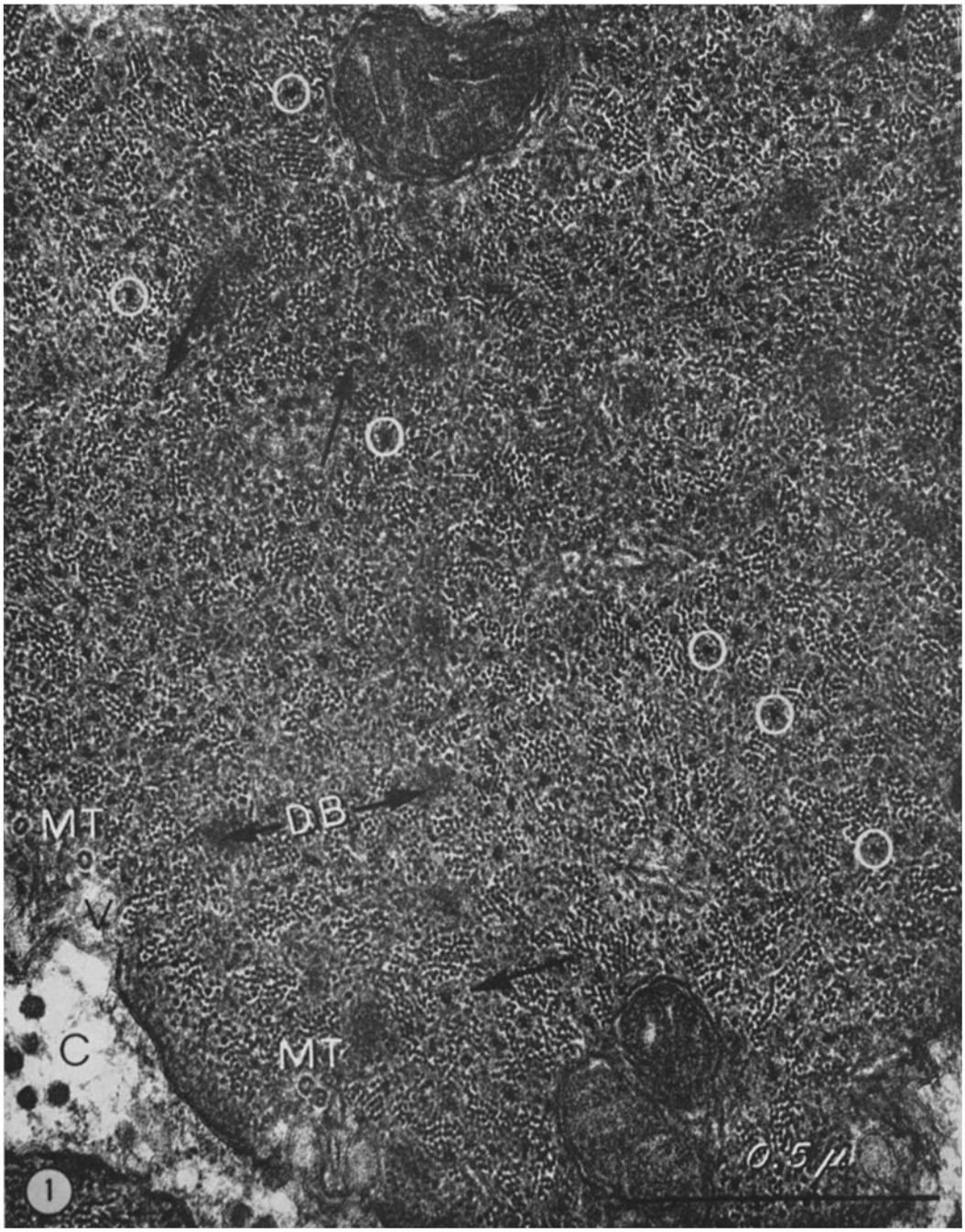

FIGURE 1 Cross-section of contracted guinea pig taenia coli. Arrays of thin filaments are found throughout the section. Arrows indicate thick filaments at the center of curvature of partially circular thin-filament arrays. Thick filaments with triangular shapes are enclosed in white circles. Dense bodies (*DB*), microtubules (*MT*), collagen (*C*), and vesicles (*V*) are common. All electron micrographs are of glutaraldehyde-fixed smooth muscle, post-stained in OsO_4, block-stained in 2% uranyl acetate, and embedded in Epon-Araldite. Sections were stained in lead citrate and uranyl acetate. × 93,000.

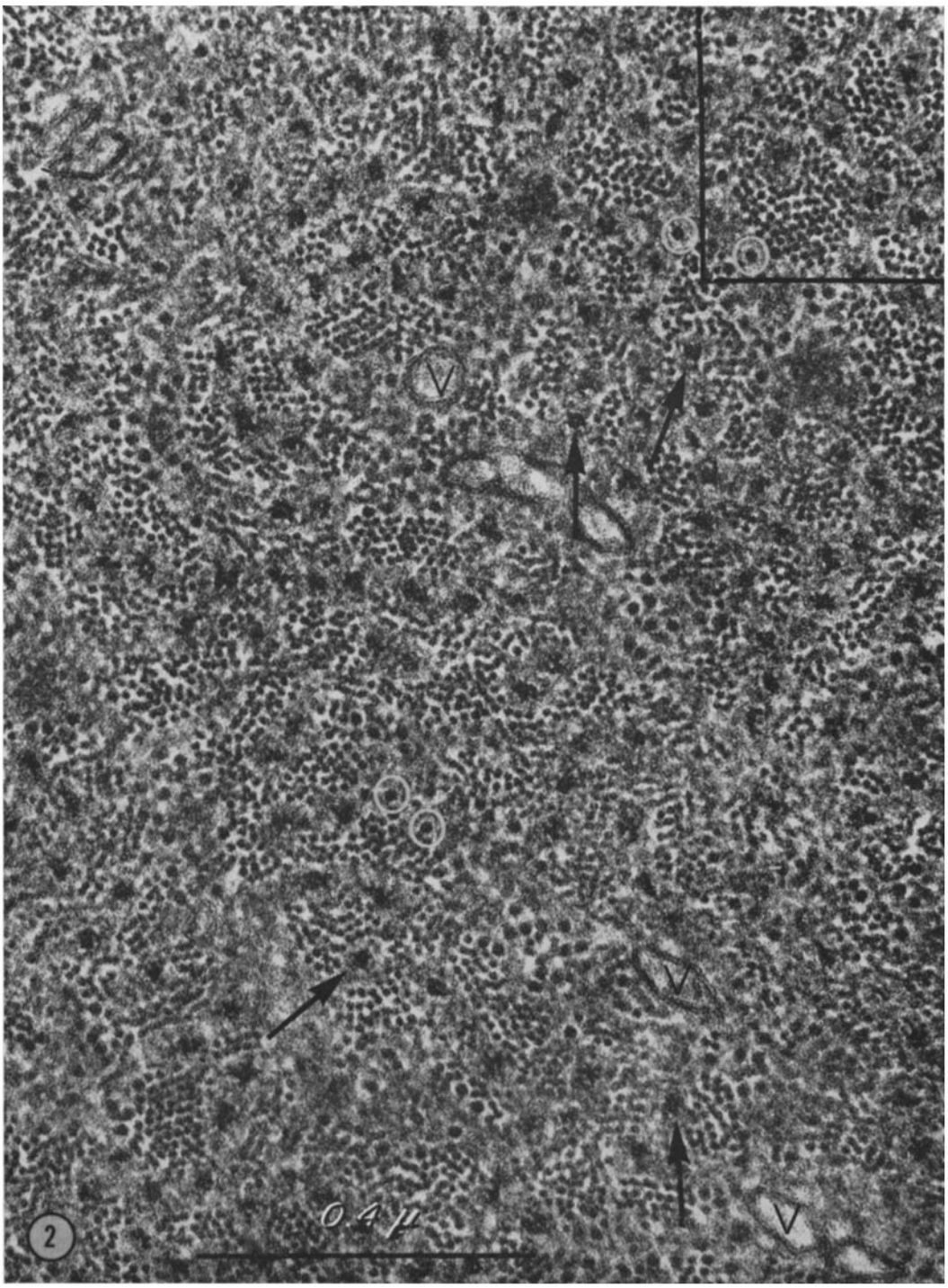

FIGURE 2 Cross-section of contracted guinea pig taenia coli showing numerous, regular, close-packed arrays of thin filaments. Thick filaments with diameters varying from 120 A (white circles) to 200 A (arrows), and vesicles (*V*) are seen in both cross- and oblique sections. Fixed as in Fig. 1. × 126,000.

size and morphology, these two organelles are distinctly different from the thick filaments. Many thick filaments tend to have a distorted triangular shape (circles), and many are from 150 to 200 A diameter. The thin filaments are about 65 A in diameter.

Thin filament arrays vary from circular packings of 15–50 filaments, with and without associated thick filaments, to hexagonal arrays of 100 or more thin filaments. The average of center-to-center distances of thin filaments in arrays is about 120 A, from measurements of electron micrographs from cross-sections.

Fig. 2 is an electron micrograph of taenia coli showing a relatively large number of ordered arrays. At least ten of the arrays show varying degrees of hexagonal packing. One area of Fig. 2 is shown at a higher magnification in Fig. 3. At this magnification, it is readily apparent that many of the filaments are far out of position for true hexagonal packing.

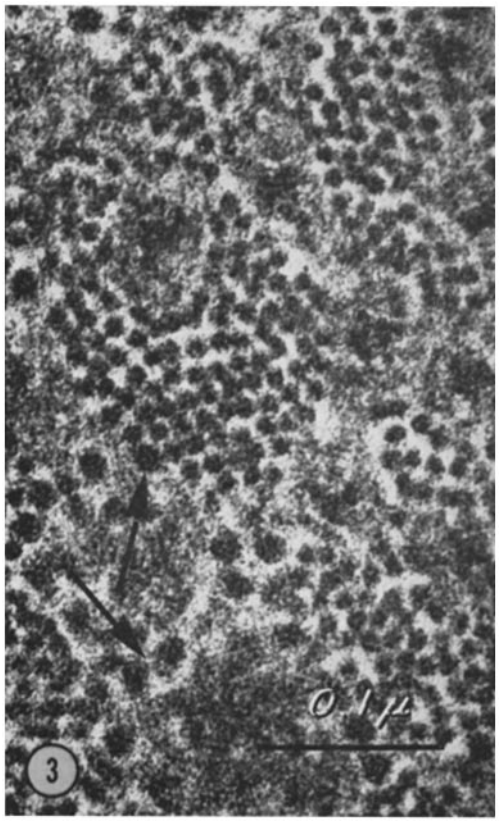

FIGURE 3 Enlargement of outlined area in Fig. 2 showing a large hexagonal array of thin filaments and associated 120 A thick filaments (arrows). × 250,000.

Fig. 4 shows the usual appearance of ordered arrays of thin filaments along with a population of thick filaments. This micrograph is more nearly representative of the degree of order found in contracted taenia coli. Several arrays of about 100 thin filaments are seen, but the filaments in the arrays are not arranged with much regularity.

The populations of thick filaments seen in Figs. 1–4 are larger than those that have usually been found in the past. An inspection of the shapes and sizes of thick filaments shows that solid circles, hollow circles, and triangles are present along with more irregular shapes. Thick filaments have diameters ranging from as small as 100 to as large as 200 A. Most of the irregular shapes are of the larger diameters. Only a few thick filaments can be resolved into a substructure of three or four circles the size (65 A) of thin filaments. Many arrays of thin filaments are closely associated with individual thick filaments, and size comparisons in these cases clearly differentiate the two types. In Fig. 3, several thick filaments are found near a larger thin filament array, with one thick filament actually in a hexagonal close-packed position (arrow). These thick filaments are about 120 A in diameter and have regular outlines close to being circular. The area encircled in Fig. 4 also has a number of thick filaments (about 120 A in diameter) close to much smaller thin filaments. Some of the smaller thick filaments have a less dense center, thus appearing to be hollow. Some larger filaments also appear to be hollow, and several are indicated by arrows in Fig. 4.

Mitochondria, vesicles, and dense bodies are all present in Figs. 1–4. These organelles have been found consistently in smooth muscle (2–5, 12, 17). In the present study, microtubules may be visualized somewhat more clearly than previously. They are 200–300 A in diameter and have walls consisting of globular subunits about 50 A in diameter. Characteristically, only a few microtubules are found in the myofibrillar regions. Their distinctive morphology easily differentiates them from thick filaments. Collagen fibrils are found in intercellular regions.

Fig. 5 is a longitudinal section of contracted guinea pig taenia coli which shows several areas of thin-filament arrays. The filaments within the array are about 65 A wide. One array with a repeat spacing of 100 A extends for at least 1 μ.

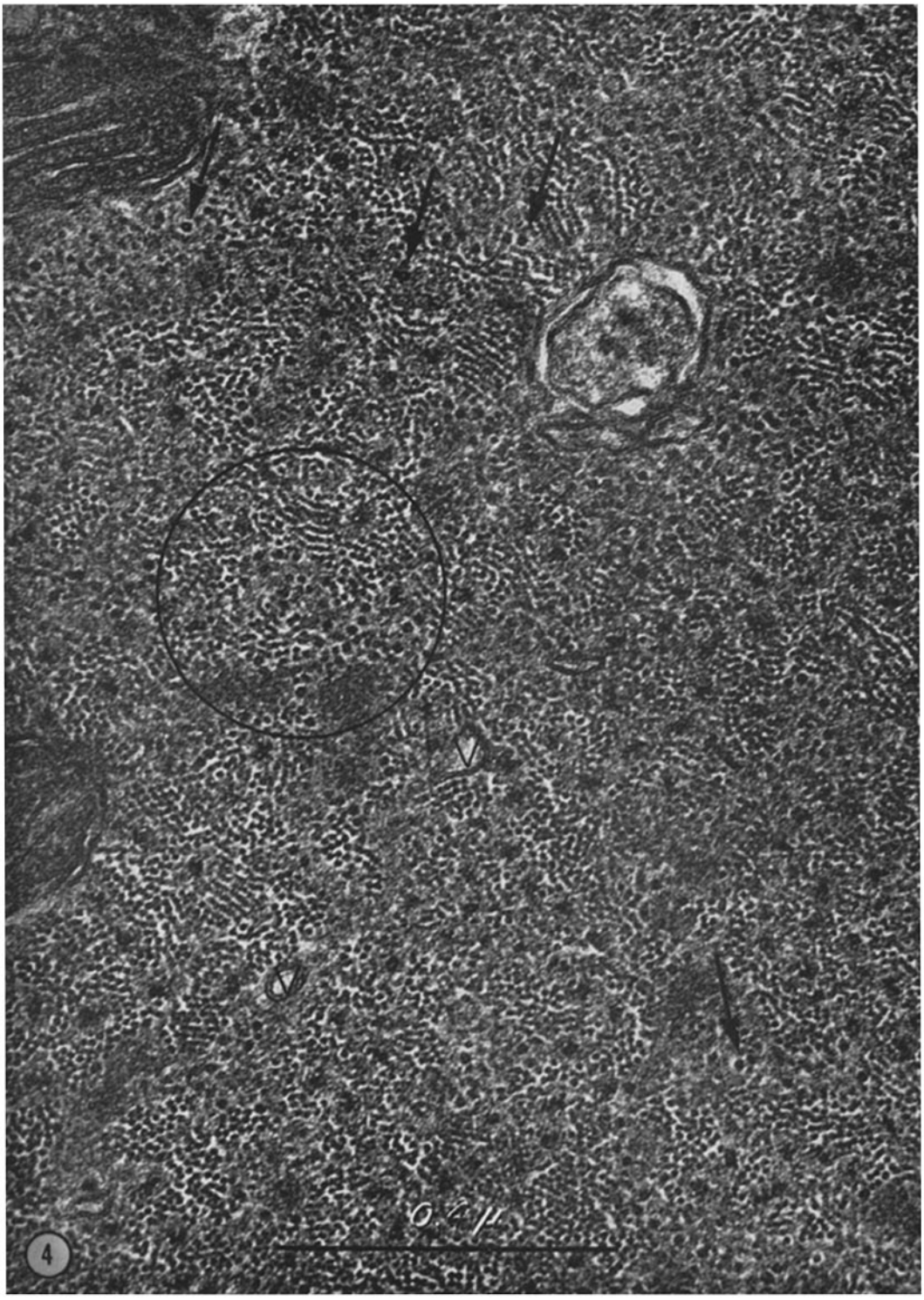

FIGURE 4 Cross-section of contracted taenia coli. Arrays of thin filaments appear throughout the myofibrillar region along with many thick filaments. Some thick filaments appear hollow (arrows), and a group of 120 A thick filaments which are closely associated with thin filaments is encircled. Fixed as in Fig. 1. × 130,000.

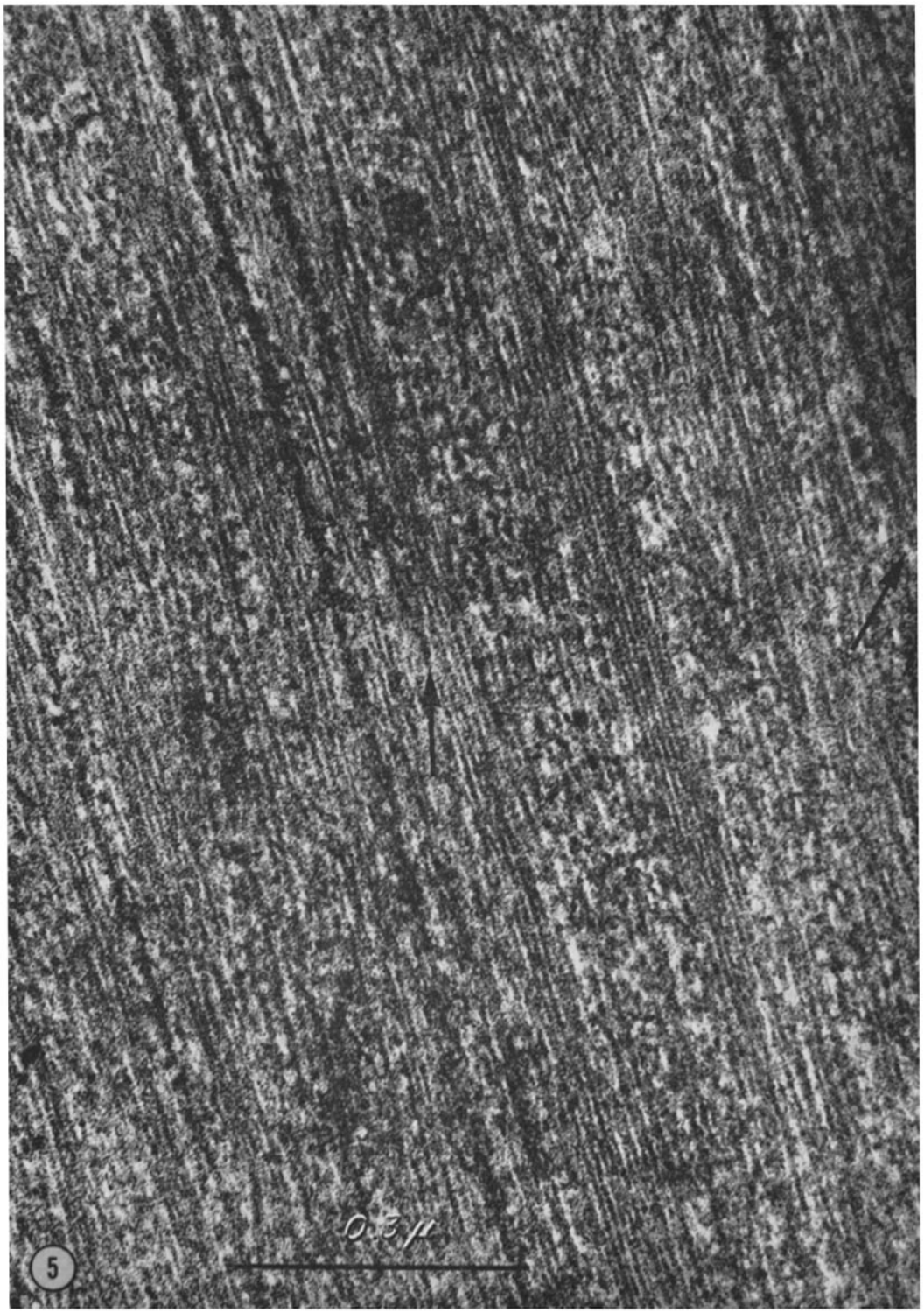

FIGURE 5 Longitudinal section of contracted guinea pig smooth muscle. Several thin-filament arrays extend for more than 1 μ. One array has two thick filaments within the thin filaments. Two thick filaments appear to have tapered ends (arrows). Thick filaments have protrusions. Orientation for this section was obtained by 90° rotation of a block which showed good cross-sections of arrays. Fixed as in Fig. 1. × 150,000.

Several thick filaments are also present. These are about 140 A in diameter and appear to have many projections. In two of these thick filaments, one tapered end is visible. Two of the thick filaments are in the midst of a thin-filament array. Although linear arrangements of smooth muscle such as taenia coli are much more convenient than nonlinear muscles such as gizzard, it is still technically difficult to cut sections parallel to the filament long axis. This is most easily done by surveying cross-sections for good alignment and then turning the block 90° in order to cut longitudinal sections (2, 5). Even when this procedure has been followed, it has not been possible to locate both ends of thick and thin filament groups as has been done for striated muscle. These difficulties are most likely due to the lack of M and Z lines which hold filaments rigidly in place for striated muscle. Lower magnifications of longitudinal sections show many thick filaments but the lengths visible in the sections are about one-half that of the longest filament shown in Fig. 5, or 0.5 μ. Until both tapered ends can be visualized, the length of smooth muscle thick filaments is unknown. However, from the lengths of the filaments in Fig. 5, it would appear that some thick filaments are at least 1 μ long.

Fig. 6 shows a cross-section of taenia coli which was completely relaxed before fixative was introduced into the cell. When the glutaraldehyde was added, slow contraction started but reached a maximum tension only 20% of that obtained before relaxation. Outer regions of the tissue strip showed few thick filaments, although the inner regions had the usual population of thick filaments. Fig. 6 is from the segregated outer region and probably represents a state near complete relaxation. In this micrograph, some relatively poorly arranged thin-filament arrays are found but only one possible large thick filament. Several smaller thick filaments about 120 A in diameter (encircled) are seen, and these include some that appear to be hollow. Microtubules are found closely associated with vesicles; in Fig. 6, all vesicles except one are near a microtubule.

Fig. 7 is the optical transform (light diffraction pattern) of the electron micrograph shown in Fig. 1. The dark ring superimposed on the Airy disc of the individual holes is characteristic of a random arrangement of holes with a popular (nonrandom distribution) nearest-neighbor spacing. This transform was calibrated by comparison with the transform of a mask with a known lattice spacing. From a densitometer trace of the negative of Fig. 7, the ring diameter (at half-width of the diffraction peak) was found to correspond to a filament spacing of 135 A. Optical transforms of several other similar micrographs gave the same results. Since the diffraction pattern is due not only to randomly arranged filaments but also to filaments in approximately regular arrays of random size and at random orientations, a mask of 88 holes was punched from the large array shown in Fig. 3. The optical transform of this pattern is shown in Fig. 8: it corresponds to a partially ordered hexagonal lattice of dimension 120 A.

DISCUSSION

The presence of ordered arrays in sections of taenia coli is striking when compared to previous electron microscopic examinations of mammalian smooth muscle. Although the arrays are limited in area, they are large and numerous enough to be immediately apparent even at relatively low surveying magnifications. The arrays are randomly located, with a slight tendency to be located near mitochondria. The greatest variation among the ordered arrays is in the size of the arrays, that is, the number of filaments within each array. The micrographs shown in Figs. 1–5 contain relatively large arrays, but all sections prepared from rapidly fixed tissue show many arrays. We have arbitrarily adopted a lower limit of two rows of four filaments as exhibiting enough order to designate an array.

The presence of numerous thick filaments which appear more electron opaque than the larger numbers of thin filaments is also readily apparent in Figs. 1–5. All of these micrographs are of contracted taenia coli, whereas few thick filaments are found in relaxed smooth muscle as shown in Fig. 6 and previous work (2, 5). The ratio of numbers of thin to thick filaments in Fig. 1 is less than 50 to 1, but it is not precisely known because of the difficulty of deciding on the lower diameter of thick filaments. The ratio may be as low as 25 to 1, due to the poor population statistics of the thick filaments. This will be discussed further when considering the size and shape of thick filaments.

The only other report of ordered arrays of thin filaments in mammalian smooth muscle was from an X-ray diffraction study of taenia coli (9). Although the diffuse equatorial reflection indicated an orderly side-by-side packing with

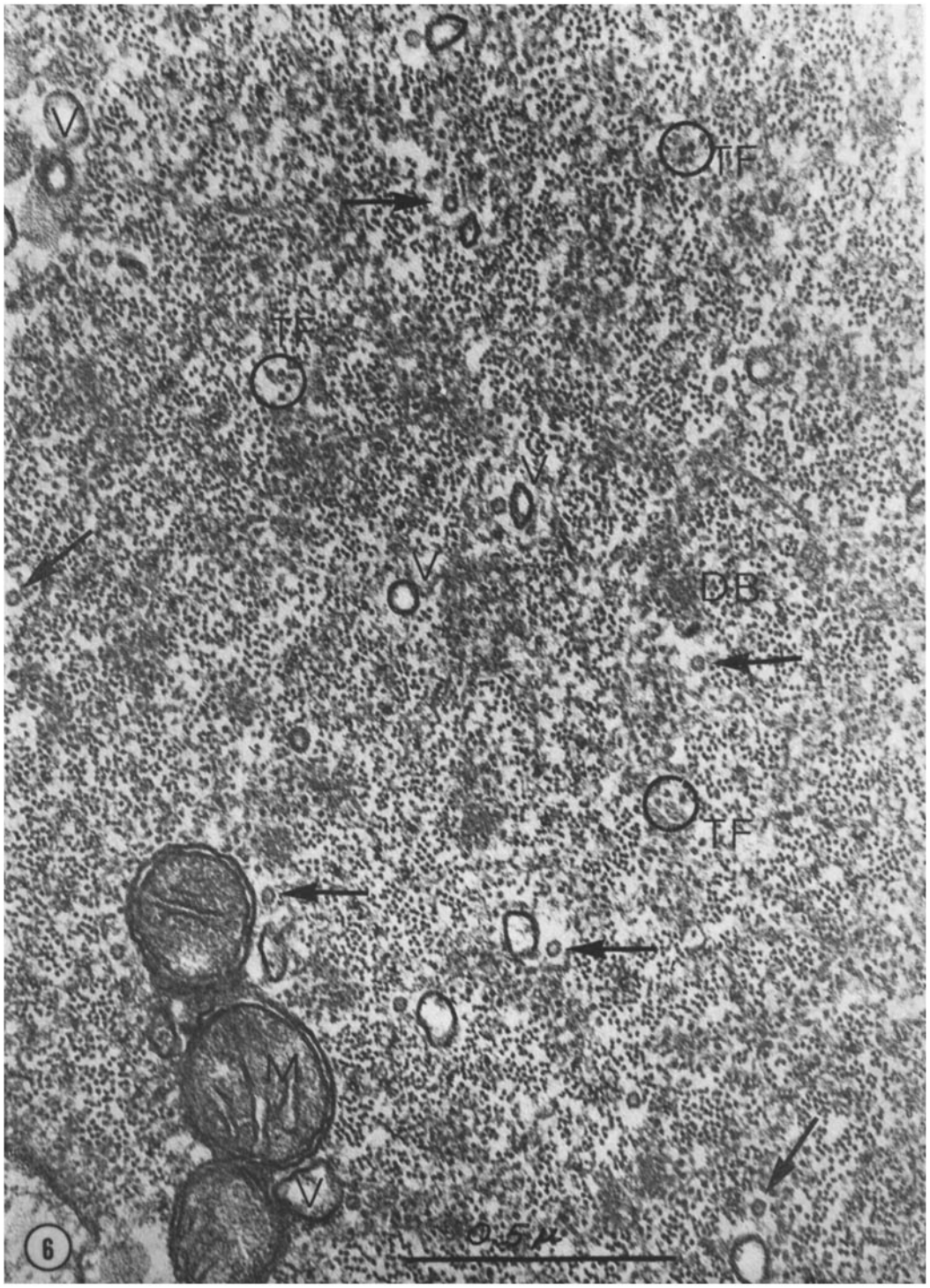

FIGURE 6 Cross-section of partially relaxed guinea pig taenia coli. Thin filament arrays are present, but relatively few thick filaments. Mitochondria (*M*), vesicles (*V*), and dense bodies (*DB*) are present; more microtubules (arrows) are seen than are usual in contracted smooth muscle. All vesicles except one are associated with a microtubule. Relaxed with adrenaline and prepared as described in text. Stained as in Fig. 1. × 82,000. Several groups of small thick filaments (120 A) are encircled (*TF*).

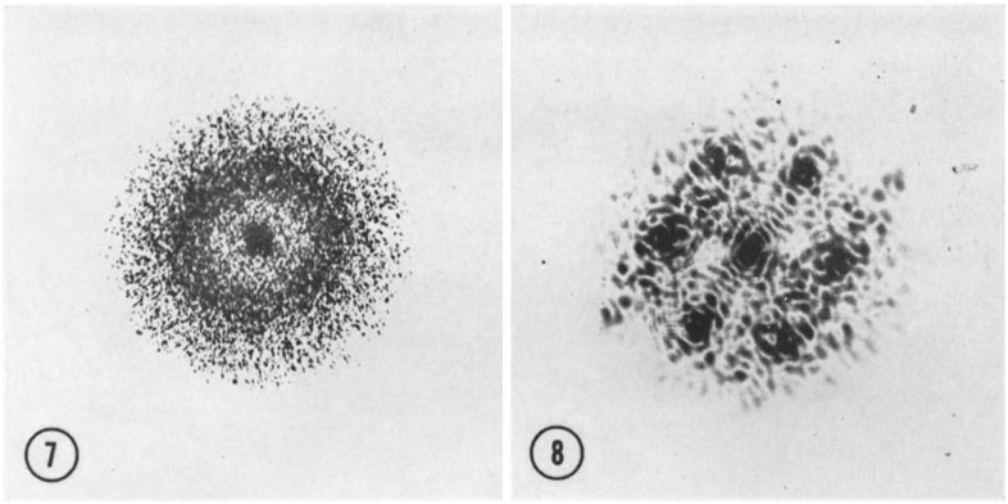

FIGURE 7 Light diffraction pattern (optical transform) of a mask prepared from the electron micrograph of Fig. 1. The diameter of the ring is 135 A. Note that only one ring is present, indicating only strong nearest-neighbor correlations.

FIGURE 8 Light diffraction pattern of the large thin-filament array found in Fig. 3. The pattern is characteristic of a disordered hexagonal lattice, with a mean lattice spacing of 120 A.

115 A separation, no information could be obtained as to the type of packing, e.g., the number of arrays or the number of thin filaments per array. That is, the X-ray reflections could have arisen from a random arrangement of thin filaments with a highly popular spacing of 115 A. In the present electron microscopic study, several modes of close-packed, thin filaments are visible. Most arrays are composed of several rows of thin filaments with order extending for a longer distance in one direction. Many rows are curved, and thick filaments are frequently found near the center of curvature. However, rosettes of thick filaments surrounded by definite numbers of thin filaments are rarely seen. Some areas, such as Figs. 2 and 3, contain arrays of thin filaments in approximate hexagonal packing. These arrays are not common. The beautiful hexagonal mode of packing apparently is restricted to striated muscles. Neither the present electron microscopic results nor the X-ray diffraction study can be interpreted in terms of a hexagonal packing (or any other regular packing) of actin filaments over large areas of taenia coli. On the other hand, it is obvious that many of the thin filaments are in quasi-regular arrays. The most common arrangement is a partially closed circular array. The significance of such arrays, especially those found near thick filaments, will be discussed later.

It is important to know the lengths of thin-filament arrays. In principle, either serial cross-sections or longitudinal sections should provide such three-dimensional information. Serial cross-sections have not been attempted as yet, but Fig. 5 represents a preliminary study of longitudinal sections. This micrograph suggests that both thin-filament arrays and thick filaments are at least as long as 1 μ. The great difficulty of finding organized structures properly oriented over several microns distance prevents a more definite determination of the third dimension, as yet. The arrangements seen in Fig. 5 agree with the results of Nonomura who distinguished two sets of filaments in longitudinal sections of taenia coli (3).

Most of the sections shown in a recent study of turkey gizzard (6) are reported as longitudinal even though gizzard smooth muscle fibers are less well oriented than are taenia coli fibers. Unfortunately, the micrographs presented show few filaments of any size, and so are difficult to compare with other work. The micrograph (Fig. 7 of reference 6) presented to show an array of thin filaments is of such low contrast that none of the filaments can be followed for more than a few tenths of a micron, and their diameters cannot be estimated accurately. In a few cases in which two or three filaments can be found side-by-side, they are about 100 A apart. Another

longitudinal section of gizzard (Fig. 4 of reference 6) purports to show actin subunits in a helical double filament to which myosin molecules are attached. Only four filaments are shown, and these are spaced at 1000 A intervals, or at ten times the regular interfilament spacing, which the authors list as their own number-one criterion for smooth muscle morphology. (One further point must be made in considering this recent turkey gizzard study [6]. The study of glycerinated gizzard reported from our laboratory is misquoted. Kelly and Rice [5] did not "attribute the presence of thick filaments . . . during prolonged storage . . . in glycerin at pH 5.8–6.6." All their glycerination was done at pH 7.0 by the usual Szent-Gyorgyi procedure. Subsequent to glycerination, the specimens were exposed to various pH values for 1–2 hr before fixation. The procedure was clearly stated by Kelly and Rice [5] in the first paragraph of their "Materials and Methods".)

The good agreement of the light diffraction patterns of electron micrographs with X-ray diffraction patterns of living taenia coli is significant. Both kinds of patterns are diffuse, and only one ring was obtained by light diffraction and one reflection by X-ray. These results indicate that extensive regular arrays (such as hexagonal close packing, square lattices, etc.) are not present either in electron micrographs or in "living" taenia coli as examined by X-ray diffraction. In general, it can be expected that arrays such as found in Fig. 1 would give the low-angle X-ray diffraction results previously reported (9). The clear hexagonal pattern, from light diffraction, of one of the limited partially hexagonal arrays by electron microscopy illustrates that light diffraction would detect hexagonal packings if they were extensive. This would be expected from X-ray diffraction also.

Thick myosin filaments have been identified by their morphology by negative staining on smooth muscle homogenates by numerous laboratories (5, 13, 18, 19, 30–33). These results might be taken as only further evidence that myosin is present in the tissue, but they do not necessarily give information concerning the organization of myosin in living muscle. The disruption of tissue necessary for this technique makes it virtually impossible to relate the results to the in vivo conditions.

A comparison of the shapes of thick filaments from smooth muscle reported here and from striated muscle (34–36) shows that various similar outlines are found in both muscles. In the region of the H zone, lacking M line bridges, striated muscle thick filament cross-sections exhibit circular and triangular outlines. Some of the striated muscle outlines are distorted from these regular shapes, although smooth muscle outlines are more distorted and of less regular size. The major difference between myosin filaments in smooth and striated mammalian muscles is in the more uniform size of striated muscle thick filaments. The size of smooth muscle thick filaments varies in the same section, although most values fall within the range of values published for other muscles. The thick filament diameters reported in the present study agree with the range from 100 to 200 A published by Nonomura (3). The lengths of thick filaments remain undetermined. From Fig. 5, the lengths appear to be longer than 1 μ, but this value is tentative because of the difficulty of finding tapered ends. It is interesting that Kaminer (31) found an optimum length of 0.6 μ for synthetic thick filaments prepared from purified myosin of gizzard. This value is very close to that observed in micrographs of taenia coli at lower magnifications. In the latter observations, one could not clearly see tapered ends, and so its significance is not certain. Hagopian and Spiro have tabulated the diameters of glutaraldehyde-fixed thick filaments from a variety of striated muscles (34). Thick-filament diameters vary from as small as 100 to as large as 230 A. Although myosin thick filaments are tapered, it is unlikely that tapering alone could account for the size range either in different striated muscles or in the present study of smooth muscle. Some of the smaller thick filaments in taenia coli are encircled in Fig. 4. These are in close proximity to definite thin filaments which are clearly of smaller diameter (55–65 A) and appear to be less electron opaque than the nearby thick filaments. Their closeness would seem to argue against the notion that staining artifacts increase the size of thin filaments to approximate thick ones. Limited penetration of stain might account for a hollow-appearing core, but sections which were not poststained with lead and uranium salts also show hollow thick filaments as do sections in which block staining was eliminated. Hollow thick filaments have been observed in several insect muscles (34, 37), and recently a substructure of 12 globules forming a wall has been demonstrated in insect muscle examined by high-voltage

electron microscopy (38). Figs. 1–4 clearly show numerous, hollow thick filaments.

It appears to us that an explanation for the variable size and shape of thick filaments in smooth muscle may be found in the suggestion that the thick filaments form prior to contraction and disaggregate during relaxation (2, 19, 33). If such a process does occur, one might expect different sizes of thick filaments, and their outlines might not be so regular as they are in muscle not undergoing such a process. Similarly, the frequent occurrence of a thick filament at the center of curvature of several rows of thin filaments might be the result of a thick filament which, in the process of aggregating, simply pushed an array of thin filaments into a distorted lattice. The tendency for thin filaments to take up a partial circular array might be promoted by the interaction of myosin heads with the actin filaments during the aggregation process. The most compelling evidence for this unusual aggregation-disaggregation cycle is the paucity of thick filaments in relaxed smooth muscle, such as shown in Fig. 6 and a recent publication from this laboratory (2), and their consistent appearance in contracted smooth muscle (2–5).

The observation of smaller (120 A) thick filaments in partially relaxed taenia coli, such as appear in Fig. 6, might suggest that larger filaments are formed by aggregation of myosin molecules onto the smaller thick filaments. This suggestion seems unlikely, but it cannot be eliminated as a possibility until fully relaxed smooth muscle is fixed without any indication of contraction. Since the tissue from which Fig. 6 was obtained showed definite slow contraction, we cannot, as yet, answer the question.

Although microtubules have been observed in smooth muscle (12), Fig. 6 illustrates the relatively large numbers found in partially relaxed taenia coli. Microtubules do not seem to be found with the same frequency in contracted tissue, although a few are usually seen at comparable magnifications. Microtubules are 250–300 A in diameter and, therefore, easily differentiated from hollow-appearing thick filaments which are less than 200 A in diameter. The characteristic globular walls of microtubules also serve to identify these organelles.

After our results had been submitted for publication, a letter by Lowy et al. (41) on X-ray diffraction of taenia coli appeared. These recent results are important, for they demonstrate the first 143 A meridional reflection found in vertebrate smooth muscle. The 115–120 A equatorial reflection assigned to "actin" arrays (9) and several other spacings were also seen. Lowy et al. suggest (because of narrow width of the 143 A reflection) that the myosin filament diameter is larger than that in other muscles. They also refer to unpublished electron microscope results which purport to show myosin in the form of "ribbon-like elements."

The meridional reflections or layer lines from striated muscle have been studied for over a decade and were first reported as arising from transverse projections by Worthington (42). Later, Elliott (8) assigned the layer lines from frog sartorius muscle to helically arranged myosin heads or cross-bridges. This assignment was made in the same report which noted the lack of myosin reflections from taenia coli. This interpretation of the layer lines has been adopted by Huxley and Brown (29). These authors also attribute certain characteristics of the 143 A reflection ("small transverse spread") to the extent of the lattice of thick filaments, and they also acknowledge the contribution of the thick filament backbone (light meromyosin packing) to the 143 A layer line. Because of the difficulties of deducing thick filament diameters from the width of the meridional X-ray reflections, we are inclined to rely on electron microscopy. Our present study clearly shows variable thick filament diameters (100 to 200 A), in agreement with Nonomura (3). Figs. 1–6 show various structures which could be interpreted as "ribbon-like elements." Since thick filaments are clearly seen, we have assumed that they were the locations of ordered aggregates of myosin.

The importance of the Lowy et al. article is that *both* contracted and relaxed taeniae coli were reported to show the 143 A reflection, and its intensity was greater in the relaxed state just as in striated muscle (29). If these results are confirmed, we suggest that the smaller thick filaments (120 A) are the location of ordered aggregates. The 120 A filaments are present in all taeniae coli studied so far, whereas the larger thick filaments do not appear in relaxed smooth muscle. We consider that, until procedures are developed which permit fixation of relaxed smooth muscle without any indication of contraction, electron microscopic results cannot be directly compared to X-ray diffraction results. Because thick filaments are scarce in smooth muscle fixed

under relaxing conditions, it is important that future X-ray experiments be undertaken under carefully monitored conditions. The pulsing of X-ray exposures with contraction and the use of adrenaline (23–25) in addition to lower temperatures for relaxed muscle would be helpful. However, this recent X-ray study (41) is a welcomed confirmation of electron microscopic observations on thin sections of smooth muscle which have lately (since 1967) been consistent in reporting both thick and thin filaments (2–5). The exception to this as observed by one group (6, 7) was discussed previously.

ADDED IN PROOF

Lowy and Small have now published electron micrographs of taenia coli fixed at 0°C under load (Lowy, J., and J. W. Small. 1970. *Nature.* **227**:46). These micrographs clearly show ribbons in addition to thin filament arrays and isolated thick filaments. They reported the diameter of the elements comprising their ribbons to average 80 A which is somewhat less than the diameter of the smallest thick filaments (100 A) seen in our micrographs, although direct measurements from their published figures suggest a width nearer 100 A. Measurement of the diameter of their isolated thick filaments also results in a range from 100 to 200 A. Thus, Lowy and Small appear to have found thick filaments in relaxed taenia coli; however, their conditions of fixation were nonphysiological. It remains to be determined whether or not *ribbons* of thick filaments exist in actively contracting taenia coli or are a result of particular fixation conditions. Unpublished results from our laboratory clearly show thick filaments, but no ribbons when taenia coli is fixed in 6.25% glutaraldehyde at 37°C in normal Ringer solutions at pH 7.0 while the mechanical activity clearly remained near the peak of contraction.

CONCLUSIONS

If we assume that the X-ray diffraction study of "living unstimulated" taenia coli detected thin filament arrays in unaltered tissue, then the present electron microscopic preparations are of the in vivo or unaltered condition. We come to this conclusion since the arrays of thin filaments seem to be the same whether detected by X-ray or electron microscopy. If this assumption is allowed, then the presence of thick filaments must also be a characterstic of the in vivo condition for contracted tissue. Since many of the thick filaments are of the same size and shape as those found in striated muscle, we expect that the contractile mechanism will be essentially the same as for striated muscle. Therefore, we expect that a mechanism of sliding (39) of thin filaments past thick filaments operates in smooth muscle. Although the details of the contractile events are not known and although "van der Waals and electrical double layer effects" (9, 40) may be important, mammalian smooth muscle appears to be only an extreme variation on the theme of all muscles.

Results of this research were presented at the Feb. 25–27, 1970 annual meeting of the Biophysical Society (Rice, R. V., J. A. Moses, A. C. Brady, and L. M. Blasik. 1970. *Abstracts Biophys. Soc.* **10**:83A).

We are pleased to thank Dr. William Johnson, Department of Biology, Rensselaer Polytechnic Institute, Troy, New York for the physiological monitoring of taenia coli in preliminary attempts to fix truly relaxed smooth muscle. We are indebted to Dr. C. R. Worthington for helpful discussions of optical and x-ray diffraction.

This research was supported in part by research grant AM-02809 from the National Institute of Arthritis and Metabolic Diseases and from General Research Support Grant Fr-54075 from the National Institutes of Health to Carnegie-Mellon University.

Received for publication 17 February 1970, and in revised form 27 April 1970.

REFERENCES

1. Somlyo, A. P., and A. V. Somlyo. 1968. *Pharmacol. Rev.* **20**:197.
2. Kelly, R. E., and R. V. Rice. 1969. *J. Cell Biol.* **42**:683.
3. Nonomura, Y. 1968. *J. Cell Biol.* **39**:741.
4. Haynes, J. F., A. L. Burnett, and L. E. Davis. 1968. *J. Exp. Zool.* **167**:283.
5. Kelly, R. E., and R. V. Rice. 1968. *J. Cell Biol.* **37**:105.
6. Panner, B. J., and C. R. Honig. 1970. *J. Cell Biol.* **44**:52.
7. Panner, B. J., and C. R. Honig. 1967. *J. Cell Biol.* **35**:303.
8. Elliott, G. F., 1964. *Proc. Roy. Soc. (London) Ser. B.* **160**:467.
9. Elliott, G. F., and J. Lowy. 1968. *Nature (London).* **219**:156.
10. Csapo, A. 1960. *In* Muscle. G. Bourne, editor. Academic Press Inc., New York. **1**:246.

11. GANSLER, H. 1961. *Z. Zellforsch. Mikrosk. Anat.* **55**:724.
12. LANE, B. P. 1965. *J. Cell Biol.* **27**:199.
13. NEEDHAM, D. M., and C. F. SHOENBERG. 1967. *In* Cellular Biology of the Uterus. R. M. Wynn, editor. Appleton-Century Crofts, New York. 291.
14. PEASE, D. C. 1968. *J. Ultrastruct. Res.* **23**:280.
15. PEASE, D. C., and S. MOLINARI. 1960. *J. Ultrastruct. Res.* **3**:469.
16. PROSSER, C. L., G. BURNSTOCK, and J. KAHN. 1960. *Amer. J. Physiol.* **199**:545.
17. RHODIN, J. A. G. 1962. *Physiol. Rev.* **42** (Suppl. 5):48.
18. SHOENBERG, C. F., and D. M. NEEDHAM. 1968. *In* Handbook of Physiology. Sect. 6: Alimentary Canal. C. F. Code, editor. American Physiological Society, Washington, D.C. **4**: 1793.
19. SHOENBERG, C. F. 1969. *Tissue and Cell.* **1**:83.
20. NEEDHAM, D. M., and C. F. SHOENBERG. 1964. *Proc. Roy. Soc. (London) Ser. B.* **160**:517.
21. CHOI, J. K. 1962. Electron Microscopy: Fifth International Congress on Electron Microscopy. Held in Philadelphia, Pa. August 29 to September 5, 1962. Sydney S. Breese, Jr., editor. Academic Press, Inc., New York. **2**:M-9.
22. KREBS, A. H., and K. HENSELEIT, 1932. *Z. Physiol. Chem.* **210**:33.
23. ABERG, A. K. G., and J. AXELSSON. 1965. *Acta Physiol. Scand.* **64**:15.
24. BULBRING, E. 1957. *J. Physiol. (London)* **135**:412.
25. BURNSTOCK, G. 1958. *J. Physiol. (London)* **143**:183.
26. STROMER, M. H., D. J. HARTSHORNE, and R. V. RICE. 1967. *J. Cell Biol.* **35**:C23.
27. REYNOLDS, E. S. 1963. *J. Cell Biol.* **17**:208.
28. TAYLOR, C. A., and H. LIPSON, 1965. Optical Transforms. Cornell University Press, New York.
29. HUXLEY, H. E., and W. BROWN. 1967. *J. Mol. Biol.* **30**:383.
30. HANSON, J., and J. LOWY. 1964. *Proc. Roy. Soc. (London) Ser. B.* **160**:523.
31. KAMINER, B. 1969. *J. Mol. Biol.* **39**:257.
32. RICE, R. V., A. C. BRADY, R. H. DEPUE, and R. E. KELLY. 1966. *Biochem. Z.* **345**:370.
33. SCHIRMER, R. H. 1965. *Biochem. Z.* **343**:269.
34. HAGOPIAN, M., and D. SPIRO. 1968. *J. Cell Biol.* **36**:433.
35. PEPE, F. A. 1967. *J. Mol. Biol.* **27**:203.
36. HUXLEY, H. E. 1968. *J. Mol. Biol.* **37**:507.
37. SMITH, D. A. 1966. *J. Cell Biol.* **29**:449.
38. HAMA, K., and K. R. PORTER. 1970. *J. Cell Biol.* In press.
39. HUXLEY, H. E. 1957. *J. Biophys. Biochem. Cytol.* **3**:631.
40. ELLIOTT, G. F. 1967. *J. Gen. Physiol.* **50**:171.
41. LOWY, J., F. R. POULSEN, and P. J. VIBERT. 1970. *Nature (London).* **225**:1053.
42. WORTHINGTON, C. R. 1959. *J. Mol. Biol.* **1**:398.