AN ELECTRON MICROSCOPE STUDY OF CANINE CARDIAC MYOSIN AND SOME OF ITS AGGREGATES

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ABSTRACT

The morphology of the canine cardiac myosin molecule has been investigated in the electron microscope with Hall's mica-replica technique. The molecule is an elongated rod (shaft) of nonuniform diameter with a globular expansion (head) on one end. Statistical analysis of the lengths of 1908 molecules showed that the mean length was 1610 ± 250 A; the mean length of the head was 210 ± 20 A; and the diameter of the head and that of the shaft were 35 to 40 and 15 to 20 A, respectively. About one-third of the molecules had single or multiple, fairly sharp, angulations along their shafts. Rarely, some details of the substructure of the molecule have been observed. Large, spindle-shaped aggregates, measuring 0.5 to 1 μ in length and 50 to 100 A in diameter, were produced by dilution of the myosin solutions. These aggregates were readily visualized in the electron microscope by means of Huxley's negative-staining technique. Projections often were visible along the length of the aggregates except at a central zone where they were frequently absent. The aggregates resembled the thick myofilaments of the myocardium and appeared similar to those produced by Huxley from skeletal myosin solutions.

INTRODUCTION

The contractile proteins of skeletal muscle (myosin, actin, and actomyosin) have been the subject of intensive investigation for the past two decades. Comparatively few physicochemical studies, however, have been performed on cardiac myosin. Estimates of its molecular weight show a wider variation than those obtained for skeletal myosin and range from 223,000 to 758,000 (2, 5, 6, 8, 18, 19). Several investigators have indicated that the molecular weights of cardiac myosin and skeletal myosin are similar (5, 8). Physicochemical studies indicate that the molecule is rod-shaped, of nonuniform diameter (19), and has a length of 1600 to 1790 A (8, 19). Electron microscope study has confirmed this description and shows that the cardiac myosin molecule is an elongated rod, 1450 A long, with a globular expansion on one end (19). Despite many similarities, there is general agreement that the molecules of cardiac myosin and skeletal myosin are not identical. Cardiac myosin has a distinctly lower adenosinetriphosphatase (ATPase) activity than has skeletal myosin (1–3, 6–8, 19). In addition, it is initially more resistant to tryptic digestion than is skeletal myosin (20). Furthermore, the low yield of cardiac myosin, as compared with skeletal myosin, on extraction with potassium chloride–phosphate buffers (18, 19), also may indicate some chemical differences between these molecules. These differences in properties probably are related to subtle variations

in the arrangement or content of amino acids in these molecules. But it is possible that these chemical differences may be associated with some dissimilarity in their gross appearance that would render skeletal myosin and cardiac myosin morphologically distinguishable.

An interesting development has been the study of aggregates and synthetic protein filaments obtained from skeletal myosin solutions (14, 21, 31). Huxley drew attention to the morphologic similarity between the synthetic aggregates he produced and the naturally occurring, thick myofilaments of skeletal muscle, and further suggested a model for the thick myofilament based on the morphology of the myosin molecule. The similarities between cardiac myosin and skeletal myosin and the similarity between the ultrastructural organization of cardiac muscle and that of skeletal muscle prompted us to attempt to produce cardiac myosin aggregates and examine them by means of the negative-staining technique.

The primary purpose of the present study was to determine whether a morphologic estimate of the length of the canine cardiac myosin molecule could be obtained that would approximate the lengths that were obtained by other investigators who used physicochemical techniques. A second objective was to determine whether there is a morphologic basis for the different chemical properties of cardiac myosin and skeltal myosin.

MATERIALS AND METHODS

ISOLATION OF MYOSIN: Myosin was prepared according to Mueller's modification of Ellenbogen's method (19). Efforts were made to complete the isolation and spraying of myosin rapidly because of a reported instability of canine cardiac myosin (2). Instability was not a serious problem in this study, since the use of Mueller's method of isolation (19) gave a relatively stable myosin. Normal dogs were anesthetized by intravenous injection of sodium pentobarbital (20 mg/kg). The beating hearts were rapidly excised, opened with a pair of scissors, drained of blood, and placed in ice cold, deionized, distilled water. All subsequent operations were carried out at 4°C in a cold room, with deionized distilled water being used for all procedures. Chilled, trimmed heart muscle was passed through a meat mincer with 4-mm holes. The inside of the mincer and the inside of the ultracentrifuge caps that were used in the preparative procedures were coated with a polyacrylic resin (Krylon, Inc., Norristown, Pennsylvania) to keep contamination with heavy metal ions to a minimum (30). Extraction, precipitation, and redissolution of myosin were carried out at 0 to 1°C. In order to avoid any contamination with actomyosin, the top two-thirds only of the myosin solution, after the final preparative ultracentrifugation, was removed by careful pipetting, and used.

PURITY OF MYOSIN: Solutions of myosin in 0.5 M potassium chloride, 0.01 M potassium monobasic phosphate, and 0.03 M potassium dibasic phosphate, pH 6.8, were examined in an analytical ultracentrifuge (Spinco Model L) and were found to have a single, hyper-sharp peak.

PREPARATION FOR ELECTRON MICROSCOPY: The procedure of Rice (19) was followed. The final precipitate of the preparative procedure was, however, subjected to further purification before dialysis against ammonium acetate solution. The precipitate was redissolved in potassium chloride and subjected to further preparative ultracentrifugation to ensure removal of any remaining actomyosin. After this step, the supernatant gave a single peak in the analytical ultracentrifuge. 1 ml of this solution (10 to 15 mg/ml) was diluted with 5 volumes of 1 M ammonium acetate and subsequently dialyzed against 1M ammonium acetate for 24 hr at 4°C. Dilute solutions of myosin (approximately 0.002%) were sprayed onto freshly cleaved mica with a Vaponefrin nebulizer (supplied by Vaponefrin Co., Inc., New York) in the cold room. Immediately prior to spraying, tobacco mosaic virus (supplied by Dr. H. Fraenkel-Conrat, University of California, Berkeley) was added to the myosin dilutions. Spraying was completed within 42 hr of commencement of the extraction procedure. The preparations were then shadowed with platinum (5:1 angle) in a vacuum evaporator and subsequently backed with a light coat of carbon. Platinum-carbon replicas were stripped in a water trough, picked up on 400-mesh copper grids (supplied by E. F. Fullam, Inc., Schenectady, New York), and examined in the electron microscope.

ELECTRON MICROSCOPY: The specimens were studied in an RCA EMU 3G electron microscope equipped with a 25 μ platinum objective aperture and operated at 50 kv. Astigmatism was reduced to less than 0.25 μ, and a resolution of better than 10 A was obtained as estimated by the fringe method. Frequent calibration was done with a 28,800 line/in. grating replica (supplied by E. F. Fullam, Inc., Schenectady, New York). Micrographs of the replica preparations were taken at a magnification of about 18,000.

MEASUREMENT OF MACROMOLECULAR PARTICLES: For mensuration purposes, a final magnification of about 75,000 was achieved by photographic enlargement. From prints, measurements of the dimensions of molecules were made with proportional dividers or with a map measure (both supplied by Keuffel & Esser Co., New York). In many instances, because of the tortuosity of the shaft of the

molecule, it was not practical to use either the proportional dividers or the map measure. Instead, fine copper wire was used to outline the molecule. This wire was subsequently cut, straightened, and measured.

PREPARATION OF AGGREGATES: The procedure used was that described by Huxley (14). Myosin in 0.6 M potassium chloride was either diluted rapidly with standard 0.15 M potassium chloride or slowly with the same solution by overnight dialysis.

NEGATIVE STAINING: The procedure of Huxley was followed (14, 16).

RESULTS

The results of shadow-casting showed that cardiac myosin has a marked tendency to aggregate. This was particularly evident at the center of large droplets and with more concentrated solutions (0.005%). The more dilute the solution of myosin, the less tendency there was for large aggregates to form but the more scattered were the individual particles. Best results were obtained with dilute solutions (0.002%) when fairly large droplets could be found since, in these circumstances, many individual particles could be seen in the same field in an intermediate area between the center and the periphery of the droplet.

Attention was directed to individual macromolecular particles. Several different types were present (Table I). The predominant group, representing more than 95% of all the particles encountered, was a long, slender rod (shaft) with a globular expansion on one end (head). Commonly, these particles were deposited in a roughly parallel fashion with their long axes directed toward the center of the droplet. Next in frequency, but accounting only for about 2% of all the particles measured, was a slender rod without a head.

TABLE I

Number and Morphologic Configuration of Macromolecular Particles in Mica-Replica Preparations of Canine Cardiac Myosin

Preparation	No. of macromolecular particles	No. of Rods		
		With globular expansion (one end)	Without globular expansion	With globular expansion (both ends)
1	408	401	5	2
2	526	505	20	1
3	491	479	10	2
4	524	523	1	0

Rodlike particles with a head at both ends were very uncommon and accounted for less than 0.5% of all the macromolecules studied. The mean length of the 1908 particles consisting of a rod with a single globular expansion at one end was 1610 A, and the standard deviation was 250 A. A histogram of the lengths at 100 A intervals (Fig. 1) shows a somewhat skewed distribution with a slight excess of shorter particles. Measurements in the range of 1700 to 1800 A showed the highest incidence. The mean diameter of the shaft, based on 588 measurements, was 16.0 ± 4.0 A.

The length of the head was determined by an analysis of 516 measurements of this segment. These lengths, grouped into intervals of 20 A, were arranged in a histogram (Fig. 2). The distribution was nearly normal, and the range was from about 120 to 300 A. The mean length of the head was 210 ± 30 A. In general, the head appeared to be a solid body, and evidence of substructure was not seen. The mean diameter of the head was 37 ± 5 A. Rarely, the head appeared to be composed of two or three globular structures (Fig. 3 *b* and *c*).

The majority of the shafts (65%) was straight or showed a slight curve (Fig. 3 *a*). The shafts of the remainder exhibited fairly sharp angulations along their length (Table II). The commonest finding was that of a single sharp bend (Fig. 4 *b*); but frequently there were two angulations (Fig. 4 *a*); occasionally there were three distinct bends, and rarely the whole shaft had a wavy appearance suggestive of multiple bends (Fig. 4 *c*). These angulations did not appear to be restricted to specific locations along the shaft but seemed to be randomly distributed.

Less than 1% of the molecules examined showed one or two distinct, transverse breaks in the shaft. The molecular fragments appeared in close proximity to one another (Fig. 5). The alignment of the fragments was unlikely to be the result of random deposition of broken off portions of several molecules. In many cases, the sum of the lengths of the individual fragments closely approximated the mean length of an intact myosin molecule. Since the total number of broken molecules was small, it was not possible to assess whether the breaks tended to occur in specific regions or whether they occurred at random.

Another very small group of molecules showed appearances suggestive of longitudinal splitting of the shaft into two strands (Fig. 6 *a*, arrow). It is difficult to be certain whether the second strand

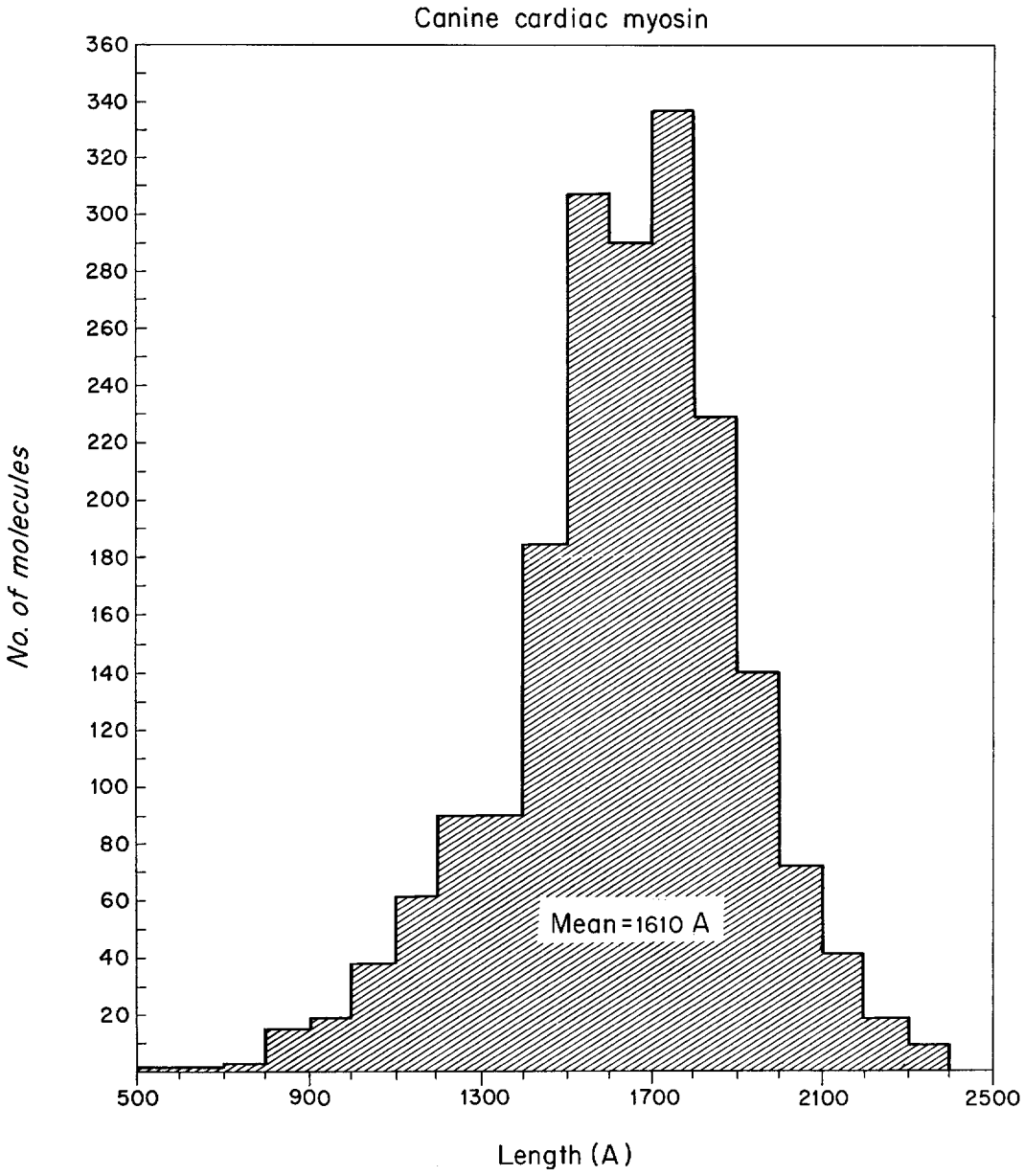

FIGURE 1 Distribution of lengths of 1908 canine cardiac myosin molecules. Only molecules consisting of a rod with a globular expansion on one end are included.

was due to random deposition of a second molecule (Fig. 6 *b*) or to a portion of a broken shaft in close proximity to the shaft of an intact molecule. However, the diameters of the strands, as indicated by their shadows, appeared in some cases to be less than that of the parent molecule. Thus the strands may well represent subunits of the parent molecule.

Aggregates were seen frequently in shadow-cast preparations of myosin even when dilute solutions in high concentration of volatile salt had been used for spraying. Usually these aggregates were small, representing the association of a few molecules at their heads (Fig. 7). Aggregates consisting of a shaft with a head on both ends, the type commonly

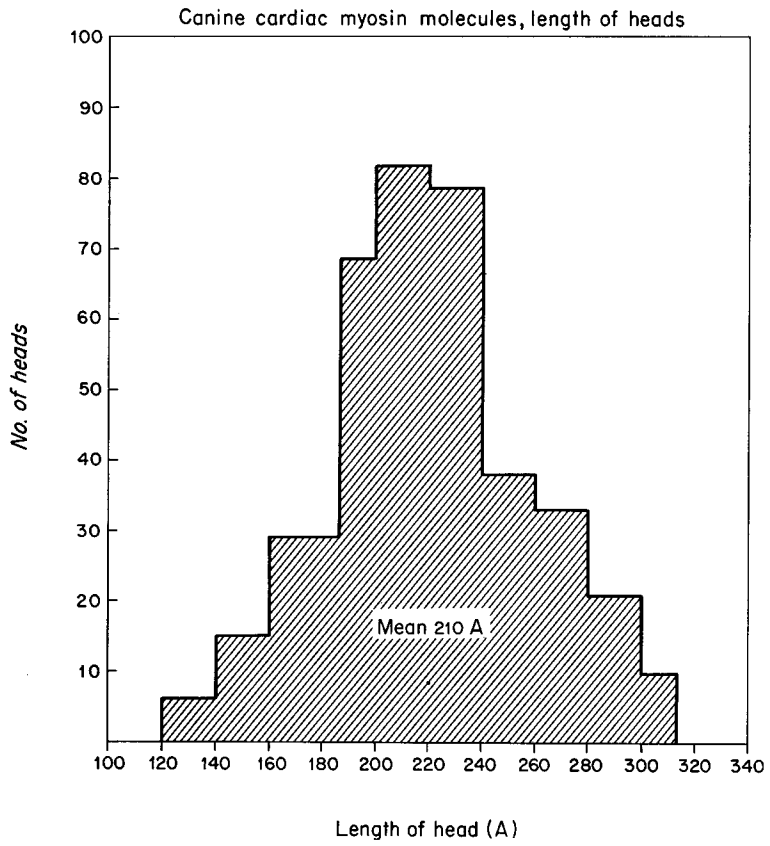

FIGURE 2 Distribution of lengths of about 500 globular expansions on canine cardiac myosin molecules consisting of a rod with a globular swelling on one end.

encountered in skeletal myosin preparations, were rarely present in the cardiac myosin specimens. Aggregates of skeletal myosin produced in solutions of low ionic strength potassium chloride have been successfully shadowed with platinum and examined in the electron microscope (31). Our attempts to visualize similar aggregates prepared from cardiac myosin by the shadow-casting technique were unsuccessful. The material shadowed in such specimens appeared amorphous without evidence of ordered structure. But, by using Huxley's negative-staining technique (14), we were able to demonstrate these aggregates effectively. Satisfactory results were obtained by dilution of a solution of cardiac myosin (0.2%) in potassium chloride (μ = 0.6) with standard potassium chloride solution (μ = 0.15). As aggregates form, the solutions become increasingly turbid. With a little practice, the opacity of the aggregate suspensions could be regulated so that the concentration of aggregates would give satisfactory results with the negative-staining technique. When aggregates produced by slow dilution were visualized in the electron microscope, they appeared as elongated structures, tapering toward the ends, and measuring about 0.5 to 1.0 μ in length and 50 to 100 A in diameter (Fig. 8). The majority of aggregates produced was straight. Occasionally, however, the long axis of an aggregate was found to be curved or actually bent. There appeared to be little tendency for the aggregates to adhere together, and their distribution in negatively-stained preparations appeared to be random. The margins of many of these spindle-shaped aggregates appeared roughened, and in places the roughening was due to a linear stippled effect (Fig. 9). Closer examination showed projections jutting from the sides of the aggregates. A significant feature of aggregates produced by rapid dilution, and a feature first pointed out by

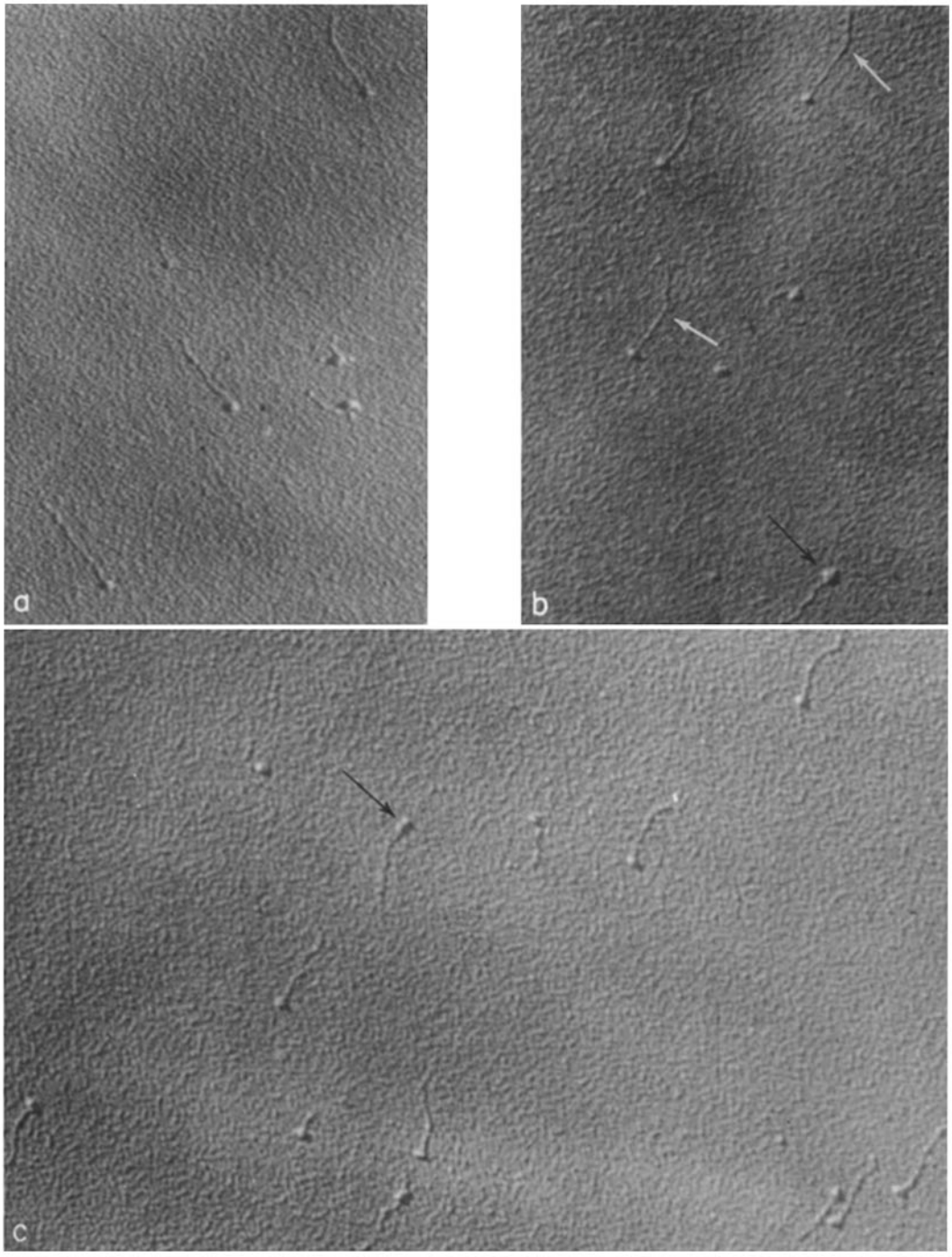

FIGURE 3 Three representative fields showing conformation of individual cardiac myosin molecules in mica-replica preparations of ultracentrifugally pure canine cardiac myosin. The majority of these dispersed particles consists of an elongated rod with a single globular expansion on one end. In general, the rod portion of the molecules is straight or slightly curved (3 *a*). A few angulated shafts can be seen (3 *b*, white arrows). Molecules with several globular structures in the head region are present (3 *b* and *c*, black arrows). × 75,000.

Huxley in his study of synthetic myosin filaments (14), was the presence of an area devoid of projections at about the center of the aggregate (Fig. 10). This bare area was 0.15 to 0.20 μ in length.

Encouraged by Huxley's demonstration, with the negative-staining technique, of individual particles measuring about 1500 A in length in solutions of myosin (15), we attempted to visualize cardiac myosin by using this technique. Initially, a stock solution of myosin in potassium chloride (μ = 0.6) was used, to which the negative stain was added. The results were poor. The stained preparations showed many amorphous aggregations but no individual molecules. Addition of the negative stain to the myosin solution involves a rapid and substantial reduction in the ionic strength of the potassium chloride solvent. It seemed possible that this rapid fall in solvent ionic strength might, in part, be responsible for the failure of the experiment and that, perhaps, if the initial concentration of potassium chloride was increased to μ = 1.0, improved results might be obtained. Unfortunately, such was not the case, and the results were again disappointing.

TABLE II

Appearance and Percentage Distribution of Shafts of Canine Cardiac Myosin Molecule

Preparation	Straight or slightly curved	Angulation		
		Single	Two	Three
	per cent	*per cent*	*per cent*	*per cent*
1	80.5	14	5	0.5
2	63	25	11	1.0
3	55.5	34	10	0.5
4	69	23	8	0.0

DISCUSSION

The size and shape of macromolecules can be studied by hydrodynamic methods and by the mica-replica technique. Each method has limitations, and consequently, it is advantageous, when possible, to use both techniques. The limitations of the mica-replica technique have been discussed by Hall (9–11). Background granularity, accumulation of shadowed metal, and local shadow-angle variance can make interpretation of results difficult. Apart from limitations inherent in the technique, forces of considerable magnitude are applied to the molecules both during the spraying procedure and subsequently during the drying of the droplets. Evidence indicating that such forces are capable of breaking molecules has been found in this study (Fig. 5). In addition, it must be remembered that measurements are made on particles which have been dried and exposed to high temperatures during evaporation of the platinum. Consequently, measurement of the length of shadowed particles is likely to be complicated by the presence of molecules that are both shorter than average (due to breakage or possibly to shrinkage) and larger than average (due to stretching or to aggregation). Subjective bias of the observer regarding particles chosen for measurement introduces a further complication. This limitation has

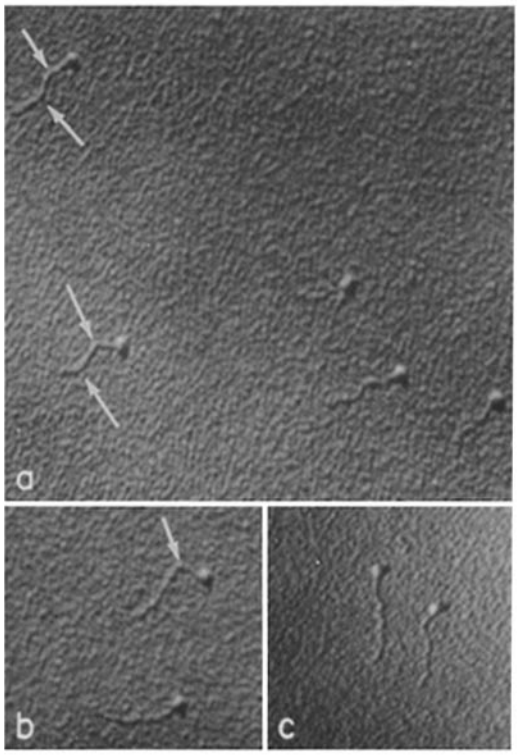

FIGURE 4 Selected fields illustrating angulation of rod portion (shaft) of several canine cardiac myosin molecules. Occasionally, two angulations in opposite directions are present on the shaft (4 *a*, arrows). The degree of flexibility of the shaft is indicated by angulation to a right angle without breakage of the shaft (4 *b*, arrow). General wavy outline of some molecules (4 *c*) is suggestive of some degree of flexibility of the entire shaft. × 75,000.

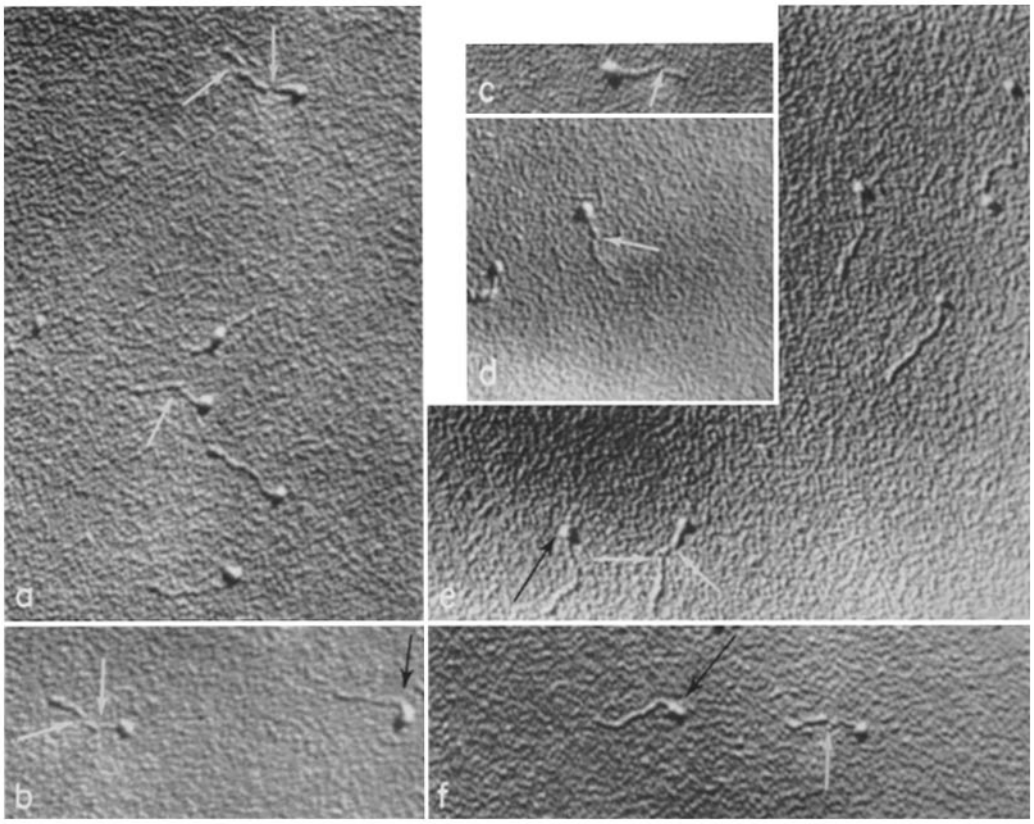

FIGURE 5 Several fields illustrating broken canine cardiac myosin molecules (white arrows). Most broken molecules show a single transverse break in the shaft (5 *c*, *d*, and *f*). Occasionally, two distinct transverse breaks are present in the shaft (5 *a*, *b*, and *e*). It is perhaps noteworthy that breaks do not seem to have occurred in angulated shafts. A few heads apparently have two globular structures in the head region (5 *e* and *f*, black arrows). Angulation of shaft in region of head is sometimes present (5 *b*, black row). × 75,000.

been recognized by other workers (14, 31). In this study, Huxley's criteria for selection of fields and of particles in these fields for measurement have been used (14).

The great preponderance of rod-shaped particles with a globular expansion on one end in mica-replica preparations (Table I) of ultracentrifugally homogeneous cardiac myosin suggests that these structures either represent myosin molecules or approximate them closely. Rods without a head and rod-shaped particles with a head on both ends were encountered so infrequently that it seems unlikely that either of these morphologic entities represents the intact myosin molecule. Our conclusions regarding the configuration of the canine cardiac myosin molecule are in agreement with those of Rice (19). Recently, we examined human cardiac myosin in the electron microscope (4), and the myosin molecule appears morphologically indistinguishable from the canine myosin molecule. Mica-replica preparations of rabbit skeletal myosin have been studied by several workers. Originally, many rod-shaped particles, varying in length, with a globular expansion on both ends of the rods were observed (22). Subsequently, it was concluded that, when higher ionic concentrations of volatile salt had been used for dissolving the myosin prior to spraying, the skeletal myosin molecule had only a single globular swelling on one end of its shaft (14, 23, 24). The low proportion of rodlike particles with a globule at both ends encountered in this investigation probably occurred because myosin was dialyzed against a high ionic concentration of ammonium acetate.

The slight amount of skewness in the histogram (Fig. 1) representing measurements of the lengths of 1908 molecules indicates a slight preponderance of shorter particles. This asymmetry can probably be attributed to breakage of some molecules, since approximately 2% of the particles measured were rods without heads (Table I). The mean length of the molecules (1610 ± 250 A) was close to, but slightly less than, the range in which the greatest concentration of molecular lengths occurred (1700 to 1800 A). However, the location of this range is somewhat dependent on the way in which the data are grouped and, further, molecular breakage (even though it did not appear to be a serious problem in this investigation) probably decreased the mean toward the shorter lengths. Rice has suggested an over-all length of 1450 A for the canine cardiac myosin molecule (19). However, the study was primarily a biochemical one, and sufficient morphologic data were not accumulated for statistical analysis. In the same communication, it was concluded from hydrodynamic studies that the molecule measured about 1800 A in length. Our estimate of the mean length (1610 A), although substantially less than that derived from those physicochemical studies, represents a closer approximation of the length as determined by morphologic and physicochemical techniques than that obtained by Rice. In a study of human cardiac myosin, in which the dimensions of about 300 molecules were estimated, the mean over-all length of the molecule was 1480 A (4). The distribution of the lengths was also moderately skewed toward the shorter lengths, and thus the significance of the mean was reduced to some extent. The real length of the human myosin molecule lies more probably, as we suggested, in the 1600 to 1700 A range where the greatest number of measurements occurred. Recent estimates of the length of the skeletal myosin molecule range from 1520 to 1590 A (14, 23, 31). Huxley reported a value of 1680 A for a limited number of selected fields where aggregation and debris were minimal (14). It appears, therefore, that the molecular lengths of cardiac myosin and skeletal myosin are approximately equal.

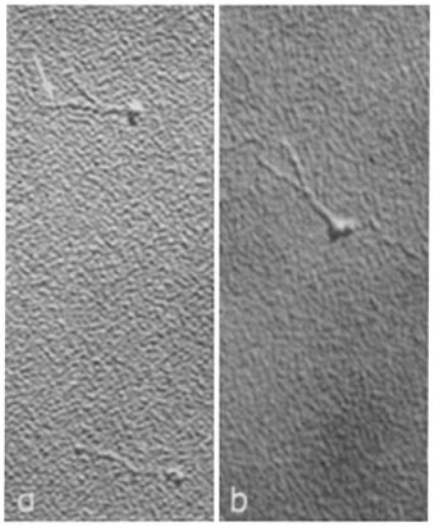

FIGURE 6 Canine cardiac myosin molecule with apparent longitudinal splitting of shaft (6 *a*). The presence of what appears to be a second head makes interpretation of appearances of shaft illustrated in 6 *b* very difficult. The second head probably represents a broken off portion of a second molecule (see Fig. 7 *a*). Alternatively, appearances are compatible with the presence of two intact molecules with their shafts lying alongside one another for a portion of their length. × 75,000.

The diameter of the shaft of the canine cardiac myosin molecule has been estimated to be about 20 A (19). Our results (16.0 ± 4 A), although somewhat lower, are in good agreement with this estimate. The rod portion of the human cardiac myosin molecule has a diameter of 15 to 20 A (4). Comparable figures for skeletal myosin, 10 to 30 A, 20 ± 5 A, and 15 to 20 A, have been reported (14, 22, 31), which indicates that this parameter of skeletal myosin and cardiac myosin is about the same. More recently, Huxley has indicated that, in negatively stained preparations of myosin in potassium iodide solution (μ = 0.6) the "molecules" have a diameter of 40 A (15). This estimate, which is considerably in excess of that derived from mica-replica preparations, may indicate either that considerable shrinkage of the molecules occurs in dried preparations or, more probably, that the negative-stained molecules examined have been denatured.

Rice estimated that the diameter of the head of the cardiac myosin molecule was 40 A (19). Our results are similar, although a diameter of 35 to 40 A is favored. A similar range of diameters was found in the human cardiac myosin molecule (4). In both species the head has a length of about 150 to 300 A (4). Similar parameters for rabbit skeletal myosin have been reported (14). Zobel and Carlson (31), however, estimated that the length of the skeletal myosin head was 200 to 700 A, with a

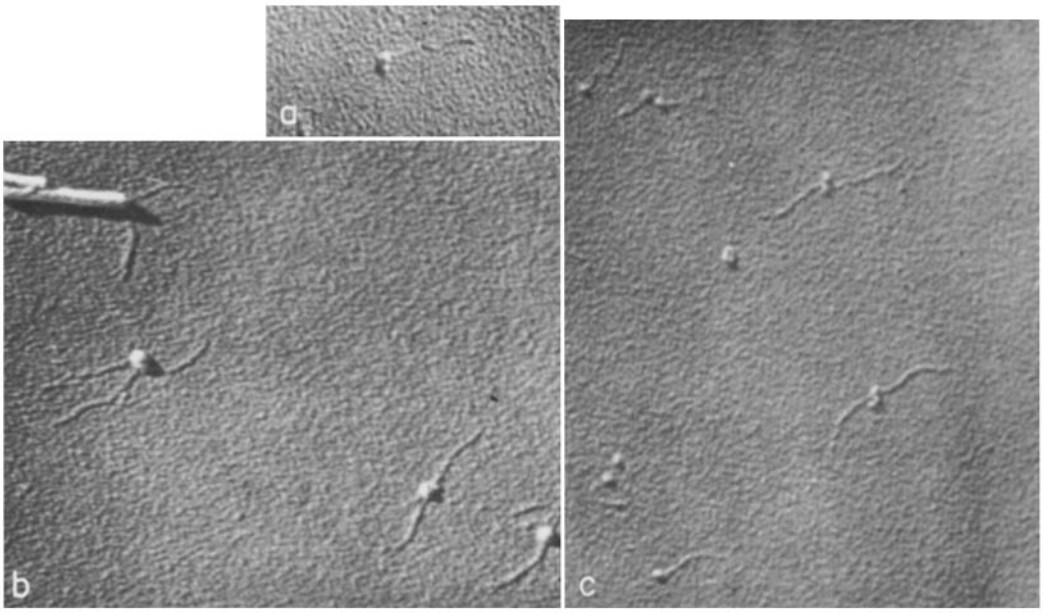

FIGURE 7 Several fields illustrating canine cardiac myosin aggregates and individually dispersed myosin molecules in mica-replica specimens prepared from myosin in 1.0 M ammonium acetate in the cold room. Growth of these aggregates can be traced from association of an apparently broken off head with head of intact molecule (7 *a*) to the association of two and three intact molecules at their heads (7 *b* and *c*). Tobacco mosaic virus is seen in 7 *b*. × 75,000.

mean of 440 A, and in their study the range of the diameter of the head was 35 to 60 A. Those investigators suggested that these increased dimensions were due to the loosening and spreading of the subunits that compose the head. Canine and human cardiac myosins in our experience show little tendency toward separation of the head substructure, and such subunits as may be present appear to be tightly bound together. The interpretation of heads, which appear to be composed of two or three smaller globules (Fig. 3 *b* and *c*; and Fig. 5 *e* and *f*), is not easy, and the significance of these appearances in regard to substructural elements in the head is unclear. Assessment is made doubly difficult, since a tendency for the molecules to associate at their heads has been shown in this investigation (Fig. 7). In some cases (Fig. 3 *c*; and Fig. 7 *a*), measurements indicate that two heads are likely present: one associated with the shaft and a second probably representing a broken off portion of another molecule. In other instances, however, the combined lengths of the two globules is within the range indicated in Fig. 2. Under these circumstances, the smaller globules could represent substructural components of the heads, or they could be separate heads.

Comparison of the results of this study with those of others (14, 23), indicates that in their gross morphology the molecules of canine cardiac myosin and rabbit skeletal myosin are the same. Measurements of the various parameters of the two types of molecule in mica-replica preparations are, with the exception of those reported by Zobel and Carlson (31) for the head, remarkably similar. The relatively few discrepancies in some parameters, for example, in the mean length of the molecules, are readily explicable on the basis of limitatations in the mica-replica technique already mentioned, particularly those limitations due to molecular breakage. In this study, attention was directed to a close scrutiny and measurement of the head region, the site of ATPase activity (20, 26), to determine whether or not there was a morphologic basis for the substantial difference in the ATPase activity of cardiac myosin and skeletal myosin. Our results indicate that in the size and shape of the head, the cardiac and skeletal myosin molecules are essentially the same. It seems that

FIGURE 8 Spindle-shaped aggregates, prepared by slow dilution of ultracentrifugally pure canine cardiac myosin in potassium chloride (μ = 0.6) with standard potassium chloride (μ = 0.15), negatively stained with 1% uranyl acetate. Aggregates are about 0.5 to 1.0 μ in length and 50 to 100 A in diameter. × 32,000.

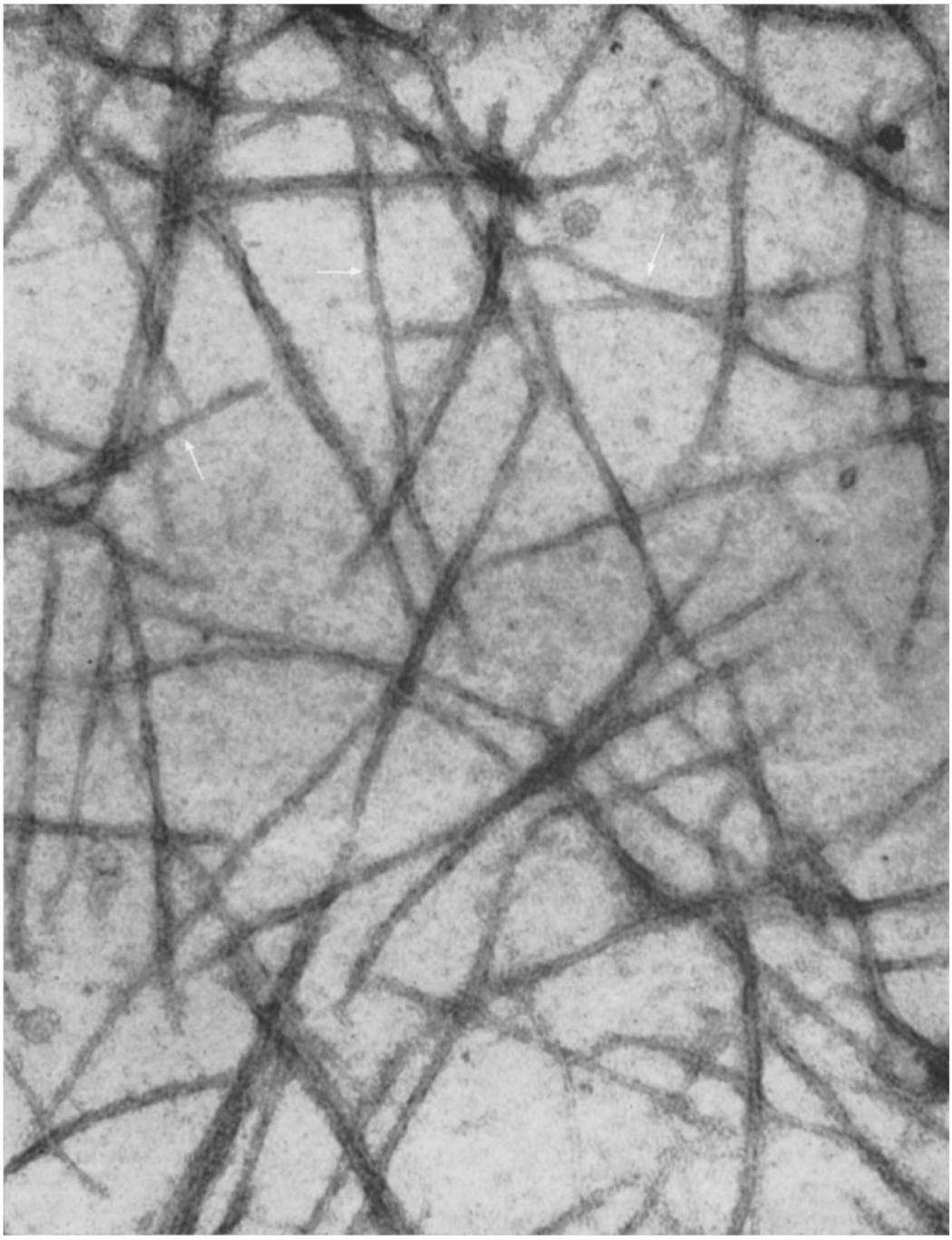

FIGURE 9 Longer, intertwined canine cardiac myosin filaments, produced by slow dilution of purified cardiac myosin in potassium chloride of ionic strength 0.6 to an ionic strength of 0.15, negatively stained with 1% uranyl acetate. Lateral projections (arrows) give aggregates a beaded appearance. Close inspection of areas where beading is prominent reveals that projections are out of register on opposite sides of filament. × 76,000.

these differences in enzymatic activity cannot yet be explained on a morphologic basis.

The finding of many molecules with single or multiple angulations along the shafts was somewhat unexpected (Fig. 3 *b*; Fig. 4 *a* and *c*). Rice has indicated that the cardiac myosin molecule is a fairly rigid structure (19). Data on skeletal myosin, however, are suggestive of a localized region of increased shaft flexibility close to the head, although the shaft is generally straight or slightly curved (31). Kinking of the shaft in the region of the head was also seen in cardiac myosin preparations, but it was not a prominent feature (Fig. 5 *b*). Huxley has pointed out that the only characteristic feature of negatively stained skeletal myosin "molecules" is that they appear curved (15). The shaft angulations frequently found in this study are very likely attributable to surface-tension forces generated during drying. Major differences in techniques are not likely responsible for the shaft angulations. These angulations indicate that the shaft of the cardiac myosin molecule can bend, sometimes even rather sharply (Fig. 4 *b*) without breaking. Skeletal myosin has an α-helical content of 56% (27). Presumably, cardiac myosin has about the same content. The α-helix is a rather inflexible structure, and in order to bend back completely on itself the α-helix must be destroyed over a segment of its length. Whether it is necessary to postulate localized destruction of the α-helix in the angulations of the shaft found in this study is not clear. In any event, there seems to be a moderate degree of shaft flexibility, permitting it to bend in several directions in response to tensions produced during the preparative procedure.

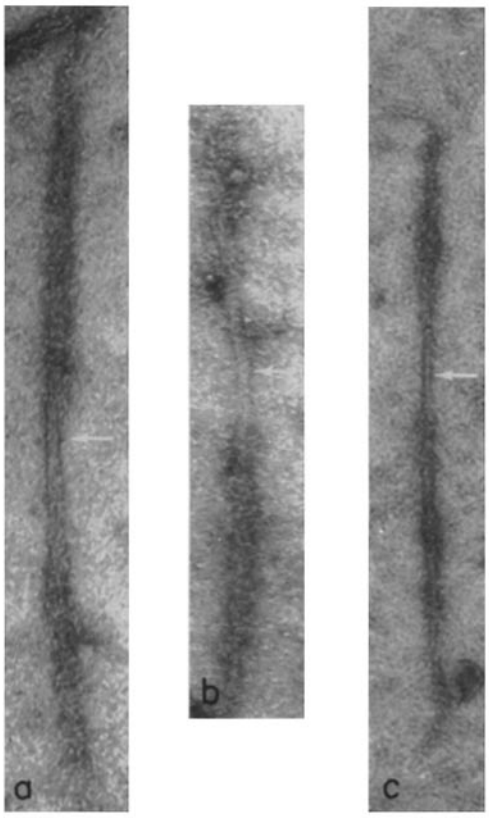

FIGURE 10 Aggregates, produced by rapid dilution of canine cardiac myosin in potassium chloride of ionic strength 0.6 to an ionic strength of 0.15, negatively stained with 1% uranyl acetate. Each aggregate shows a central area that is bare (arrow) with roughening of shaft on either side of this area. (10 *a* and *b*, × 104,000; 10 *c*, × 76,000.)

Cardiac myosin has the same capability as skeletal myosin of differentiating, after dilution, into structures having a close resemblance to the thick myofilaments of the myocardium (Figs. 8 to 10). However, these synthetic cardiac filaments frequently do not exhibit the localized area of thickening seen at the middle of the bare area of the thick myofilaments (M band) in longitudinal sections of myocardium. Huxley discussed this anomalous finding in relation to the skeletal thick myofilament and speculated on the possible presence of a small quantity of a protein, other than myosin, located at the center of the skeletal thick myofilament in vivo (14). If the thick myofilament is assumed to be composed of a greater proportion of myosin than that calculated (14), the question arises whether or not some of this deficit can be accounted for in a way which might simultaneously offer a partial explanation of the thickening present at the center of the thick myofilament. One suggestion is that since the small lateral projections on synthetic and naturally-occurring thick myofilaments are believed to correspond to the heads of myosin molecules (12–14), the localized area of thickening at the center of the thick myofilament (M band) in sectioned tissue might be due, at least in part, to an accumulation of heads of myosin molecules in this region. The description of cross-bridges linking the thick myofilaments of the myocardium together at this thickened zone (25) indicates that this possibility should be considered. Close proximity or actual contact or overlap of

heads might appear, except in ultrathin sections of tissue, as a localized thickening rather than as individual discrete heads. Some of the aggregates found in shadowed preparations (Fig. 7 *b* and *c* lower left) indicate that the heads of myosin molecules can associate together under experimental conditions of slow drying of the droplets in the cold room. It is possible that such an association of heads of myosin molecules might contribute to the formation of the localized swelling at the center of the thick myofilament, although this likelihood is reduced by antibody studies which indicate that there is an area free of myosin at the center of the thick myofilament (29).

The disposition of the fragments of the broken molecules found in this study was unexpected. In general, the broken pieces tended to lie in a straight line (Fig. 5). The main effect of the surface forces operative during drying of the droplets seemed to have been to produce angulation of the shafts. Yet, this localized stress probably did not result in many breakages, since examples of fragments, sharply angulated in relation to each other, were not found. Whatever the cause of breakage, which clearly occurred subsequent to deposition of the molecules on the mica, it does not seem to have been secondary to angulation of the shaft.

The substructure of cardiac myosin has not been investigated in detail. However, tryptic digestion has been shown to split the molecule into several fragments (20) including two that seem to be analogous to heavy meromyosin and light meromyosin obtained from skeletal myosin by brief tryptic digestion (17, 28). Mica-replica preparations offer some clues regarding the internal structure of the cardiac myosin molecule. Reference has already been made to the possible substructural organization of the head. The two-stranded molecules seen in this study (Fig. 6 *a*) could be interpreted as indicating that the shaft of the cardiac myosin molecule contained at least two strands; however, the data are meager and the possibility of artifact cannot be excluded.

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