Method for Isolation of Large Quantities of Human and Canine Cardiac Myofibrils*  

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Morphological and physiological studies have not provided an understanding of the basic mechanisms of myocardial failure. It is possible that in some instances the myocardial cell itself is defective and that the defect may involve changes in the intracellular production, transport, or utilization of energy required for efficient contraction. One factor leading to such changes may be intrinsic to the myofibrils themselves, and our attention has been directed to the isolation and study of the activity of these intracellular contractile units.

The syncytial nature of the myocardium renders the isolation of single, intact myofibrils more difficult than it is with skeletal muscle. The following method, however, has proved satisfactory.

EXPERIMENTAL

Ventricular myocardium from canine and human hearts was used. The hearts were removed as soon as possible after death and washed or perfused with 0.154 M KCl to remove blood and blood clots. The great vessels and epicardial fat were dissected away and the ventricular muscle divided into pieces weighing 30 to 50 gm. These were used at once or stored at 

In preparing myofibrils for study, the large blocks of myocardium were cut into smaller pieces. Muscle which had been kept at 

was cut up while still frozen or only partially defrosted. The tissues were placed in an Eppenbach Colloid Mill (QV6) containing 200 ml. of a 4:1 glycerol-O.01 M Tris buffer solution, pH 7.0. The mill was cooled with tap water circulating through the water jacket. During the first 5 minutes of operation of the mill, the rotor-stator space was set at 60 units and the rotor speed at 2500 r.p.m. This combination of a wide milling space and low rotor speed divided the myocardiun into fragments of connective tissue and muscle cells. The rotor-stator space was then decreased to 18 units and the rotor speed increased to 3300 r.p.m. for 60 to 90 minutes. During this time small samples were removed periodically and the progress of cellular fragmentation was followed microscopically. Milling was terminated after all cells had been disrupted and their component myofibrils released individually or in small packets. When fresh muscle or muscle which had been frozen immediately after death was used, the crude suspension was allowed to stand for 30 to 60 minutes at room temperature before further purification.

The crude suspension was purified in a cold room at 2-3°. It was diluted with 1 volume of 0.154 M KCl and divided among eight centrifuge tubes. These were centrifuged at 1300 × g for 30 minutes or until the supernatant was clear. The supernatants were discarded and the process repeated. The sediment was then resuspended in 0.154 M KCl and centrifuged at 1000 × g for 1 to 2 minutes and the upper, unpacked portion of the suspension retained. These were combined and concentrated into four 40-ml centrifuge tubes. The sediment was discarded.

The retained portion was washed six times with 0.154 M KCl for 10 to 15 minutes at 630 × g, with the sediment being re-suspended each time. Finally, the sediments were suspended in 1:4 Tris buffer (1:4 in 0.154 M KCl at pH 7) and centrifuged for 30 to 60 seconds at 1000 × g. The upper, cloudy portion was retained and stored at 0°. Before use, this purified suspension was washed twice more with Tris buffer (1:4 in KCl at pH 7).

This procedure was equally useful for isolation of myofibrils from human and canine skeletal muscle.

RESULTS

Effect of Variation of Post-mortem Period—Canine and human hearts were obtained at varying intervals after death and either milled at once or stored at 

Hearts obtained 6 hours or more after death, whether milled at once or frozen before milling, yielded myofibril suspensions characterized by: (a) numerous fragmented fibers, (b) short myofibrils, (c) a distinct granularity of myofibrils, and (d) narrower sarcomere lengths with the “I” bands generally narrower than the “A” bands. Suspensions with similar characteristics were obtained from hearts which were frozen within 4 to 6 hours after death but which were allowed to defrost at room temperature before milling.

Effect of Variation of Glycerol Content of Solution Used in Milling—When the myocardium was milled in small volumes of
buffer (a procedure satisfactory for isolation of myofibrils from skeletal muscle), numerous well preserved cardiac myofibrils were recovered. However, these disintegrated very soon after isolation. No myofibrils were obtained when small amounts of muscle were milled in large volumes of buffer. Myofibrils, obtained from fresh cardiac muscle milled in various aqueous buffers with glycerol concentrations up to 50 per cent, contracted spontaneously during the isolation procedure. When the glycerol concentration in buffer solutions was 70 per cent or more, there was a high yield of well preserved myofibrils which did not perceptibly contract during the milling process. The use of other viscous solutions containing gelatin, gum arabic, gum ghatti, and paraffin oil resulted in suspensions with few well preserved myofibrils.

Effect of Various Buffers—The 4:1 glycerol-buffer solutions were made up with phosphate-phosphate, phosphate-citrate (μ 0.154 to 0.25, pH 7.0), borate, and Tris buffers. There were no significant differences in the myofibrils suspensions obtained with these glycerol-buffer combinations. Tris buffer was selected for the following reasons: (a) it contains no phosphate which would interfere with subsequent enzyme activity determinations, (b) it is a more efficient buffer at pH 7.0, and (c) the myofibrils were better preserved on washing with 0.1 M Tris buffer than with other buffers and especially so at higher molar concentrations.

Effect of Dilution—When myofibrils were prepared from hearts obtained and milled immediately after death, dilution of the crude, unwashed glycerol suspension within 30 minutes after milling produced a prompt and irreversible contraction. This effect occurred with the use of 0.1 M Tris buffer, 0.1 to 0.15 M KCl, 0.1 to 0.25 M NaCl, phosphate-phosphate buffer (μ 0.154 to μ 0.25), phosphate-citrate buffer (μ 0.154 to μ 0.25), or 0.1 to 0.25 M sucrose as the diluent. This effect also occurred in suspensions prepared without the use of glycerol. Contraction quickly proceeded to about 40 per cent of the original length in 1:1 dilutions, whereas maximal contraction to about 5 per cent of the original length occurred in 1:10 dilutions. A similar response to dilution was noted when unwashed glycerol myofibril suspensions from hearts which were frozen immediately after death and milled without defrosting were used. Contraction upon dilution did not occur in (a) suspensions prepared from hearts removed longer than 6 hours after death; (b) suspensions from hearts obtained immediately after death but allowed to remain at room temperature for 4 to 6 hours before milling; or (c) suspensions from heart obtained and milled immediately after death but maintained at room temperature for 1 to 3 hours before dilution. This “dilution effect” was avoided in milled suspensions of fresh muscle by allowing the crude, unwashed 4:1 glycerol suspension to remain at room temperature for 30 to 60 minutes after milling and before the washing procedure leading to purification was begun.

Yield of Myofibrils—Regardless of the time which elapsed after death before the heart was obtained and milled, the yield of myofibrils was approximately 20 to 30 per cent of the weight of myocardium used.

Purity of Myofibril Preparations—Light and electron microscopy revealed that the suspensions consisted almost entirely of single myofibrils of varying length and a few packets of unseparated fibers. Occasional nuclei were seen. No mitochondria or connective tissue fibers were identified and miscellaneous tissue particles of similar dimensions were scarce.

After the preparation stood for several days, bacteria often appeared even at 0°. The growth of the bacteria could be controlled by addition of 200 units of penicillin and 1 mg. of streptomycin per ml. of preparation. The antibiotics had no demonstrable effect on the structure, contractility, or ATPase activity of the myofibrils.

Morphology of Myofibrils—The microscopic structure of myofibrils obtained from cardiac and skeletal muscle was very similar. The “A” and “I” bands were distinct, although their boundaries were somewhat blurred in fresh preparations. Studies with the electron microscope disclosed no differences between myofibrils of cardiac and skeletal origin after isolation by the method described.

Contractile Response of Myofibrils—As noted above, dilution with buffer caused contraction of myofibril suspensions prepared from fresh muscle. Contraction of purified suspensions also occurred after addition of ATP in concentrations varying from 0.5 to 4.0 mM in the presence of added 1.0 mM Mg++ at a pH of 7.0. The contraction was prompt, complete, and irreversible to approximately 5 per cent of the original length of the myofibril. In this stage all structural detail disappeared and the myofibrils appeared as dense, round dots. When ATP

![Fig. 1: Inorganic phosphate liberation by human cardiac and skeletal myofibrils.](image-url)
was added in the absence of additional ions or with added calcium ions, either no contraction or contraction to not less than 80 per cent of the original myofibril length occurred. No contraction took place upon the addition of Mg++ or Ca++ alone, without ATP. These contractile properties were identical to those observed in studies of skeletal myofibrils (1, 2).

**ATP Activity of Myofibrils**—The accumulation of inorganic phosphate in the filtrates of myofibril suspensions was used as the determinant of ATPase activity. The Fiske-SubbaRow (3) method for phosphate analysis was used. ATPase action was stopped with trichloroacetic acid at varying intervals after ATP addition. The reaction mixture was maintained at 37° in a water bath.

In general, the most rapid liberation of phosphate from ATP occurred in the presence of added 1.0 mM Mg++. Phosphate liberation was less rapid and in lesser amount in suspensions to which 1.0 mM Ca++ had been added and least rapid in control suspensions to which neither calcium nor magnesium ions were added (Fig. 1).

The effect of the duration of the post-mortem period on the ATPase activity of myofibril suspensions was studied in canine hearts. The hearts were removed after death and maintained at room temperature for up to 24 hours. Samples of myocardium were taken at once, at 6 and at 24 hours. The myofibrils were then isolated. There was no significant difference in the ATPase activity of the myofibrils obtained immediately after death and 6 hours after death. Less than a 5 per cent decrease in phosphate liberation was noted when myofibrils were prepared from muscle which had been stored at room temperature for 24 hours.

Myofibril suspensions stored at 2° for periods up to 14 days lost some ATPase activity, but the loss never exceeded 10 per cent of the initial activity.

**DISCUSSION**

Several methods are available for the study of skeletal and cardiac muscle. Among these the glycercinated fiber preparation of Szent-Gyorgyi (4) has been used extensively in the study of skeletal muscle and by Ranney (5), Benson et al. (6), and others in studying cardiac muscle. The various soluble proteins of muscle have been studied by methods developed by Weber (7, 8), Edsall (9), Banga and Szent-Gyorgyi (10), Szent-Gyorgyi (11), Bailey (12), and others. Thread models formed by extrusion of dissolved contractile proteins, as developed by Portzehl and Weber (13) and Portzehl (14) have also provided methods of approach to an analysis of muscular contraction.

We believe that a study of the isolated cardiac myofibril as described herein may contribute information unobtainable by other techniques in the investigation of the normal and pathological myocardium. Whether the preparations may prove useful in carrying out certain microchemical bioassays remains to be determined.

There are some advantages to using these preparations. The substrate in which the myofibrils react is subject to rather rigorous control. The reactive system is uniform, particulate, and easily separable from the substrate. The same method is equally applicable to the isolation of cardiac and skeletal myofibrils from human and animal sources during life or after death. The retention by the isolated myofibrils of the morphological characteristics of the myofibrils in intact muscle renders them suitable for both light and electron microscopic observation. The large quantities of myofibrils recovered by the technique facilitate the simultaneous use of several chemical determinations on a single sample. The contractile response of the isolated myofibril is easily observed and provides a means of studying this mechanical process simultaneously by physical, chemical, and morphological techniques.

**SUMMARY**

A method for isolation of large quantities of mammalian cardiac and skeletal myofibrils was developed. The myofibrils were recovered as suspensions of individual units with little admixture with other tissue elements. Studies with light and electron microscopy showed excellent preservation of structural detail. Adenosine triphosphatase activity was retained, and the influence of different substrates on this activity was measured quantitatively. Under proper conditions of substrate, this activity was accompanied by prompt contraction of the myofibrils from thin long structures into broad, short rods and thence into spherical masses. These properties were characteristic of all myofibrils studied, even though they were recovered from muscle obtained several hours after death. Thus it has become possible and practical to make comparative studies of large amounts of human cardiac and skeletal myofibrils under controlled substrate conditions. These studies permit close correlation of changes in form, chemical composition, and enzymatic activity of the contractile myofibrils with changes in the composition of the substrate from which they may be easily separated.

**REFERENCES**