# ACTIN PARTICIPATION IN ACTOMYOSIN CONTRACTION*

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## INTRODUCTION

In current muscle literature, any consideration of the mechanism of muscle contraction must seek to interpret an extensive body of information from experimentation on the so-called "muscle models" (see, for example, reviews by BUCHTHAL *et al.*[1], WEBER[2], among others). Such a mechanism must inevitably include the characteristics and reactions of the proteins actin and myosin. Generally, in the most recently proposed contraction mechanisms[2,3,4], a configurational change of one or the other of these proteins with or without an interaction between them, or an interaction without a configurational change is suggested.

Before any molecular mechanism can be accepted, clarification of a fundamental question is required: the question whether myosin alone has the capacity to undergo a configurational change, *i.e.* to contract; or whether it requires the presence of, and interaction with, the second principal structural protein, actin.

On this question, KAFIANI AND ENGELHARDT have published the results of a series of experiments[5], from which they have concluded that myosin alone is capable of contraction upon reaction with ATP, and that the role of actin seems to be to alter and to stabilize the pH dependence of the myosin ATPase. Since these results have been quoted widely (*cf.*[3,6,7]) for their obvious bearing on molecular mechanisms, and since they seemed to be in conflict with some of our earlier unpublished results, a re-examination of the question was undertaken**.

## MATERIALS AND METHODS

### 1. *Myosin*

Myosin, as actin-free as possible, was prepared according to the method of SZENT-GYÖRGYI[8]. The thrice-precipitated product, as a concentrated solution in 0.6*M* KCl, was stored in this form in ice until used, or alternatively in 50% glycerol with enough KCl added to maintain the molarity at 0.6*M*, and stored in the deep-freeze. In the latter case, the glycerol was removed before use by diluting the stock with 10 volumes of water, and the resulting myosin precipitate packed by centrifugation and re-dissolved in a suitable volume of 0.6*M* KCl.

### 2. *Actin*

The actin was prepared according to the method of FEUER, MOLNÁR, PETTKO AND STRAUB[9] from the muscle residue left after the myosin extraction. The acetone-dried muscle powder was stored in a desiccator in the deep-freeze. For each experiment, a fresh extract of G-actin was prepared and polymerized to F-actin as described by SZENT-GYÖRGYI[10]

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*References p. 8.*

3. *Other reagents*

All the chemicals used in the experiments were standard commercial products, reagent grade. The water used throughout the experiments was laboratory-distilled water de-ionized by passing through a column of Amberlite MB-3 ion-exchange resin.

The ATP used in the experiments was the disodium crystalline Sigma product. This was dissolved in water, neutralized with KOH, suitable buffers and other reagents added before bringing up to volume determining the concentration of ATP. Thus, the ATP used at a concentration of $5 \cdot 10^{-3}M$ carried a contamination of Na, about $0.01M$.

4. *Tests for the actin content of the myosin preparation*

*a. Solubility test.* The myosin, in solution in $0.6M$ KCl and clarified by centrifugation for 15 min at 8,000 *g*, was brought to low ionic strength ($0.085M$) and the protein diluted simultaneously by the addition of water. The final protein concentration was approximately 1.5 mg/ml, and the preparation exhibited a slight turbidity. Upon the addition of ATP (final concentration $4.7 \cdot 10^{-4}M$) the preparation became somewhat clearer. According to WEBER[11], this test showed the preparation to be L-myosin, or actin-free myosin. By contrast, a control preparation of re-constituted actomyosin, similarly treated, showed "superprecipitation"[10] indicating the presence of actin.

*b. Viscosimetric test.* A sample of myosin (Preparation VII) was dissolved in $0.6M$ KCl with $0.01M$ tris buffer at pH 7.6, diluted suitably until the discharge time in the Ostwald viscosimeter was approximately 2 min. For the solvent alone, the discharge time in the same viscosimeter was determined to be 71 sec, at 23°C, ± 0.1°. Using 6.0 ml of protein solution, the following data were obtained:

TABLE I

VISCOSITY TEST

| *Trial* | *Myosin* | *Myosin + 0.1 ml ATP ($5 \cdot 10^{-3}M$)* |
|---|---|---|
| 1 | 123.2 sec | 122.1 sec |
| 2 | 123.1 sec | 122.4 sec |
| 3 | 123.1 sec | 123.1 sec |
| 4 | 123.1 sec | 123.1 sec |
| Average | 123.1 sec | 122.5 sec |

From the actomyosin viscosity studies of SCHRAMM, PORTZEHL, AND WEBER[12], the "activity" of the myosin solution may be calculated from the following relationship:

$$\text{"Activity"} = \frac{\log\eta_{rel} - \log\eta_{relATP}}{\log\eta_{relATP}} \, 100$$

where $\eta_{rel}$ = relative viscosity of the protein solution without ATP and $\eta_{relATP}$ = relative viscosity of the protein solution after ATP addition.

Since an "activity" value of 150 represents 20% actin, it may be calculated that the above data show an actin content of approximately 0.1%. This represents an actin content in a range which is barely detectable. The figures given do not include the dilution effect of the small volume of ATP. For many other preparations of myosin, this was included, and gave lower values of the actin content, so that the 0.1% value is a maximum one.

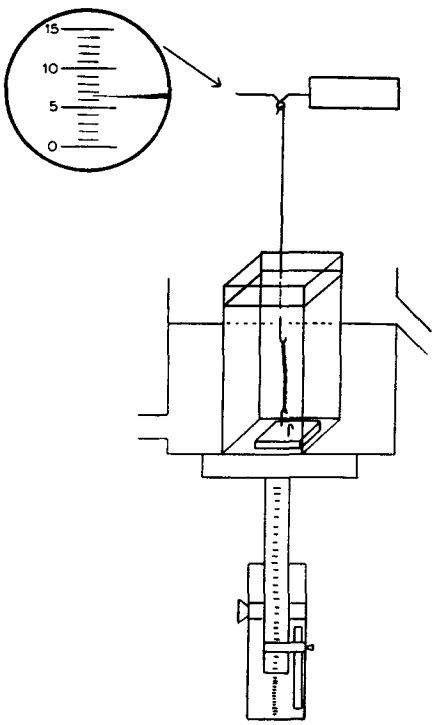


Fig. 1. Apparatus for measuring isometric tension. Description given in text. The reaction vessel does not show the stirring chamber and bubbling arrangement.

5. *Preparation and handling of fibers*

The fibers were prepared according to the method of HAYASHI[13] and isotonic contractions measured according to the same author. For the isometric contractions, a quartz lever was used, the movement of the tip of the lever magnified by the low power objective of an ordinary microscope (Fig. 1).

*References p. 8.*

This is a modification of the method originally developed by SICHEL[14]. The lever arrangement afforded a fiber movement which, in the range of the tensions measured, amounted to a maximum of 0.676 mm. Since the fibers, on the average, were approximately 20 mm in length, the maximum amount of shortening was 3.4% of the total length. A tension of 0.4 mg could be accurately measured.

The suspension (see Fig. 1) connecting the protein fiber to the lever consisted of an upper thin rod of quartz to which was fused a short length of 3.5 mil platinum wire. This wire passed through the surface film of the reaction mixture, to minimize surface tension effects, and to its lower end was attached a small glass hook. The fiber itself was tied to this hook, the lower end being tied to a second hook fixed to a lead block coated with paraffin which thus served as a fixed attachment.

Stirring with a minimum of disturbance of the fiber was accomplished by means of a small bubbling chamber separated from the main body of the reaction vessel by a plastic partition. Openings at the top and bottom of the partition permitted circulation of the aqueous solution of the reaction chamber, the circulation being driven by the bubbles.

Before each measurement of tension, a few preliminary stretches were applied to the fiber, in order to extend all parts of the fiber uniformly. The fiber was then allowed to come to equilibrium at a low initial tension before the addition of ATP. All tension values given therefore are "developed tensions" over and above the initial small tension (usually about 2 mg).

## EXPERIMENTS AND RESULTS

### 1. *Isometric contraction of myosin and actomyosin at pH 9.0 and 7.6*

According to KAFIANI AND ENGELHARDT, the ATPase activity of pure myosin is maximum at 9.0, whereas in combination with actin as actomyosin, the maximum ATPase activity is shifted to pH 7.0*. For this reason they tested for the contractile activity at the higher pH.

In the present experiments, fibers of myosin were tested for ATP-induced contraction at both pH 9.0 and 7.6 (see footnote), and their behavior compared to fibers of actomyosin under the same conditions. In all experiments, the pH of the reaction vessel was tested electrometrically both before and after the contraction. To check specific buffer effects, three different buffers were used: 0.025 $M$ tris(hydroxymethyl)-aminomethane buffer, 0.05 $M$ borate buffer, and 0.03 $M$ glycine buffer. All three buffers yielded exactly the same results, so that the results using the tris buffer are presented, since this buffer was used most extensively in other experiments.

Fig. 2 presents the typical and unfailing result from this group of experiments. It shows clearly that myosin without actin does not contract at either pH 7.6 or pH 9.0. Actomyosin does not contract at pH 9.0, but at pH 7.6 it contracts strongly as shown.

In contraction experiments of this type, there is another variable, namely the presence of $Mg^{++}$ or $Ca^{++}$. In the experiment presented, the $Mg^{++}$ was omitted for the myosin fiber at pH 9.0 since it has been reported[15] that $Mg^{++}$ inhibits the enzyme activity of myosin. In other experiments, however, both of these divalent ions were tested for their effects on myosin contraction with the same results; the myosin fiber does not contract at either pH 9.0 or 7.6.

### 2. *Isotonic contraction of myosin and actomyosin at pH 9.0 and 7.6*

Tests made under isotonic conditions with a load of 10.1 mg confirmed the above results (Fig. 3). In contrast to the actomyosin fiber at pH 7.6, the myosin fiber at pH 9.1 did not shorten, but lengthened slightly with the application of ATP.

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* The authors state that the fibers "contract maximally at pH 7.0," and refer this statement to one of us[13]. This is in error, since the reference states that the maximal contraction takes place at pH 7.6, although the fibers are *formed* at pH 7.0.

*References p. 8.*

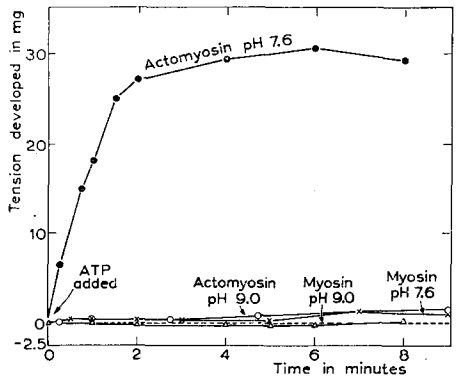


Fig. 2. Myosin and actomyosin: isometric contraction. Reaction mixtures: pH 7.6. 0.05*M* KCl and 0.0001*M* $MgCl_2$ in 0.025 *M* tris buffer. Final concentration of added ATP = $5 \cdot 10^{-3}$ *M*.

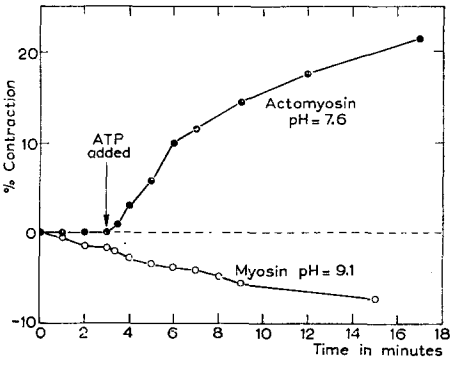


Fig. 3. Myosin and actomyosin: isotonic contraction. Rection mixtures: as in Fig. 1. Load 10.1 mg in water.

pH 9.0. 0.05*M* KCl and 0.0001*M* $MgCl_2$ (no $MgCl_2$ for the myosin fiber) in 0.025*M* tris buffer. Final concentration of added ATP = $5 \cdot 10^{-3}M$.

### 3. *Enzymic activity of myosin and actomyosin fibers at alkaline and acid pH.*

Since contraction in muscle and muscle models is often correlated with ATPase activity[2,5], the argument might be advanced that the failure of the myosin fibers to contract could be due to a lack of enzymic activity of these fibers because of denaturation of the protein in preparation. Tests were made, therefore, of the fibers used in the present investigation for their enzymic activity concurrently with the tension measurements. A fiber was suspended in the specified reaction mixture at 25°C, and aliquots were removed at timed intervals and analyzed for inorganic phosphate by the method of FISKE AND SUBBAROW[16] while tension measurements were being made. Tests were made for actomyosin fibers at pH 7.6 in 0.06*M* KCl and 0.0001*M* $MgCl_2$; for myosin fibers at pH 10–9.5 in 0.06*M* KCl and 0.001*M* $CaCl_2$, and at the acid pH 6.4 in 0.06*M* KCl and 0.04*M* $CaCl_2$. The pH and ionic composition of the reaction mixtures were in accordance with the conditions specified by MOMMAERTS AND GREEN[15]. The fibers were exposed to the reaction mixtures only during the course of the run.

The results showed that the fibers possess a definite ATPase activity in all three determinations; that is, myosin fibers as well as actomyosin fibers can split ATP, but only the actomyosin fiber contracts. The results demonstrate, therefore, that the ability to hydrolyze ATP by itself does not confer the property of contraction to myosin fibers.

### 4. *pH dependence of actomyosin contraction*

The foregoing results show that myosin does not contract at either pH 7.6 or 9.0, and further tests (not shown in figures) have established that fibers of myosin alone do not contract at all within this range. However, actomyosin contraction varies with the pH in this range, a fact already published for isotonic contractions[13]. Since the pH dependence of contraction is a crucial point in the present argument, a complete pH dependence curve for isometric contraction for the present material was established. The result is presented in Fig. 4, and it may be seen that, whereas the optimum pH is

*References p. 8.*

about 7.6 and very little contraction occurs above pH 8.8, considerable tension may be developed at pH 8.5.

5. *The effect of actin on contraction*

The results thus far presented show a clear contradiction to the results reported by KAFIANI AND ENGELHARDT. In an effort to explain the cause of this discrepancy, a series of experiments was run using fibers made of myosin and graded amounts of actin. These fibers were made by preparing separate solutions of myosin in 0.6 *M* KCl, and F-actin in 0.1 *M* KCl. An aliquot was removed from each solution for protein nitrogen determination. Mixtures were then made in various proportions of the two proteins, the actin content being expressed as the actin/total protein ratio based on the protein nitrogen determinations. An aliquot was removed from each mixture for

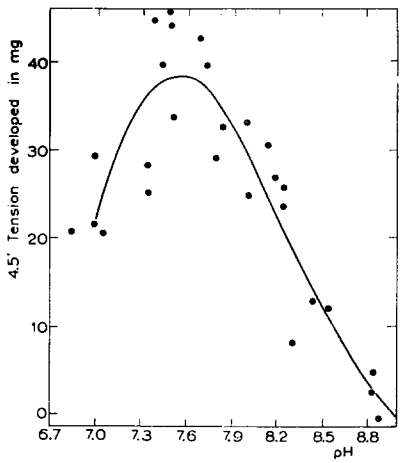


Fig. 4. pH dependence of isometric contraction of actomyosin fibers. All contractions in 0.05 *M* KCl and 0.0001 *M* $MgCl_2$, buffered with 0.025 *M* tris buffer, $5 \cdot 10^{-3}$ *M* ATP.

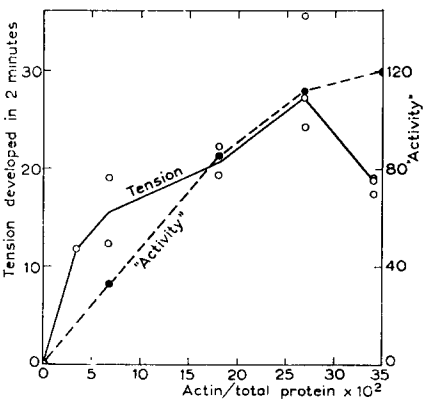


Fig. 5. Comparison of tension development and viscosity change with varying actin content. All contractions in 0.05 *M* KCl and 0.0001 *M* $MgCl_2$, in 0.025 *M* tris buffer at pH 7.6, final ATP concentration $5 \cdot 10^{-3}$ *M*. All viscosity tests on 6 ml of actomyosin in 0.6 *M* KCl, add 0.1 ml of $5 \cdot 10^{-2}$ *M* ATP.

the determination of "activity" viscosimetrically. Each mixture was then used for the formation of fibers and the determination of contractile properties.

For uniformity, the same preparation of myosin from the glycerinated stock and the same actin preparation were used throughout the series. Since five different actin–myosin mixtures were made, each of which was tested for fiber contraction and viscosity changes, the total number of tests run in any one series was naturally limited by time. The comparison of performance by different fibers cannot as yet be made absolutely quantitative since the fibers cannot be made uniform along their length, nor can the cross-sectional area be accurately determined. Nonetheless, previous experience[13] has shown that fibers formed on a trough of constant area, with standardized spreading and compression technique, will give reasonably uniform results. For these reasons, the performances of the different fibers employed in the experiment can be compared as to their relative contractile activity. All fibers were run in 0.05 *M* KCl containing 0.0001 *M* $MgCl_2$ at pH 7.6, since the preceding experiments had shown that actomyosin does not contract at pH 9.0.

*References p. 8.*

The "activity" of each mixture of myosin and actin in solution in 0.6 *M* KCl and pH 7.6 was determined viscosimetrically.

The results are presented in Fig. 5 in summary form. The contractile performance of the fibers is given as the tension developed in 2 minutes (left-hand ordinate), while the "activity" values are given by the right-hand ordinate. The actin content (abscissa) is expressed as the ratio actin/total protein in percent. For the tension curve, each point represents a different fiber, and all of the fibers used in the experiment are presented.

The data show that, at least up to about 27% actin, the tension development is dependent upon the presence of actin, as is the "activity". At a higher actin content, there is an apparent decrease in the contractile activity. However, it must be noted that since all the fibers were about the same size, an increase in the actin content means also a decrease in the absolute myosin content of the fibers. This fact probably accounts for the decrease in contractility. From this, it would seem that the "true" optimal content of actin lies at a value somewhat higher than 27%.

*6. The effect of temperature on the behavior of myosin and of actomyosin fibers*

All of the foregoing experiments involved the formation of fibers, their suspension, and contraction at temperatures of about 25°C. Since LAKI AND CARROLL[17] have shown that myosin exposed to room temperature for two hours undergoes a reversible change of the sedimentation properties, which they suggest may be related to the contractile properties of myosin, experiments were done to test whether such changes affecting the contractile properties of the fiber system were occurring under the temperature conditions of the previous experiments. Specifically, fibers were tested for contraction which, during their formation and subsequent handling, were never exposed to temperatures higher than 3–4°C.

A myosin fiber was formed on the trough in a cold room at 0°C and kept at this temperature during all the subsequent handling. After suspending the fiber from the isometric lever and preliminary stretching, etc., had been completed, ATP was added to the reaction vessel, still at 0°C. Tension measurements were begun and, after some time, the temperature was raised to 25°C. The results are presented in Fig. 6.

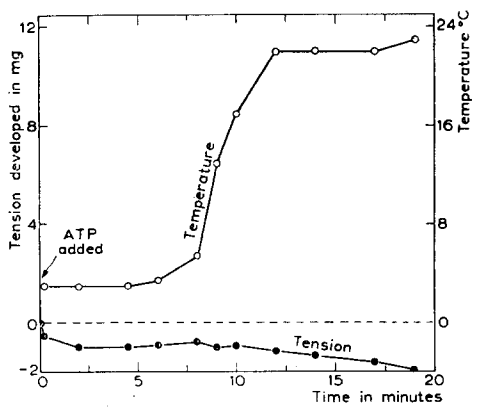


Fig. 6. Effect of temperature change on myosin fiber + ATP. Solution: 0.05 *M* KCl and 0.0001 *M* $CaCl_2$ in 0.025*M* tris buffer pH 9.1 at 25°C, final ATP concentration $5 \cdot 10^{-3} M$.

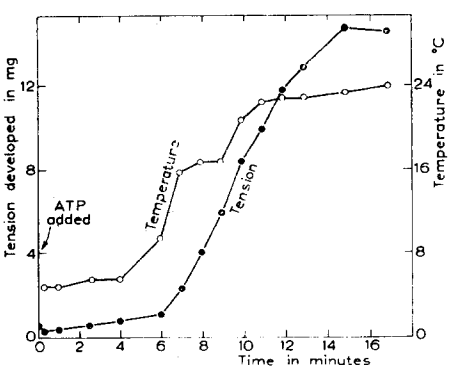


Fig. 7. Effect of temperature change on actomyosin + ATP. Solution: 0.05 *M* KCl and 0.0001 *M* $MgCl_2$ in 0.025 *M* tris buffer pH 7.6 at 25°C, final ATP concentration $5 \cdot 10^{-3} M$.

*References p. 8.*

The open circles trace the progress of temperature change. The solid circles indicate that the myosin fiber showed no tendency to develop tension.

As a control, the same procedure was carried out for actomyosin, which did not spread very well at 0°C, so that the fiber was rather poorly "structurated"[5]. Nevertheless, when the temperature was raised, the fiber contracted unmistakably (Fig. 7), thus showing that the failure of the myosin to contract was not due to an already contracted condition due to exposure to room temperature. As a further control, tension–time curves were determined for both myosin and actomyosin fibers without the addition of ATP (not shown in figures). Save for small tensions of the order of one mg, the temperature change left the fibers unaffected.

## DISCUSSION AND CONCLUSIONS

The results presented in Figs. 2 and 3 show clearly that a condition for contraction for fibers formed from muscle protein is the presence of actin. The results are in sharp contradiction to those presented by KAFIANI AND ENGELHARDT[5]. A precise clarification of this disagreement is not possible because of lack of detail in their published account, but there are certain points in the experimental procedure by which this difference in result might be explained.

The viscosimetric test for the actin content of the myosin solution depends on the drop in viscosity upon the addition of ATP. Since this drop in viscosity reverses upon the depletion of ATP with enzymic hydrolysis, the duration of the lowered viscosity is dependent on the amount of ATP added. Thus, the drop in viscosity must be detected reasonably quickly after the addition of an adequate amount of ATP in a small volume to minimize the dilution effects. If these conditions are not met, an actual actin content of 1% or more may not be detected.

Since our measurements show that a myosin fiber does not contract at either pH 7.6 or pH 9.0, whereas an actomyosin fiber contracts only at the lower pH, the factor of hydrogen ion concentration must be considered. The complete pH-dependence curve for contraction (Fig. 4) indicates that if the buffering is not adequate at the upper limits of the pH curve so that the pH should drop to, say 8.5, a sizable tension is developed. The myosin fibers at the alkaline pH possess a high enzymic activity resulting in a considerable production of acid. The figures of KAFIANI AND ENGELHARDT show weak tensions (*ca.* 10 mg) at pH 7.0 and 9.0, a result to be expected with fibers contaminated with actin and insufficient buffering at the higher pH. Further evidence in support of this explanation comes from the finding that fibers initially exposed for 15 min to pH 9.0, and then brought to pH 7.6, contract strongly. That is, at least on the alkaline side, Fig. 4 represents a true pH curve uncomplicated by irreversible denaturation at the high pH.

The effect of increasing the amounts of actin in the fiber shown in Fig. 5 lends support to the above argument. It may be seen from Fig. 5 that actin present to the extent of only a few percent can result in the development of considerable tension in the fiber, even though the "activity" in solution may be quite low.

The role of actin in the contraction process is most probably a highly specific one. This is indicated by the fact that the fiber contraction dependence on the actin present parallels that of the ATP-induced viscosity drop of actomyosin solutions, a well-known specific reaction. Further, the optimal actin/total protein ratio of 27% or

*References p. 8.*

slightly higher for both contraction and viscosity drop is in good agreement with the indicated stoichiometric complex formation of these proteins from the ultracentrifuge studies of SNELLMAN AND ERDÖS[18]. Likewise, another specific actin–myosin reaction, that of the ATP-induced "super-precipitation"[10], shows an optimal actin/total protein ratio in agreement with those given here, as shown by SPICER AND GERGELY[19]. These authors obtained an actin/total protein ratio of 20% after correcting for the non-actin protein contamination in the actin preparation. The same correction applied to the present data and to that of SNELLMAN AND ERDÖS[18] brings the optimal actin/total protein ratio from all three investigations to about 20%.

It may be concluded that myosin alone does not have the ability to contract, and that actin is a specific factor for contraction. It is acknowledged that, while the present experiments show that the presence of actin is a sufficient condition for contraction, they do not prove that it is a necessary condition. However, in the light of the specific nature of actin–myosin interactions, and the oft-demonstrated occurrence of these proteins in large amount in muscle, one may infer that the actin–myosin complex is instrumental in muscle contraction.

## SUMMARY

1. Pellicular fibers made of actin-free myosin show no ability to contract, at either pH 7.6 or 9.0.

2. Fibers of actomyosin contract strongly and optimally at pH 7.6, but do not contract at pH 9.0.

3. With increasing actin content, up to approximately 27%, the actomyosin fibers exhibit an increasing contractile activity.

4. Fibers formed of actomyosin and of myosin hydrolyze ATP enzymically, the former at 7.6, the latter at pH 6.4 and 9.5.

5. Myosin fibers, formed and maintained in the cold, do not contract when the temperature is raised in the presence of ATP, whereas actomyosin fibers under the same conditions, contract strongly with the rise in temperature.

6. It is concluded that myosin without actin does not contract, and that actin is a specific requirement for contraction in the muscle protein model system.

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