Some Properties of Contractile Proteins of the Heart as Studied on the Extracted Heart Muscle Preparation

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The physiologic properties of the contractile elements (actomyosin) have been studied on the extracted heart muscle of the dog. It was found that many properties of fresh heart muscle are due to inherent qualities of the contractile proteins. For example, the speed of contraction decreased with increasing loads, the work increased with rising tension up to an optimal value, and increased fiber length resulted in greater muscle work.

There appears, now, convincing evidence that the main protein associated with muscular contraction is actinomyosin, and that shortening of the muscle fibers results from the interaction of actinomyosin with adenosinetriphosphate (ATP). The basic process of muscular contraction can be reproduced in vitro. Threads of actinomyosin can be prepared from a pure actinomyosin solution. These threads contract in a 0.1 M potassium chloride solution when ATP is added. However, this technic fails to preserve the structural elements of muscle. Szent-Györgyi has recently described a preparation of skeletal muscle which contracts only in the presence of ATP and electrolytes; electric stimulation fails to elicit a contraction. This indicates that the contractile process is no more initiated through the physiologic process of stimulation but occurs as a result of a combination of actinomyosin with ATP. In this “extracted” or “washed” skeletal muscle, the histologic structure of fresh muscle is essentially preserved. It appeared of interest, therefore, to prepare extracted muscle fibers from the myocardium and to study their contractility. This paper deals with some of the physiologic properties of washed heart muscle and the factors which may influence its work performance.

Methods

The chest of dogs anesthetized with Nembutal (35 mg. per kilogram weight) was opened under artificial respiration. The heart was quickly removed from the body and the beat was immediately stopped by placing the organ into ice cooled distilled water. Muscle strips measuring about 2 mm. in diameter and 2 cm. in length were then dissected from trabecular muscle of the left ventricle and tied at both ends to wooden sticks in order to prevent shrinkage. Only strips from parallel arranged fibers were used and stretching of the fibers during dissection was avoided. The strips were extracted in 50 per cent glycerol for three days at 0 C., followed by extraction for 10 to 14 days at —20 C. according to the method described by Szent-Györgyi for skeletal muscle. Thinner strips measuring 0.4 to 0.7 mm. in diameter and 2 cm. in length were then dissected from the extracted strips; their diameter was microscopically determined. Isotonic and isometric contractions of these strips were studied following immersion in isotonic salt solution with a cation composition identical to that of heart muscle* and an ATP (sodium salt)† content of 0.5 per cent. When the fibers were immersed in the ionic solution alone, no contraction took place: however, as soon as ATP was added, shortening occurred. More uniform results were obtained by immersing the extracted preparation in a cation solution already containing ATP. All experiments were performed at room temperature (26 to 28 C.).

Since extracted muscle preparations fail to relax following the ATP induced contraction, it was necessary to use a new preparation for each experiment. Only a limited number of preparations could be obtained from the same heart. Therefore, strips from several hearts had to be compared. In order to avoid any error resulting from variations in the

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* NaCl 4.68 Gm., KCl 4.75 Gm., MgCl₂·6H₂O 2.85 Gm., CaCl₂ 0.57 Gm., distilled water 1000 ml.
† Sigma Chemical Company, St. Louis, Mo.
size of the strips, the work performed by the isotonically contracting muscle preparations was related to their initial length and diameter.

\[
\text{Work} = \frac{\text{mm. contraction after 30 seconds} \times \text{load}}{\text{initial length} \times \text{diameter}}
\]

The tension applied on the strips by various loads was calculated as follows:

\[
\text{Tension} = \frac{\text{load}}{\text{cross section of strip}}
\]

A strain gage manometer (Statham), calibrated with loads varying from 0.5 to 2 Gm. was used to measure the tension of the isometrically contracting muscle preparation.

**RESULTS**

**Comparison between Extracted and Fresh Heart Muscle**

The histologic structure of extracted heart muscle was identical with that of the fresh preparation. In contrast, the extraction had greatly altered the mechanism of excitation: epinephrine, acetylcholine, or single and tetanic electric stimuli induced no contraction. Contraction could only be initiated when ATP in a proper ionic solution was added. The ATP-induced contraction of extracted heart muscle, progressed much slower than electrically induced contraction of fresh heart muscle. In contrast to fresh muscle preparations, extracted heart muscle did not relax following contraction. These findings are in line with those obtained on extracted skeletal muscle.1-4

**Effect of ATP**

In 25 experiments the influence of various concentrations of ATP on the isometrically developed tension of washed heart muscle was studied. It could be shown that a minimal concentration of 0.1 to 0.2 per cent ATP in the cation solution was required to initiate contraction. An increase in ATP concentration was accompanied by a rise in isometric tension. Maximal isometric tensions were recorded with an ATP content of about 1 per cent. These findings illustrate that the supply of ATP determines within certain limits, the force of contraction of the extracted myocardial preparation (fig. 1). Similar findings were obtained by Korey on washed skeletal muscle.4

**Speed of Contraction of Extracted Heart Muscle**

Figure 2 illustrates that the degree of shortening of extracted heart muscle was directly proportional to the logarithm of time. Therefore, the slope of the contraction curve could be expressed as a function of the logarithm of time.
time and the degree of shortening. In order to make it possible to compare the individual results obtained on a variety of preparations, the slope was calculated for 1 mm. of the strip.

\[ \text{Slope (s)} = \frac{\Delta \text{mm. contraction}}{\Delta \log \text{time} \times \text{initial length of the muscle strip}} \]

The slope, at any given time, is directly proportional to the speed of contraction.

The slope of contraction and its relationship to the load were investigated in a series of experiments on isotonically contracting extracted heart muscle preparations. Figure 3 illustrates the relation between the slope of contraction and the tension exerted on the muscle strips. Each point in figure 3 represents the contraction of one preparation. The results of these measurements showed that the slope decreased as the tension rose. Similar results were obtained by Fenn on fresh frog muscle, who found that the speed of contraction decreased with the log \((W + kv)\), where \(W\) represents the load, and \(v\) the speed of contraction.\(^5\) Fenn’s results were later confirmed by Hill.\(^6\) However, the findings described in this report on washed heart muscle cannot be directly related to those obtained by these investigators who maintained the initial length of the muscle, while in our experiments the initial length increased with rising tension.

**The Relationship between Work, Load and Initial Length**

In order to investigate whether the relationship between initial length or initial tension and work could be demonstrated on the extracted heart muscle preparation, extracted cardiac muscle strips were made to contract isotonically against different loads. The results of these experiments are illustrated in figure 4 in which the work per unit fiber is plotted against the tension exerted by the load. Each point in figure 4 represents the contraction of one preparation. It may be seen that the work increased with rising tension up to a maximal value, and decreased as this tension was exceeded. The maximal work performance equalled 100 mg. per millimeter fiber length per millimeter diameter; this maximal work was accomplished when the preparation was made to carry a load of 800 to 1000 mg. per square millimeter. This represented about 50

\[ \text{maximal work} = \frac{\text{ATP} \times 0.8\%}{\text{diameter}} \]

**Fig. 3.** Graph showing isotonic contraction of extracted heart muscle after addition of 0.8 per cent ATP. It may be seen that the speed of contraction expressed by its logarithmic slope decreases with rising tension. To assure equal rate of diffusion, the diameter of the muscle strips used in these experiments was relatively constant (0.5 to 0.6 mm.).

**Fig. 4.** This figure shows isotonic contraction of extracted heart muscle after addition of 0.8 per cent ATP. The work of the isotonically contracting muscle strip calculated per unit fiber (millimeter length per millimeter diameter) is related to the tension exerted on this preparation. It may be seen that the work performance increases with rising tension up to an optimal value and decreases when the tension becomes excessive.

Work on the whole heart has demonstrated that a moderate rise in initial fiber length results in increased work of the heart. There-
fore, experiments were performed to investigate the effect of an increase in length of the muscle strips, which was obtained by a prolonged period of stretch. Strips of heart muscle prepared as mentioned above, were stretched to 20 to 30 per cent of their initial length and tied on heavy wooden sticks in order to prevent shortening. Some unstretched strips from the same heart were used as controls. Both stretched and unstretched strips were then extracted in 50 per cent glycerol as described above. Thinner strips (0.4 to 0.7 mm. diameter) were again dissected from these extracted myocardial strips. Due to a marked decrease in elasticity of the extracted strips, only slight shortening of the muscle was observed upon release of the stretch prior to the initiation of the loaded isotonic contraction. The work-load relationship of these fibers was determined as described above, and the work was again calculated per unit fiber. The results of these experiments are illustrated in figure 5. It may be seen that similar to muscles which had undergone no stretching during the extraction, the work of the prestretched preparation increased with rising load up to an optimal value; it decreased as the tension became excessive. However, the work performed by the prestretched strips at any given load definitely exceeded that of the unstretched preparation (fig. 5). These findings demonstrate that a prolonged period of stretch leading to an increase in initial length results in a greater work performance of the extracted heart muscle. When, on the other hand, the washed strips were stretched to 50 to 60 per cent of their initial length, a diminution of the work was observed. It is possible, however, that this was the result of mechanical injury, since tears in the fibers were observed after excessive stretching.

DISCUSSION

The results reported here demonstrate that washed heart muscle preparations do not respond to the usual electrical or chemical stimulation and contract only after addition of ATP in a proper ionic medium. This indicates that the physiologic mechanism of stimulation has been lost and that the contraction process is initiated directly by the interaction of ATP and the contractile proteins, probably actomyosin. Despite these functional differences, the histologic structure of the extracted myocardium is similar to that of fresh unextracted heart muscle and the contraction process in the washed heart muscle occurs in an essentially unchanged muscle structure. Consequently, due to the loss of the normal excitatory stimulus for contraction and the permanent association of actin with myosin to actomyosin, some physiologic properties of muscular contraction can be studied without interference from excitation and relaxation.

A comparison between the extracted and fresh heart muscle reveals that the ATP induced contraction of the former is much slower than the electrically induced twitch of fresh muscle. Since, under the microscope, individual extracted heart muscle fibers were observed to contract very rapidly following addition of ATP, the rate of diffusion of ATP into the preparation was probably an important factor for this difference.

The importance of a sufficient supply of ATP for muscular contraction is illustrated in experiments in which it could be shown that
within certain limits, an increase in ATP supply results in greater force of contraction (fig. 1). This finding could have been anticipated because ATP appears to be the main source of energy directly available for muscular contraction.\(^1\)\(^-\)\(^8\) The question whether changes occur in myocardial ATP content or its turnover with different degrees of cardiac work, has not yet been conclusively answered.\(^9\)\(^-\)\(^10\)

Similar to the results obtained by Fenn and Hill, the speed of contraction, expressed by the logarithmic slope, decreases with rising loads. The resemblance between the speed-load relationship of isotonically contracting heart muscle preparations and the fresh skeletal muscle is so great as to suggest that the properties of muscle responsible for this relationship are inherent qualities of the contractile proteins.

The work of the extracted heart muscle increases with rising tension up to a maximal value and then decreases as this load is exceeded. In this respect, extracted heart muscle reacts similarly to fresh heart or skeletal muscle, or, for that matter, to the whole heart.\(^7\)\(^-\)\(^11\)\(^-\)\(^12\) The similarity in the response to increased load of extracted heart muscle and of fresh skeletal muscle or the whole heart suggests that this response of muscle is the result of fundamental properties of the contractile proteins.

It has been found by Ramsey and Street that the length-resting tension relationship of muscle fibers is mainly due to properties of the sarcolemma.\(^13\) The extracted heart muscle preparation shows a similar length-resting tension relationship to fresh muscle, namely a progressively larger rise in tension with increase in length (fig. 6). The properties of the sarcolemma are therefore at least partially preserved in the extracted muscle fiber. However, in contrast to fresh muscle, the return values show a relatively greater muscle length at corresponding tensions (larger hysteresis) (fig. 6). It is likely that this finding explains the partial loss of elasticity, observed in the preparations which had undergone prolonged stretch during extraction. The increase in initial length observed in this preparation appeared to be responsible for the larger amount of work performed at any given tension.

Astbury suggested that "myosin" in the resting muscle exists in a half-folded "\(\alpha\) form." As the muscle is stretched, the "myosin" molecules are first oriented, then as stretch increases further, "myosin" is transformed into "\(\beta\) myosin."\(^11\) The findings on the prestretched heart muscle preparation, reported above, suggest that changes in "initial length," which occur during prolonged stretch, may lead to similar changes in the orientation or organization of the contractile elements. These changes then could be responsible for the larger work performance of the prestretched preparation.

Studies on the whole heart in vitro demonstrated that the cardiac work is determined by the initial fiber length and/or initial tension.\(^7\)\(^-\)\(^15\) The present findings suggest that increase in fiber length alone can lead to a greater muscle work. However, whether in the whole heart an increase in length alone can occur without any increase in tension, is not known. It is possible that in the whole heart any increase in length is secondary to a rise in tension.

**SUMMARY**

Some of the physiologic properties of the contractile elements (actomyosin) have been studied on the extracted heart muscle of the dog. Contractions could be elicited only with
ATP in a proper ionic medium. The contraction process was initiated by direct interaction between ATP and the contractile proteins.

The force of contraction of extracted heart muscle was dependent on the ATP supply. Similar to fresh muscle, the speed of contraction decreased with increasing loads and the work of the isotonically contracting preparation increased with rising tension and decreased after an optimal load was exceeded. The work performed by extracted fibers which had undergone a prolonged period of stretch, was greater at any given load than that of the unstretched preparation. The increased work output observed appeared to be the result of increased initial fiber length.

These observations were interpreted as evidence that the molecular orientation of the contractile proteins of heart muscle during stretch may determine its work performance. The essential similarities between extracted and fresh heart muscle suggest that many of the physiologic properties of fresh heart muscle are due to inherent qualities of the contractile proteins.

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Circ Res. 1953;1:129-134
doi: 10.1161/01.RES.1.2.129

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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