Physicochemical studies on cardiac myosin of rabbits and dogs have shown that the myosin molecule is highly asymmetric, measuring about 1600 to 1800 Å long and having a molecular weight of approximately 500,000.\(^1\)\(^2\) Electron micrographs of canine cardiac myosin have confirmed this general morphology, although the molecule appears to be several hundred angstroms shorter than the physicochemical measurements suggest.\(^2\)

Detailed studies of human cardiac myosin, normal or pathologic, have not been made because of the practical problems involved in obtaining relatively large amounts of fresh human myocardium. However, quantities of human myocardium sufficient to yield enough myosin for electron microscopic study are frequently available at open heart surgery. Advantage was taken of this circumstance in the present study.

**Methods**

Two specimens of human myocardium, weighing approximately 1 g each, were obtained at operation, one from the right ventricle during the correction of a tetralogy of Fallot, and the other from a papillary muscle of the left ventricle of a patient with stenosis of the aortic and mitral valves. Each specimen was chilled immediately in ice-cold, de-ionized, distilled water. Myosin was prepared by the method of Mueller and co-workers\(^2\) with the following modifications necessitated by the small quantity of tissue available: 1. The chilled myocardium was chopped with a razor blade instead of being minced. 2. After the extraction, the steps of filtration through cheesecloth and paper pulp were omitted, and insoluble material was removed by centrifugation (2000 g for 5 min). 3. Myosin was precipitated twice instead of three times.

The chopped myocardium was extracted at 0 to 1°C for 10 minutes with four volumes of 0.3 M KCl, 0.075 M KH\(_2\)PO\(_4\), 0.075 M K\(_2\)HPO\(_4\), and 0.5 mM ATP, pH 6.6. Myosin was precipitated at an ionic concentration of \(\mu = 0.04\) and subsequently redissolved by adding solid KCl to raise ionic concentration to \(\mu = 0.5\). Actomyosin was removed by ultracentrifugation (96,000 g for four hours). Myosin was precipitated a second time; and, after it was dissolved in 0.5 M KCl, 0.01 M KH\(_2\)PO\(_4\), 0.03 M K\(_2\)HPO\(_4\), pH 7.0, any actomyosin remaining was removed by further ultracentrifugation (151,000 g for three hours). The myosin solution was dialysed against a 1 M ammonium acetate solution at 4°C for 24 hours. Dilute solutions (0.002%) were sprayed onto freshly cleaved mica in the cold room (tobacco mosaic virus was added as an internal standard). Platinum-carbon replicas were prepared by means of the technic of Mueller and co-workers\(^2\) and were examined subsequently in an RCA EMU-3G electron microscope, operating at 50 kv. Some specimens, having been shadowed, were rotated through 180 degrees and reshadowed.

**Results**

The yield of myosin was less than 1 mg in each case; consequently, no effort was made to characterize it by physicochemical procedures. Electron micrographs of the shadowed preparations showed that the predominant particle in each of the two myosin preparations was a slender rod of uniform diameter except for a bulbous expansion at one end (fig. 1). A histogram based on measurement of the length of 334 such particles is shown in figure 2. The average length of the particles was 1480 Å (SD = ±180 Å), and the shafts were about 15 to 20 Å in diameter. The bulbous expansion...
Dispersed molecules of human cardiac myosin prepared from 1 M ammonium acetate solution, at shadow angle of about 5/1, vary in length and generally have an expansion at one end. Tobacco mosaic virus (diameter = 150 Å) was used for internal standard (electron micrograph; X 78,000).

Histogram shows distribution of lengths of 334 molecules of human cardiac myosin, according to electron microscopic observations.

(head) was approximately 200 Å long and had a diameter of about 40 Å. The shafts of approximately 60% of the particles were more or less straight, but those of the remaining molecules showed one or several definite curves, sometimes even a sharp bend (fig. 3).
Micrographs obtained from specimens shadowed in two directions, 180 degrees apart, suggest that the end of the molecule opposite the head was tapered in some instances (fig. 4). Sufficient data were not accumulated to determine how frequently this occurred.

Comment

Major alterations in cardiac myosin obtained from dogs with chronic congestive cardiac failure have been reported and subsequently refuted. Although neither of our patients had chronic congestive cardiac failure, the question of whether the molecules examined in this study show changes resulting from heart disease cannot be resolved until myosin from normal human hearts is examined.

The molecule of human cardiac myosin is morphologically similar to that of canine cardiac myosin and also to that of rabbit skeletal myosin. The average length for the human cardiac molecule (1480 Å) compares favorably with that reported by Mueller and co-workers for the canine cardiac molecule (1450 Å)² and with Huxley’s mean measurement for rabbit skeletal myosin (1520 Å).⁶ The distribution of the molecular lengths in the histogram (fig. 2) is moderately skewed to the left; this is to be expected if distortion due to molecular breakage is added to the normal distribution of myosin molecules. In fact, the most frequent measurement in our experience was 1660 Å. It seems likely, therefore, that the actual length of the molecule of human cardiac myosin is closer to the median (1660 Å) than to the mean (1480 Å). Huxley, working with rabbit skeletal myosin, found that in his best fields the average molecular length was 1680 Å.⁶

The globular expansion measured about 200 Å long and about 40 Å in diameter. The globular expansions in molecules of canine cardiac myosin also measured 200 Å,⁶ and Huxley reported this same value for the globular end of molecules of rabbit skeletal myosin.⁶ Zobel and Carlson, however, reported a considerably larger value (440 Å) for the length of this portion of the molecule in skeletal myosin.⁷ The diameter of the shaft (15 to 20 Å) was about the same as that of the shaft of molecules of canine cardiac⁶ and of rabbit skeletal myosin.⁶

A large proportion (40%) of the molecules examined had distinct single or multiple angulations along the shaft (fig. 3). We have noted this also in studies of canine cardiac myosin (unpublished data); however, Mueller and co-workers did not describe this in their report on canine cardiac myosin.² The published electron micrographs of skeletal myosin indicate that this molecule is a relatively straight or slightly curved structure. Our findings suggest that the cardiac myosin molecules, human and canine, may be more flexible than their skeletal counterparts. Studies are in progress to determine whether the sharper bends tend to have specific localization or tend to be randomly distributed along the molecule. Specific localization might pinpoint areas of greater flexibility along the shaft.

Summary

Solutions of human cardiac myosin contain macromolecules which were visualized in the electron micrographs.
electron microscope by the shadow-casting technic. The dominant particle was observed to be rod-shaped, to have a globular expansion at one end, and to have a mean length of about 1500 Å. The molecule of human cardiac myosin was found to be morphologically similar to myosin molecules from canine cardiac muscle and rabbit skeletal muscle.

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