ATPase Activity of Myosin Correlated with Speed of Muscle Shortening

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ABSTRACT Myosin was isolated from 14 different muscles (mammals, lower vertebrates, and invertebrates) of known maximal speed of shortening. These myosin preparations were homogeneous in the analytical ultracentrifuge or, in a few cases, showed, in addition to the main myosin peak, part of the myosin in aggregated form. Actin- and Ca^{++}-activated ATPase activities of the myosins were generally proportional to the speed of shortening of their respective muscles; i.e. the greater the intrinsic speed, the higher the ATPase activity. This relation was found when the speed of shortening ranged from 0.1 to 24 muscle lengths/sec. The temperature coefficient of the Ca^{++}-activated myosin ATPase was the same as that of the speed of shortening, Q_{10} about 2. Higher Q_{10} values were found for the actin-activated myosin ATPase, especially below 10°C. By using myofibrils instead of reconstituted actomyosin, Q_{10} values close to 2 could be obtained for the Mg^{++}-activated myofibrillar ATPase at ionic strength of 0.014. In another series of experiments, myosin was isolated from 11 different muscles of known isometric twitch contraction time. The ATPase activity of these myosins was inversely proportional to the contraction time of the muscles. These results suggest a role for the ATPase activity of myosin in determining the speed of muscle contraction. In contrast to the ATPase activity of myosin, which varied according to the speed of contraction, the F-actin–binding ability of myosin from various muscles was rather constant.

It is generally accepted today that contraction of all muscles is brought about by the interaction of actin, myosin, and ATP, regardless of the type of muscle (e.g. skeletal or smooth) or the difference in its physiological behavior (e.g. speed of contraction or maximum tension produced). In the past two decades, studies on actin and myosin have led to the accumulation of considerable knowledge about these proteins: thus both actin and myosin can be isolated in a pure form, their molecular weight, size, and shape are well understood, and their amino acid composition has been determined; moreover, there is some fragmentary information on their primary structure (for review see reference 1). Of course, the main question remains as to how these proteins function in muscle, and in this connection certain properties of actin

and myosin are of special importance. Perhaps the most important is the interaction of ATP with both. Actin in its G-form contains bound ATP, and, in its F-form, bound ADP. Myosin or actomyosin hydrolyzes ATP into ADP and P_i.

Recent studies in this laboratory have shown that the physiologically important nucleotide of actin, the bound ADP of F-actin, is not involved in the characteristic properties of F-actin or actomyosin (2) and thus it appears that, in the interaction of actomyosin with ATP, the myosin molecule plays the key role. It is the object of this paper to show that the ATPase activity of myosin varies from muscle to muscle and that it is the ATPase activity of myosin which ultimately controls the speed of muscle contraction.

EXPERIMENTAL

The individual muscles were carefully dissected from the animals and stored in beakers, chilled in ice, until enough material (at least 2.5–3 g) had been collected. To isolate the largest possible amount of myosin from the muscles, the total actomyosin was extracted first, and from this the myosin was prepared by the dissociation procedure of A. Weber (3). It was also necessary to prepare myosin from actomyosin, since in a few cases (see below) only glycerinated muscle was available; thus the classical methods of preparing myosin from fresh muscle could not be used. Preparing both actomyosin and myosin from the same muscle has the added advantage that, by comparing the ATPase activities of actomyosin and myosin, one can detect any denaturation of myosin taking place during its isolation.

Preparation of Myosin

The muscles were weighed, cut with scissors into small pieces, and washed exhaustively with cold 0.04 M KCl, pH 7, to remove the soluble proteins. The washed residue was blended with the 0.04 M KCl solution for 3 min; then KCl, Tris-HCl buffer, pH 7.5, and ATP were added from appropriate stock solutions to obtain final concentrations in the homogenate of 0.6 M, 0.05 M, and 1.5 mM, respectively. The final volume of extracting solution was 20–25 ml/g of fresh muscle. Extraction was performed at 4°C for about 20 hr. Insoluble residues were removed from the extract by centrifugation at 13,000 *g* for 20 min in the cold, and the actomyosin in the supernatant was precipitated by dilution with 15 volumes of cold distilled water. The precipitate was collected in a refrigerated centrifuge at 2500 *g*, dissolved in 0.6 M KCl, pH 7, and reprecipitated once more. In the case of actomyosins from *Mytilus* posterior adductor and rabbit uterus muscles (Table IV), two reprecipitations were performed at ionic strength 0.27, pH 7.5, to remove contaminations of tropomyosin A (4). The final precipitates were dissolved in 0.6 M KCl.

The actomyosin solution was diluted with 0.6 M KCl to 5 mg/ml, chilled in an ice bath, and then brought to final concentrations of 0.02 M Tris-HCl buffer, pH 7.5, 0.01 M $MgSO_4$, and 0.01 M ATP. The mixture was immediately centrifuged in the chilled rotor 50 of the Spinco preparative ultracentrifuge at 150,000 *g* (average force) for 3 hr. The supernatant solution was dialyzed against 300–400 volumes of 0.02 M

KCl, pH 7, in the cold overnight; the precipitated myosin was collected, washed with 0.04 M KCl, pH 7, and dissolved in 0.6 M KCl, pH 7. The protein concentration of the myosin solution was adjusted to 6 mg/ml, and remaining actomyosin was removed from the solution by centrifugation at 150,000 *g* for 1 hr. The upper two-thirds of the supernatant solution, containing the pure myosin, was used to determine ATPase activity, ATP sensitivity, and homogeneity in the analytical ultracentrifuge.

By this method myosin was prepared from the following muscles, which were kept in glycerol at −15°C for 2–4 weeks: cat and sloth extensor digitorum longus and gastrocnemius medialis (Table II); squid mantle, *Limulus* abdominal, *Thyone* longitudinal retractor (Table III); and dogfish mesenteric (Table III) and coracohyoid (Table IV). The cat muscles were used to demonstrate that the glycerol treatment mentioned herein does not decrease the ATPase activity of myosin. Furthermore, Fig. 2 shows that myosin homogeneous in the analytical ultracentrifuge can be isolated from glycerinated muscles.

Determination of ATPase Activity

ATPase activity was determined under magnetic stirring in a temperature-controlled water bath in a final volume of 4 ml for 1–3 min. The conditions were as follows: *Actin-activated ATPase*: 30 mM KCl, 20 mM Tris-HCl buffer, pH 7.4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 1 mM ATP; 0.4 mg of F-actin/mg of myosin. *Ca^{++}-activated ATPase*: 30 mM KCl, 20 mM Tris-HCl buffer, pH 7.4, 10 mM $CaCl_2$, 1 mM ATP (Table IV). The same assay medium was used to determine Ca^{++}-ATPase activity in the presence of 0.05 M KCl and 0.50 M KCl (Tables I–III) when KCl in concentrations of 50 and 500 mM was substituted for the 30 mM KCl. *EDTA-activated ATPase*: 0.50 M KCl, 20 mM Tris-HCl buffer, pH 7.4, 1 mM EDTA, 1 mM ATP. The reactions were initiated by the addition of ATP and stopped by the addition of 1 ml of 10% trichloroacetic acid to the incubation mixtures. After filtration, 3 ml samples were used to determine inorganic phosphate according to the method of Rockstein and Herron (5). All determinations of ATPase activity were performed in duplicate under conditions wherein the ATPase activity was a linear function of either the enzyme concentration or the reaction time.

No denaturation of myosin was observed when ATPase activity was determined at high temperatures, i.e. at about 37°C (Tables II and IV). This conclusion is supported by the close agreement between the ATPase activities measured directly or extrapolated to the experimental temperature by use of the Q_{10} values of the ATPase activity.

Actomyosin Formation

The reaction system contained 1.6 mg of myosin/ml, 0.4 mg of rabbit skeletal F-actin/ml, 0.6 M KCl, 20 mM Tris-HCl buffer, pH 7.4, and 1 mM $MgSO_4$, in a total volume of 4 ml. The actomyosin formation was expressed according to H. H. Weber and Portzehl (6) as ATP sensitivity. To determine the viscosity of the actomyosin solution in the presence of ATP, an ATP concentration of 1.2 mM was used. Viscosity determinations were performed in a temperature-controlled water bath.

Preparation of Actin

Actin was prepared from the skeletal muscle of rabbit (7) and purified according to Mommaerts (8). In a few cases, actin was prepared and purified by the same procedures from other skeletal muscles, e.g. frog or *Pecten*. The source of F-actin had no effect on either the extent of actomyosin formation or the activation of myosin ATPase.

Other Methods

Protein was determined by the biuret method of Gornall et al. (9) as described earlier (7).

Sedimentation was performed in the Spinco model E ultracentrifuge at a constant temperature of 20°C.

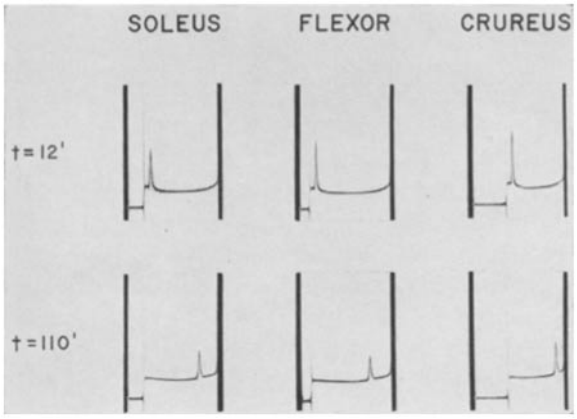

FIGURE 1. Ultracentrifugal patterns of cat myosins in 0.6 M KCl, pH 7.0. Protein concentration, 2.3 mg/ml; speed, 59,780 rpm.

The sloth muscles were generously donated by Professor M. Goffart, University of Liége, Belgium; the *Limulus* abdominal, dogfish mesenteric, and coracohyoid muscles were generously donated by Professor C. L. Prosser, University of Illinois, Urbana; the human elbow flexor muscle was generously donated by Dr. J. Hagestrom, Cornell Medical School, New York.

RESULTS

Relationship between Contraction Time and ATPase Activity of Myosin of Muscles

It was shown previously in this laboratory (10) that myosin from the slow muscles of the rabbit (soleus and crureus) has ATPase activities 2 to 3 times lower than those from the fast muscles (gastrocnemius and extensor digitorum longus). Since the speed of contraction of rabbit muscles had not been studied, these experiments were repeated with cat muscles of known contraction times (11).

Fig. 1 shows the homogeneity of these myosin preparations in the analytical ultracentrifuge. They sediment as a single component during the 2 hr of the experiment. Table I compares the contraction time of cat muscles with the ATPase activity of their myosin and with the ability of the myosins to combine with F-actin (see "ATP sensitivity" in Tables I and II). The flexor hallucis longus muscle contracts 2.6 times faster than the crureus, and 2.8 times faster than the soleus muscle. The actin-activated ATPase activity of myosin from the flexor muscle is 2.3 times higher than that of myosin from the crureus, and 3.2 times higher than that of soleus muscle, whereas the Ca^{++}-activated ATPase activity of myosin from the flexor is 2.2 times higher than that from the crureus and 2.4 times higher than that from the soleus. Thus, the ratios of myosin ATPase activities are about the same as the ratios of the contraction speeds for the muscles. In contrast to the differences in the ATPase activ-

TABLE I
RELATIONSHIP BETWEEN CONTRACTION TIME AND ATPASE ACTIVITY OF MYOSIN IN CAT MUSCLES

Muscle	Contraction time at 37°C*	ATPase activity at 25°C in the presence of		ATP sensitivity at 25°C
		Actin	Ca^{++}+0.5 M KCl	
	msec	*μmole P_i/mg/min*	*μmole P_i/mg/min*	%
Flexor hallucis longus	27	0.55	0.36	133
Crureus	70	0.24	0.16	144
Soleus	75	0.17	0.15	138

* Reference for contraction time: Buller et al. (11).

ities of myosin from fast and slow muscles, the abilities of these myosins to combine with F-actin are the same, as evidenced by the virtually identical ATP sensitivity values.

It seemed of interest to follow up the possible relationship between ATPase activity of myosin and speed of muscle contraction by comparing the behavior of the same muscle from different species. The muscles of the sloth (*Choloepus hoffmanni* Peters), a known sluggish animal, were used. Goffart and his collaborators (12) determined the contraction times of the sloth muscles and concluded from their studies that at body temperature (34–35°C) the sloth muscles are 4–6 times slower than their homologues in the cat (body temperature, 37–38°C). We isolated the myosin from the extensor digitorum longus and gastrocnemius medialis muscles of the sloth and cat and compared their ATPase activities at 34.5° and 37.5°C, respectively. The homogeneity of these myosin preparations in the analytical ultracentrifuge is demonstrated in Fig. 2.

From the data in Table II it appears that the extensor and gastrocnemius muscles of sloth are 6.7 and 4.4 times slower than the corresponding muscles of the cat. Actin-activated ATPase, Ca^{++}-activated ATPase (in the presence

of 0.05 M KCl and 0.5 M KCl), and EDTA-activated ATPase activities of myosins from the extensor and gastrocnemius muscles of the sloth are lower by factors of 5.6 and 5.6, 3.7 and 3.4, 3.7 and 3.3, and 3.6 and 3.0, respectively, than the corresponding ATPase activities of myosins from the extensor and

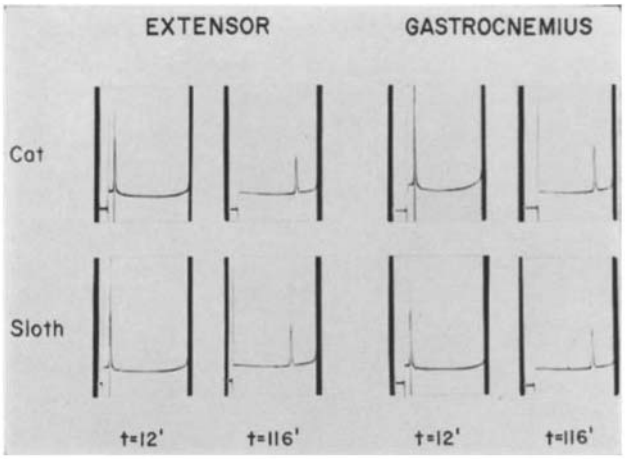

FIGURE 2. Ultracentrifugal patterns of cat and sloth myosins in 0.6 M KCl, pH 7.0. Protein concentration for cat extensor, sloth extensor, and sloth gastrocnemius, 3.0 mg/ml; for cat gastrocnemius, 3.2 mg/ml; speed, 59,780 rpm.

TABLE II

RELATIONSHIP BETWEEN CONTRACTION TIME AND ATPASE ACTIVITY OF MYOSIN IN MUSCLES OF CAT AND SLOTH

Muscle	Contraction time*	ATPase activity‡ in the presence of: Actin	Ca^{++}+0.05 M KCl	Ca^{++}+0.5 M KCl	EDTA	ATP sensitivity‡
	msec	*μmoles P_i/mg/min*	*μmole/P_i/mg/min*	*μmole P_i/mg/min*	*μmole P_i/mg/min*	%
Cat extensor digitorum longus	19–19.5	1.46	0.67	0.45	0.61	131
Sloth extensor digitorum longus	122–135	0.26	0.18	0.12	0.17	100
Cat gastrocnemius medialis	22.5–27	1.41	0.68	0.39	0.58	128
Sloth gastrocnemius medialis	109	0.25	0.20	0.12	0.19	122

* 37–38°C and 34–35°C for muscles of cat and sloth, respectively. References for contraction times: cat extensor digitorum longus, Gordon and Phillips (13, 14); cat gastrocnemius, Wills (15) and Buller et al. (11); sloth muscles, Goffart et al. (12).
‡ 37.5°C and 34.5°C for myosin of muscles of cat and sloth, respectively.

gastrocnemius muscles of the cat. In considering a certain variation in the contraction time of the cat gastrocnemius, determined by two different authors (see Table II), a proportionality appears between the ratio of contraction time of sloth and cat muscles and ATPase activity of their myosin. The data of Table II show, furthermore, that the actin-binding abilities of myosins from cat and sloth muscles are roughly the same.

The differences in the ATPase activities between myosins from sloth and cat muscles are revealed over the temperature range 5–35°C (Fig. 3). The curves for Ca^{++}-activated ATPase, at low and high ionic strength, and for EDTA-activated ATPase follow a straight line with an identical slope. The curve for actin-activated ATPase is biphasic, showing a break at 15°C; its slope throughout is greater than that of the aforementioned ATPases. The Q_{10} calculated from these curves varies from 1.9 to 2.0 for Ca^{++}-activated

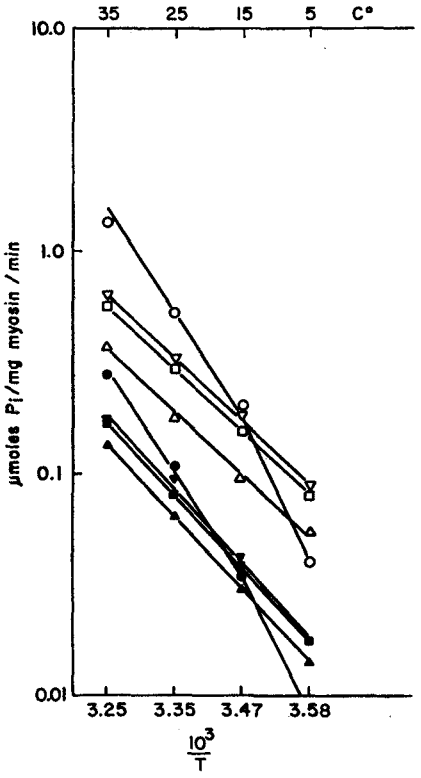

FIGURE 3. Arrhenius plot of ATPase activities of myosins from cat and sloth muscles. Actin-activated: ○ —— ○, cat; ● —— ●, sloth. Ca^{++}-activated in the presence of 0.05 M KCl: ▽ —— ▽, cat; ▼ —— ▼, sloth. Ca^{++}-activated in the presence of 0.5 M KCl: △ —— △, cat; ▲ —— ▲, sloth. EDTA-activated: □ —— □, cat; ■ —— ■, sloth.

ATPase at low and high ionic strength, and from 2.0 to 2.1 for EDTA-activated ATPase. For the actin-activated ATPase, the Q_{10} varies from 2.9 to 3.0 in the temperature range of 15–35°C, and from 4.2 to 4.6 in the range of 15–5°C. There is no systematic variation in the Q_{10} between myosins of cat and sloth muscles. The Q_{10} for the contraction time of cat muscles is 1.5 (14), and the Q_{10} for the contraction time of sloth muscles varies from 1.5 to 1.7 (16). These Q_{10} values for the contraction time of the muscles are closer to the Ca^{++}-ATPase or EDTA-ATPase activities of cat or sloth myosins than they are to their actin-activated ATPase activities.

A relationship between speed of contraction and ATPase activity of myosin was also found in the muscles of lower vertebrates and invertebrates. A few

examples are given in Table III. The contraction times of squid mantle, *Limulus* abdominal, *Thyone* longitudinal retractor, and dogfish mesenteric muscles increase in a ratio of 1:2.9:46:260. The actin-activated ATPase activities of myosins from the same muscles decrease in a ratio of 1:2.4:72:310, and the Ca^{++}-activated ATPase activities of the myosins decrease in a ratio of 1:3.3:56:414 at low ionic strength and in a ratio of 1:2.8:53:264 at high ionic strength. These data show that the isometric twitch contraction time of these muscles is inversely proportional to the ATPase activity of their myosin.

TABLE III

RELATIONSHIP BETWEEN CONTRACTION TIME AND ATPASE ACTIVITY OF MYOSIN IN MUSCLES OF LOWER VERTEBRATES AND INVERTEBRATES

Muscle	Contraction time at 18°C*	ATPase activity at 20°C in the presence of		
		Actin	Ca^{++}+0.05 M KCl	Ca^{++}+0.5 M KCl
	msec	*μmole P_i/mg/min*	*μmoles P_i/mg/min*	*μmole P_i/mg/min*
Squid mantle	68	0.94	1.24	0.53
Limulus abdominal	197	0.40	0.38	0.19
Thyone longitudinal retractor	3,100	0.013	0.022	0.010
Dogfish mesenteric	15,000–20,000	0.003	0.003	0.002

* Reference for contraction time: Prosser and Brown (17). Professor Prosser kindly informed me that the contraction time was determined at 18°C.

Relationship between Maximal Speed of Shortening and ATPase Activity of Myosin in Muscles

The contraction time in the isometric twitch is dependent upon the properties of the series elastic component, the duration of the active state, and the force-velocity properties of the contractile component (e.g. reference 18). Of these parameters only the last one can be related to the ATPase activity of myosin. Therefore, a relationship between contraction time and ATPase activity of myosin may be purely coincidental. The speed of muscle contraction is also characterized by the maximal speed of shortening (V_0), "intrinsic speed," which represents the velocity of shortening of unloaded muscle and can be determined from the force-velocity curve. The maximal speed of shortening is a true property of the contractile material, which ultimately reflects the speed of shortening at the sarcomere level. Therefore, it seemed of interest to compare the ATPase activities of various myosins with the intrinsic speed of shortening of their muscles.

Table IV lists the maximum velocity of shortening of various muscles, along with the actin-activated and Ca^{++}-activated ATPase activities of the myosins

of these muscles. The ATPase activities were determined at the same temperature as the speed of shortening. The muscles used are of mammals, lower vertebrates, and invertebrates; they represent striated and smooth muscles. The myosins from mammalian muscles were homogeneous in the analytical

TABLE IV

RELATIONSHIP BETWEEN MAXIMAL SPEED OF SHORTENING (V_0) AND ATPASE ACTIVITY OF MYOSIN OF VARIOUS MUSCLES

Muscle	Temp	V_0*	ATPase activity in the presence of		K‡	
			Actin	Ca^{++}	Actin	Ca^{++}
	°C	*muscle lengths/sec*	*μmoles P_i/g/sec*	*μmoles P_i/g/sec*		
Mouse extensor digitorum longus	35–36	24.0	30.03	21.81	0.80	1.10
Rat extensor digitorum longus	35–36	17.2	27.95	20.64	0.62	0.83
Mouse soleus	35–36	12.8	14.38	14.93	0.89	0.86
Rat diaphragm	37	11	15.32	13.56	0.72	0.81
Cat fast¶	36	10.4	20.27	12.12	0.51	0.86
Frog sartorius	22	10	16.91	11.55	0.59	0.87
Rat soleus	35–36	7.2	10.74	9.62	0.67	0.75
Human elbow flexor	37	6	11.38	9.89	0.53	0.61
Cat soleus	36	4.2	7.50	7.37	0.56	0.57
Pecten striated adductor	14	3	4.03	3.14	0.74	0.96
Frog sartorius	0	2	2.04	3.03	0.98	0.66
Dogfish coracohyoid	0	2	1.83	2.77	1.09	0.72
Tortoise iliofibularis	20	0.4	2.02	0.88	0.20	0.45
Rabbit uterus	37	0.2	0.23	0.47	0.87	0.43
Tortoise iliofibularis	0	0.1	0.13	0.20	0.77	0.50
Mytilus posterior adductor	14	0.1	0.15	0.17	0.67	0.59

* References for V_0 values: rat diaphragm, frog sartorius (22°C), human elbow flexor, *Pecten* striated adductor, tortoise iliofibularis (20°C), and rabbit uterus, Hanson and Lowy (19); frog sartorius (0°C), dogfish coracohyoid, and tortoise iliofibularis (0°C), Ritchie and Wilkie (20); *Mytilus* posterior adductor, Abbott and Lowy (21). V_0 values for mouse extensor digitorum longus, rat extensor digitorum longus, mouse soleus, cat fast, rat soleus, and cat soleus were calculated from the data of Close (22) by dividing the maximum speed of shortening per sarcomere ("V_s" in his Table III) by the sarcomere length, 2.5 μ.

‡ $K = \dfrac{\text{muscle lengths} \times \text{g of myosin}}{\mu\text{moles } P_i}$.

¶ Mixture of quadriceps, gastrocnemius, and sartorius muscles.

ultracentrifuge; those of the vertebrate and invertebrates showed, in addition to the main myosin peak, part of the myosin in aggregated form. In the case of myosins from vertebrates and invertebrates, the presence of contaminating protein, e.g. tropomyosin A or actomyosin, was excluded. ATPase activities were determined at physiological pH and at low ionic strength; the latter condition was chosen to obtain maximal enzymic activity. ATPase activities at high ionic strength, in the presence of Ca^{++} or EDTA, showed proportion-

ally lower activities than those given in Table IV. An example of this proportionality may be seen in the data of Table II.

The data of Table IV show that actin- and Ca^{++}-activated ATPase activities of 14 different myosins decrease generally in the same order as the speed of shortening of their respective muscles. A 240-fold change in the speed results in 130- to 200-fold change in ATPase activity. By dividing the speed of shortening, expressed as muscle lengths per second, by the ATPase activity of myosin, expressed as micromoles of P_i per g of myosin per second, the proportionality factor K can be obtained. [K has the units (muscle lengths $\times$ g of myosin)/(μmoles P_i).] The values for K, related to the actin-activated myosin ATPase, vary from 0.51 to 1.09, with one exception (tortoise iliofibularis at 20°C), when a value of only 0.20 is found. With the Ca^{++}-activated ATPase activity of myosin, the K value varies from 0.43 to 1.10. In consideration of the wide range in which both the speed of shortening and ATPase activity were studied, the 2- to 3-fold variation in the K values would suggest that K tends to be a constant. This assumption gains more weight in view of the fact that (1) the values for V_0 were determined by several authors and (2) the changes in the ATPase activity of myosin during its isolation cannot be ruled out even when determined within the same laboratory. However, there is a possibility of a small variation in the K values, depending on the intrinsic nature of the contractile system in different muscles.

In the case of frog sartorius and tortoise iliofibularis, one can compare the effect of temperature on the speed of shortening and on the ATPase activity of myosin (Table IV). Decreasing the temperature from 22°C to 0°C decreases the speed of shortening of frog sartorius 5-fold, and a decrease in temperature from 20°C to 0°C results in a 4-fold decrease in the speed of tortoise iliofibularis. Thus, the Q_{10} for the speed of shortening is close to 2. A Q_{10} of about 2 also appears for the Ca^{++}-activated ATPase activity of frog and tortoise myosins. However, the Q_{10} values for the actin-activated ATPase activity of these myosins are higher than 2, since the temperature change from 22°C to 0°C decreases the ATPase activity of frog myosin 8.5-fold, and the change from 20°C to 0°C decreases the ATPase activity of tortoise myosin 15.5-fold. With frog myosin more detailed determinations were performed on the temperature dependence of the actin-activated ATPase activity. In the temperature ranges of 30–20°C, 20–10°C, and 10–0°C, Q_{10} values of 2.9–3.0, 3.4–3.5, and 6.9–8.1, respectively, were obtained. In contrast, the Q_{10} values for the Ca^{++}-activated frog myosin ATPase were 2.0–2.1 throughout these temperature ranges.

Hasselbach (23) was the first to show Q_{10} values above 2 for the Mg^{++}-activated actomyosin ATPase below 20°C. Perry (24) later noted anomalously high Q_{10} values for the Mg^{++}-activated myofibrillar ATPase. In a more systematic study, Bendall (25) pointed out that the apparent energy of activa-

tion is always much higher for the Mg^{++}-activated myofibrillar ATPase than for the Ca^{++}-activated ATPase, and that with decreasing temperature the activation energy increases for the Mg^{++}-activated enzyme. One would expect that the temperature coefficient of a myosin ATPase activity, if it is related to the speed of shortening, would reflect the speed of shortening; i.e. its Q_{10} would be close to 2. If any enzymic reaction involved in the shortening of muscle should have a Q_{10} above 2, this reaction would control the temperature dependence of the speed of shortening, since it would require the highest energy of activation.[1] Therefore, a Q_{10} higher than 2 for the actin-activated myosin ATPase does not support the concept that this ATPase activity is intimately involved in determining the speed of shortening.

Because all studies in vitro suggest that the Mg^{++}-activated actomyosin ATPase represents the true model of muscle contraction, additional experiments were performed on the temperature dependence of the actin-activated frog myosin ATPase. Hasselbach (23) noted that below 10°C the ATPase activity of Mg^{++}-activated actomyosin is similar to that of pure myosin alone. From the data of Bendall (25) it appears that the Mg^{++}-activated myofibrillar ATPase is very much reduced between 18.5°C and 0.6°C, when the ionic strength is increased from 0.04 to 0.15. These observations suggest that the nonproportional decrease of the Mg^{++}-activated actomyosin ATPase at lower temperatures is due to the dissociation of actomyosin; in other words, what is observed is not a Mg^{++}-activated actomyosin ATPase but mainly a Mg^{++}-inhibited myosin ATPase. It follows, if one can overcome the dissociation of actomyosin at a low temperature, that its Mg^{++}-activated ATPase should give a linear Arrhenius plot. This assumption was substantiated in the following experiment.

The leg muscles of the frog were chopped with scissors, then blended at 0°C for 2 min with 25 volumes of a solution containing 0.04 M KCl and 1 mM Tris-HCl buffer, pH 7.4. The suspension was filtered through gauze, to remove connective tissue and unbroken muscle cells, and centrifuged in the cold at 600 *g* for 10 min. The residue was washed with 5 volumes of the 0.04

[1] A simple example may illustrate this. From the rate of ATP hydrolysis at various temperatures for myosin of cat muscles (Fig. 3), the activation parameters ΔH^*, ΔS^*, and ΔF^* can be calculated from plots of log (k/T) vs. $1/T$ with the Eyring equation (26),

$$k = \frac{RT}{Nh} e^{\frac{-\Delta F^*}{RT}}$$

where k is the pseudo-first order rate constant for ATP hydrolysis catalyzed by myosin. ΔH^* values are 10.9 and 11.5 kcal/mole for Ca^{++}-activated ATPase activity at low and high ionic strength, and 18.4 for the actin-activated ATPase activity, whereas the ΔS^* values are -0.17 and -1.6 cal/degree/mole for Ca^{++}-ATPases, and $+24.6$ cal/degree/mole for actin-activated ATPase. The corresponding ΔF^* values are 11.4, 12.0, and 17.6 kcal/mole. Thus, it can be shown that actin-activated ATPase activity has the highest free energy of activation.

M KCl and 1 mM Tris-HCl solution and centrifuged as before; the final residue was resuspended in a small volume of 1 mM Tris-HCl buffer solution, pH 7.4. The Mg^{++}-activated ATPase activity of these myofibrils was determined at 30°, 20°, 10°, and 0°C, in the presence of either 0.01 M Tris-HCl, pH 7.4, 1 mM $MgSO_4$, and 1 mM ATP (total ionic strength = 0.014), or 0.01 M Tris-HCl, pH 7.4, 1 mM $MgSO_4$, 1 mM ATP, and 0.05 M KCl (total ionic strength = 0.064). Fig. 4 demonstrates that the ATPase activity of freshly prepared myofibrils at ionic strength of 0.014 gives a straight line in the Arrhenius plot

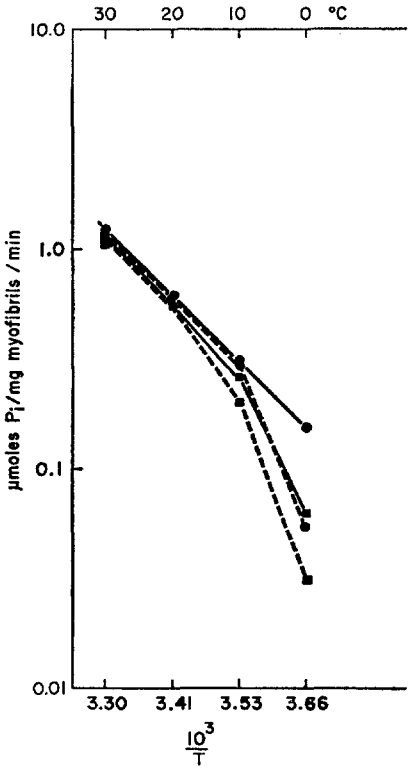

FIGURE 4. Arrhenius plot of Mg^{++}-activated ATPase activity of frog myofibrils. Fresh myofibrils at 0.014 ionic strength, ● —— ●; fresh myofibrils at 0.064 ionic strength, ■ —— ■; 2 day old myofibrils at 0.014 ionic strength, ● - - - ●; 2 day old myofibrils at 0.064 ionic strength, ■ - - - ■. For other experimental details, see the text.

with a Q_{10} of 2 from 30°C through 0°C. However, at ionic strength of 0.064, a sharp break occurs in the ATPase activity curve of the same myofibrils at 10°C, giving a Q_{10} of 4.2 from 10°C to 0°C. 2 day old myofibrils show this break in their ATPase activity at 10°C and the consequently higher Q_{10} value, even at 0.014 ionic strength. The deviation from the linearity in the ATPase activity of the 2 day old myofibrils occurs even at 20°C, when the ionic strength is 0.064.

The data of Fig. 4 show that, under favorable conditions, Q_{10} values of 2 can be obtained for the actin-activated ATPase activity of frog myosin even at low temperatures, i.e. 10–0°C. These conditions were fulfilled by using

extremely low ionic strength and myofibrils instead of reconstituted actomyosin, both of which minimize the dissociation of actomyosin. In addition, the data of Fig. 4 indicate that a certain structural organization is necessary to produce unimpaired Mg^{++}-activated myofibrillar ATPase activity at 0°C, since this ATPase activity is considerably decreased even after 2 days of aging. Clearly, the ionic strength of 0.014, at which the Q_{10} of 2 may be obtained, is nonphysiological. However, the actomyosin concentration is 10% in the frog muscle at 0.12 ionic strength, but there is only 0.025–0.1% actomyosin in the ATPase assay media at ionic strength of 0.014. It seems likely that in the muscle actomyosin is less sensitive to dissociation, induced by changes in temperature or ionic strength, than it is in the test tube, and therefore that the Mg^{++}-activated actomyosin ATPase can work with a Q_{10} of 2 in muscle.

Similarity of F-actin–Binding Ability of Myosin of Various Muscles

In contrast to the ATPase activity of myosin, which may vary according to the speed of contraction, the F-actin–binding ability of myosin from various muscles is rather constant. It has been shown already that myosins of the fast and slow muscles of the cat and myosin of the slow muscles of the sloth have different ATPase activities but similar ATP sensitivities (Tables I and II). Table V shows that the ATP sensitivities of myosins of skeletal muscles of the rat, human, frog, and tortoise and myosin of the smooth muscle of the rabbit vary only slightly, although the ATPase activities of these myosins and the speed of shortening of these muscles vary greatly (see Table IV). It was tempting to determine the ATP sensitivities of myosins at the same temperatures (Table V) used to measure their ATPase activities and the speed of shortening of their respective muscles (Table IV). Table V demonstrates that the ATP sensitivities of these myosins show no dependence on temperature.

TABLE V

THE SIMILARITY OF ATP SENSITIVITIES OF MYOSINS OF MUSCLES WITH DIFFERENT SPEEDS OF SHORTENING

Muscle	Temp	ATP sensitivity
	°C	%
Rat extensor digitorum longus	35–36	98
Rat diaphragm	37	91
Frog sartorius	22	115
Rat soleus	35–36	127
Human elbow flexor	37	132
Frog sartorius	0	103
Tortoise iliofibularis	20	125
Rabbit uterus	37	86
Tortoise iliofibularis	0	128

ATP sensitivities were determined at the temperatures indicated. The speed of shortening of muscles at the same temperatures is listed in Table IV.

It will be noted, however, that in the ATP sensitivity determinations one does not measure the velocity of the actin and myosin combination, but only the extent of this combination. Therefore, the lack of temperature dependence of the ATP sensitivity does not mean necessarily that the rate of actomyosin formation is the same at various temperatures.

The ATP sensitivity determination refers to the F-actin binding of myosin at high ionic strength. However, similar results were obtained when the binding was determined at low ionic strength (27). Thus, when F-actin was added to myosin in a weight ratio of 1:4, in the presence of 0.05 M KCl and 1 mM $MgSO_4$ at pH 7.4, and the resulting suspension was centrifuged, essentially all the added F-actin sedimented together with the myosin, which indicates a complete combination of actin and myosin.

DISCUSSION

It appears that the intrinsic speed of muscle contraction is a characteristic property of the ATPase activity of myosin in the muscle. Although the intrinsic speed of shortening represents the speed of shortening of unloaded muscle, this speed is related to the velocity of shortening under different loads. The relationship between velocity and load is described by the "characteristic equation" of Hill (28) and follows a hyperbolic curve. The force-velocity relation has always the same general form in a wide variety of muscles (29). Therefore, it appears that the ATPase activity of myosin is related to the speed of shortening both with and without load. When muscle shortens under load, it performs work which can be directly related to the free energy of hydrolysis of ATP catalyzed by myosin.

It has been shown recently by Close (22) that the intrinsic speed of shortening of rat muscles is inversely proportional to the isometric twitch contraction time; in other words, the product of the intrinsic speed and contraction time is constant. Since the intrinsic speed of shortening is proportional to the ATPase activity of myosin, it appears that the ATPase activity multiplied by the contraction time is also a constant. The data presented in Tables I–III give a clear demonstration for the validity of this constant. The inverse relationship between the contraction time of the muscles of the cat, sloth, squid, crab, sea cucumber, and dogfish and the myosin ATPase activity argues strongly for a role of myosin ATPase in determining the speed of muscle contraction.

A relationship between speed of shortening and ATPase activity of myosin can be shown for a number of activators, e.g. actin and Mg^{++}, or Ca^{++}, or EDTA. Clearly, of these ATPase activities only that activated by actin and Mg^{++} bears physiological significance, since in the ionic milieu of living muscle (Mg^{++} excess over Ca^{++}) the Ca^{++}-activated myofibrillar ATPase is very much depressed (25), and EDTA is not a likely candidate to activate

myosin ATPase in muscle. To bring the actin-activated myosin ATPase into the basic events of muscle contraction is in general agreement with studies in vitro which show that, whenever the enzymic interaction between actin and myosin is inhibited, model contraction is also inhibited (30).

Bendall (31) studied the Mg^{++}-activated myofibrillar ATPase activity of various muscles at the natural ionic strengths and body temperatures of the animals. Of the animals used by Bendall, only the rat, frog, and tortoise were employed in the present work. At comparable temperature and ionic strength, the myofibrillar ATPase activity of the leg muscles is in the same range as the actin-activated myosin ATPase activity of comparable muscles [cf. Fig. 1 in reference 31 and Table IV of the present work]. In the case of the frog and tortoise, Bendall found a satisfactory agreement between the energy liberated by the muscles, determined by heat measurements (28, 32), and the energy liberated from the hydrolysis of ATP catalyzed by the myofibrils. These data show that under optimal conditions the actin-activated myosin ATPase can fulfill the requirement of contraction.

The temperature dependence of the actin-activated myosin ATPase in the reconstituted actomyosin system does not follow the same pattern as that of the speed of shortening. Nevertheless, conditions can be found where Q_{10} values of 2 are obtained for Mg^{++}-activated frog myofibrillar ATPase from 30°C to 0°C (Fig. 4), which are in agreement with the Q_{10} of about 2 for the speed of shortening of frog muscle (Table IV). To establish a role for the actin-activated myosin ATPase in determining the speed of shortening, one has to assume that the dissociation of actomyosin at 0°C in vitro (25) does not take place in the muscle. This assumption is based on the 100–400 times higher concentration of actomyosin in vivo than in vitro, on the 30–100 times lower molar ratio of ATP to actomyosin in the muscle than in the test tube, and on the evidently greater structural integrity of actomyosin in the living than in the artificial system.

It is well accepted today that the Mg^{++}-activated actomyosin ATPase requires about 10^{-5} M Ca^{++} to obtain its full activity (33–36). According to current views, activation of contraction in the living muscle fiber is brought about by Ca^{++}, released from the sarcoplasmic reticulum by the electrical stimulus, and this liberated Ca^{++} causes contraction by acting directly on actomyosin (for review, see reference 37). Close (22), when discussing the possibility that the speed of shortening is proportional to the ATPase activity of myosin, also raised the question whether the speed is proportional to the amount of Ca^{++} utilized during contraction. Indeed, observations have been made which suggest a control of the speed of shortening by Ca^{++}. The pattern of the sarcoplasmic reticulum differs significantly in fast and slow muscles: the reticulum is well developed in muscles that perform a complete contraction cycle in a few milliseconds, but very rudimentary in slow striated fibers, and

absent in some smooth muscles (for review, see reference 35). Furthermore, pronounced differences in Ca^{++} uptake exist between fast and slow rabbit muscle grana (38). However, data are also available that contradict these observations. Insect visceral muscles have a highly developed sarcoplasmic reticulum system, although they contract slowly (39); on the other hand, the asynchronous flight muscles of certain insects have a reduced sarcoplasmic reticulum, although they contract with extreme speed (40). The difference in Ca^{++} uptake in grana prepared from fast and slow muscles of man is not so pronounced as that found in the case of rabbit muscle (41).

According to Weber and Herz (42), 1–2 moles of Ca^{++} have to be bound per mole of myosin to activate the actomyosin ATPase maximally. This amount of Ca^{++} (0.15–0.30 μmole/g of muscle) is only a small fraction of that available in various muscles. And if, as seems possible, only half of the muscle's total Ca^{++} is stored in the vesicles of the sarcoplasmic reticulum, then the Ca^{++} needed to saturate the actomyosin ATPase may be only 10–20% of that stored in the vesicles. Thus, it is quite possible that the Ca^{++} released from the vesicles during excitation-contraction coupling is sufficient essentially to saturate the contractile system and thus to cause the speed of shortening to be determined by the intrinsic rate of the actomyosin ATPase activity. And, in view of our results showing that the actin-activated myosin ATPases of different muscles vary widely in their speed of action, it seems most likely that this variation determines the speed of contraction, in general, rather than the differences among the various muscles in the amount of Ca^{++} that may be released to activate contractions. It should be noted, however, that, at least in the case of the responses of the frog sartorius muscle, the intrinsic isotonic shortening speed and rate of isometric tension development are not fixed (i.e. at a given temperature), but can be increased under the influence of various potentiating substances, and that such increases may be due to potentiator-induced modifications of excitation-contraction coupling which enhance the release of activator Ca^{++} (43, 44). Such increases in contraction speed, however, are small (about 10–20%), and this suggests that, although the primary determinant of the speed of contraction is the rate of ATPase activity, a secondary regulatory role may be played by the amount of Ca^{++} made available to activate the contractile enzyme. This concept is supported by recent results of Costantin et al. (45), which show that a large excess of Ca^{++} cannot transform the "skinned" slow fibers of the frog iliofibularis muscle into fast fibers. This finding indicates that the speed of muscle contraction in the frog is an inherent property of the actomyosin ATPase and is not determined by the amount of available Ca^{++} released from the sarcoplasmic reticulum.

Earlier studies by H. H. Weber and Portzehl have shown that the speed of shortening of water-glycerol–extracted skeletal and smooth muscle fibers is related to the rate of ATP hydrolysis catalyzed by these fibers (46). Attempts

were also made at the same time to show a proportionality between the power of the extracted fibers of frog sartorius muscle and the ATPase activity of their actomyosin. The data of the present work permit a further step to be made toward the living system. Since the actin-activated ATPase activity of myosin appears to be related to the speed of shortening, the rate of extra energy liberation by the muscle, $(P + \alpha)v$ (see reference 47), is also related to this myosin ATPase. This relation suggests that the energy required for the working muscle results from the breakdown of ATP catalyzed by actomyosin. Davies and his collaborators provided evidence that ATP is broken down during both tetanic contractions and single twitches, and that the amount of ATP utilized is largely related to the work done by the muscles (48–51).

The data of the present work also indicate that myosins that show great differences in their ATPase activities are similar in their actin-binding abilities. The behavior of ATPase activity has its counterpart in the great variation of the speed of shortening, but the actin-binding ability is reflected in the maximal tension produced by muscles. Thus, muscles of man, rat, tortoise, frog, toad, dogfish, snail, and *Mytilus* develop tension from 0.6 to 5 kg/cm^2, whereas their velocity of shortening varies from 0.06 to 11 muscle lengths/sec (20). The tension of frog sartorius is 2.1 kg/cm^2 at 20–22°C and 1.7 kg/cm^2 at 0°C (20). Close (22) has surveyed the literature on the lack of temperature dependence of maximum tension in muscles of amphibian, 0–20°C; locust flight, 25–45°C; and hamster diaphragm, 24–38°C. It is pertinent that the extent of actin and myosin combination is also independent of the temperature (Table V). From these data it appears that tension generation in muscle is controlled by actomyosin formation. The differences in the maximal tension of various muscles may reflect differences in actomyosin concentration or, more precisely, in the number of cross-linkages in 1 cm^2 of cross-section of muscle.

It is tempting to associate two basic features of muscle contraction, namely, speed of shortening and tension output, with the ATPase activity and actin-binding ability of myosin, respectively. The results presented favor these correlations.

Note Added in Proof After this work was completed, Mr. Roy E. Larson, in the laboratory of Dr. R. E. Davies, determined the temperature coefficient of the rate of P_i liberation by sartorius muscles of *Rana pipiens*, treated with 2,4-dinitrofluorobenzene, during isometric contractions in tetanus (52). Under these conditions, the P_i liberated by the muscles has its origin in the ATPase activity of myosin, with a contribution (of about one-third) from the calcium-pumping system. Therefore, these experiments provide the first example of the temperature dependence of the ATPase activity of myosin in vivo. Larson has found Q_{10} values of 1.84 and 1.89 in the temperature ranges of 20–10°C and 10–0°C, respectively. These Q_{10} values are closer to the Ca^{++}-activated ATPase activity of frog myosin than to its actin-activated

ATPase activity. However, as shown under "Results," under certain conditions Q_{10} values of 2 can also be obtained for the actin-activated ATPase activity of frog myosin. Thus, the temperature coefficient of the P_i liberation by the sartorii, during isometric contractions, may reflect the participation of the actin-activated myosin ATPase in contraction.

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Discussion

Dr. Pringle: May I perhaps suggest to Dr. Bárány that it would be easier for people who are not biochemists to remember these results of his if he would express the ATPase activity of the actomyosin in terms of moles of ATP turned over per mole of

myosin per second? This would then give numbers which are quite small and easy to remember and could be compared easily from preparation to preparation. Now that we have reasonable certainty that the molecular weight of myosin is about 500,000, this seems a reasonable further calculation to make.

A second suggestion: it would be very interesting to know whether an even closer correlation between maximum speed of shortening and ATPase activity could be obtained if account were taken of the difference in sarcomere lengths of the muscles.

The speed of shortening of a whole length of muscle is related to the speed of movement at each cross-bridge in a way which has to take account of the extent to which the cross-bridges are acting in parallel or acting in series. If the sarcomere length is long, then more cross-bridges are acting in parallel in each half-sarcomere. If the sarcomere length is short, then effectively over a given length of muscle fiber more cross-bridges are acting in series. The length of the sarcomere is therefore a factor which would have to be taken into account if you are going to relate speed of movement at the cross-bridge to over-all speed of shortening of the muscle.

Dr. Bárány: As far as the first suggestion is concerned, one of the slides showed the turnover of ATP by several myosins. If all of our data were to be expressed in terms of moles of ATP per 500,000 g myosin per second, as suggested by you, the turnover would vary from 0.1 to 20. Concerning your second suggestion, Dr. Close in his paper [*J. Physiol.*, (*London*), 1965, **180**:542] expressed the maximum speed of shortening per sarcomere. It turns out that muscles with identical sarcomere length show great differences in their speed of shortening. This suggests to me that the main factor in determining the speed of shortening is not the sarcomere length of the muscle but the ATPase activity of the myosin.

Dr. Perry: We too have been interested in the differences in the myosin from various muscles, both red and white, and also from fetal muscle. In fact, the differences are perhaps even more striking in myosin from fetal tissue. The specific ATPase of myosin from developing muscle increases at a fairly rapid rate along with development.

There are certain problems in explaining these facts, and I'd like to hear your comments on our working hypothesis that myosin exists as isoenzymes. It's not easy to pick up myosin isoenzymes by the standard procedures because myosin is not amenable to the conditions of electrophoresis normally used. I believe this hypothesis helps us to understand how each tissue has a myosin of slightly different ATPase activity because it can be postulated that each tissue has a particular isoenzyme complement. I wouldn't like to say how many isoenzymes there are, but I look upon fetal myosin as the most primitive type with the lowest ATPase activity, whereas the most highly specialized myosin is that associated with fast skeletal muscle. Cardiac and red skeletal muscle myosins could then represent appropriate combinations of these extreme types.

So far as the fetal myosin is concerned, we believe we have good evidence that there are tertiary structure differences and suggestions that primary structure differences also exist in the molecule.

Dr. Bárány: Concerning your comment on fetal myosin, I would like to add the following. Based on your finding of a low ATPase activity of myosin from fetal rabbit and based on the findings of Buller, Eccles, Eccles, and Close that muscles of newborn mammals are physiologically slow, we isolated and compared the ATPase

activities of myosin from newborn and adult rabbit muscles. The ATPase activities of myosin from newborn rabbits were about one-half those of myosin from adult rabbits. This result also shows that myosin from slow muscles has lower ATPase activity than myosins from fast muscles.

It is more difficult to answer the question how these differences in the ATPase activities of various myosins are brought about. Clearly, one possibility is that myosin occurs in the form of isoenzymes, as suggested by you, although, as far as I can recall, isoenzymes do not show great differences in their enzymic activities. The second possibility is that the over-all structure of various myosins is different, as for example fetal and adult hemoglobin, which differ from each other in one polypeptide chain. The third possibility is that the amino acid sequence of the ATPase site of the myosins is different. Finally, we cannot exclude the possibility that differences in the tertiary structure of various myosins are the ultimate cause of the differences in the ATPase activities.

Dr. Perry: I think we already have suggestions of active site modifications in the lactic dehydrogenases, judging from the differences in enzymic behavior of the isoenzymes, and I see no reason why similar differences could not occur in the myosins.