The Journal of Biochemistry, Vol. 40,No. 1.

MECHANISM OF MUSCULAR CONTRACTION. I. INTERACTIONS BETWEEN ACTOMYOSIN AND ADENOSINE TRIPHOSPHATE‡

By YUJI TONOMURA, SHIZUO WATANABE and KOICHI YAGI

(From the Research Institute for Catalysis, and Chemical Department, Faculty of Science, Hokkaido University, Sapporo)

(Received for publication, September 25, 1952)

INTRODUCTION

It is well known that upon the addition of adenosine triphosphate (ATP) to actomyosin solution, the viscosity (*1*), the double refraction of flow (*2*), the light-scattering (*3*) *etc.* of actomyosin solution are changed while ATP is split to adenosine diphosphate (ADP) and inorganic orthophosphate (P) (*4*).

In other words, the deformation of actomyosin particles and coincidently the adenosine-triphosphatase (ATPase) action are then observed.

These two reactions being thought to represent fundamental mechanisms of muscular contraction have so far been studied by many investigators but their mechanisms have been poorly clarified owing to the difficulty of the exact measurements of these phenomena.

The present writers have estimated the inorganic orthophosphate split in consequence of ATPase action using a precise electrophotocolorimeter and they have, on the other hand, investigated the deformation of actomyosin particles by means of measurements of the light-scattering. On the basis of these experiments, the mechanisms of these two phenomena were clarified respectively and the interrelation of them was established; further, it became possible to interpret many facts that have already been learned about muscular contraction.

‡ The contents of this paper were presented at the 4th (August 1951, Tōkyō) and the 5th meeting of the Symposia on Enzyme Chemistry (July 1952, Ōsaka), and the 5th annual meeting of the Chemical Society of Japan (April 1952, Tōkyō). Parts of them have already been published in *Nature* 169, 112 (1952) and in *Symp. Enz. Chem.*, (in Japanese), 7, 46, (1952).

PREPARATION OF MATERIALS

Preparation of ATP—Fresh muscle cut from rabbits anaesthetized rapidly (within about 2 or 3 minutes) with chloroform was dried by the acetone treatment. This acetone dried muscle was extracted with hot water. The extracted solution was brought to 0.3 per cent by volume acetic acid solution by the addition of glacial acetic acid and the precipitates formed were removed. ATP was isolated from the resulting clear fluid as Hg-salt and then as Ba_2-salt according to Kerr's method (*5*).

The yield of the Ba_2-salt is about 2 gram per Kg of fresh rabbit muscle and its purity is 75—80 per cent. But the raito of P:N is 1:1.30 and the ratio of the quantity of phosphorous split by 7 minutes-hydrolysis in N-HCl, at 100° (7′ P) to that of phosphorous liberated in the case of hydrolysis with H_2SO_4-H_2O_2 (Total P) is about 2:3 in our ATP preparations. Therefore, its impurities should be inorganic Ba-salts and were probably removed as Ba_2SO_4 in the case of the converting Ba_2-ATP into a neutral K-ATP which was used for our experiments.

Preparation of "Purified Myosin B"—The minced striated muscle of rabbit hind leg was suspended in ice-cold Weber's solution (0.6 *M* KCl, 0.04 *M* $NaHCO_3$, 0.01 *M* Na_2CO_3). The suspension was kept in the ice box (0—5°) for about 24 hours. After this time, the suspension was centrifuged.

The resulting clear supernatant fluid was adjusted to pH 6.5 by adding dilute acetic acid and was diluted with ice-cold distilled water, about 5—6 volumes being added for every volume of the supernatant. The flocculent precipitate formed was allowed to settle overnight in a cold room and then the clear supernantant fluid was siphoned off. The settled precipitates were centrifuged, washed twice with ice-cold distilled water. The "Myosin B" precipitate was dissolved in 0.5 *M* KCl solution and the insoluble matter was removed by centrifugation.

The myosin B solution was again diluted with ice-cold distilled water, three volumes being added at this time. The precipitate was again centrifuged, washed with cold distilled water and redissolved in KCl. "Myosin B" was purified by two or three times repeating this procedure.

Finally, the myosin B was obtained as a solution containing 0.5 *M* KCl.

ATPase action

Methods of Measuring Enzyme Activity—The enzyme reaction was started by adding 0.5 ml myosin B solution (1—3 mg. protein per ml.) to the mixed solution which is composed of 1.5 ml buffer solution (0.1 *M* glycine—0.1 *M* KOH, 0.1 *M* veronal-acetate, or 0.1 *M* citrate), 0.5 ml. K-ATP solution, 0.25 ml. $CaCl_2$aq. or H_2O and 0.25 ml. $MgCl_2$-aq. or H_2O (the potassium content in this reaction mixture is about 0.15—0.18 *M*; when the concentration of potassium was desired to be 0.4—0.5 *M*, concentrated KClaq. was used instead of H_2O). After certain times (usually 1,2 and 3 minutes), the reaction was stopped by adding 1.0 ml. of 10 per cent CCl_3COOH or 20 per cent $HClO_4$.

This digest was filtered through the filter paper and an aliquot of this filtrate (1 or 2 ml.) was then analyzed for free ortho-phosphate liberated according to Briggs' method (*6*) or Youngburg-Youngburg's mothod (*7*). The analysis was carried out colorimetrically using an electrophotometer. The electrophoto-colorimeter was constructed according to Müller *et al.*(*8*) and was schematically shown in Fig. 1. In this apparatus the electrophoto-tube PG-50-G (Matsuda) and the filter VR-2 (Matsuda) were used.

Then, the concentration of ATP was calculated from the quantity of 7′ P (acid labile phosphate), presuming 7′ P to be two-thirds of Total P in ATP. The content of protein was determined by the Kjeldahl method using a factor of 6.25 and pH values reported were measured with a Beckman pH-meter (G-type).

Results and Discussion—The purified myosin B used splits off only one phosphate residue from one molecule of ATP.

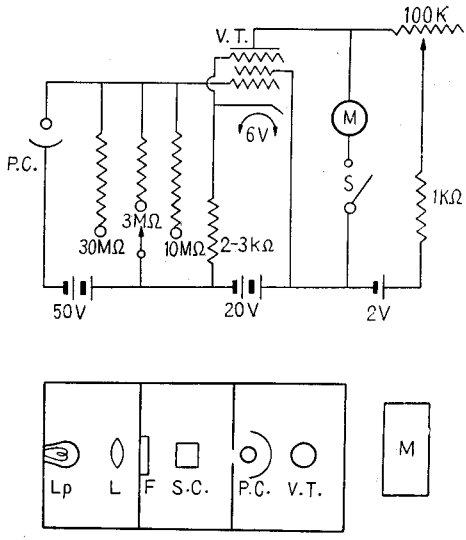


FIG. 1. Electrophoto-colorimeter. P.C.: electrophoto tube PG-50-G, V.T.: vacuum tube 6D6, M: μ-ampere meter 300 μA, LP: lamp 6V 10W, L: lens, F: filter VR-2 S.C.: sample vessel, S: switch.

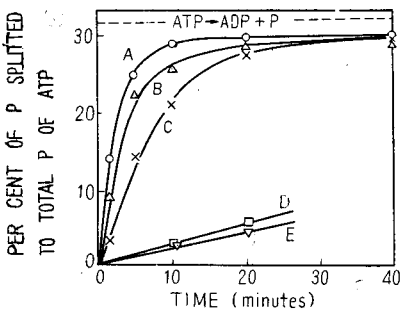


FIG. 2. ATPase action of purified myosin B. 10°, [ATP] 5×10^{-3} *mole/lit.* [$CaCl_2$] 5×10^{-3} *mole/lit.* and [$MgCl_2$] 5×10^{-5} *mole/lit.* A: glycine buffer at pH 9.2 (Ca^{2+} addition), B: veronal-acetate buffer at pH 9.2 (Ca^{2+} addition), C: veronal-acetate buffer at pH 6.3 (Ca^{2+} addition), D: glycine buffer at pH 9.2 (Ca^{2+} and Mg^{2+} additions), E: glycine buffer at pH 9.2 (Control)

Its ATPase activity is strongly activated by Ca^{2+} ion (without addition of Ca^{2+}, its activity is very slight and therefore all enzymatic experiments reported below were made in the presence of $CaCl_2$) and this activating effect is inhibited by the addition of $MgCl_2$ thereto (see Fig. 2).

Estimation of Michaelis Constant: Michaelis constant (K_m) of ATPase has not yet been determined on account of the difficulty of measuring the micro amount of phosphorous. We made an attempt to estimate K_m using Youngburg's method (#). Two examples from among the results obtained are shown in Fig. 3a–b. Our results

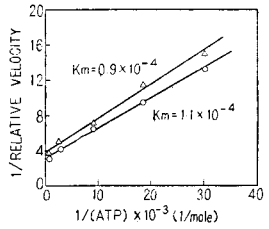


FIG. 3a. Initial velocity-substrate concentration relationship in the presence of 1.6×10^{-2} *mole/lit.* $CaCl_2$ (circle) or $CaCl_2$ plus 2.5×10^{-5} *mole/lit.* $MgCl_2$ (triangle). Temperature at 6°. Veronal-acetate buffer at pH 6.3.

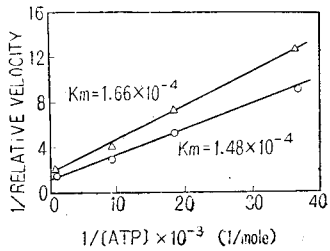


FIG. 3b. Initial velocity-substrate concentration relationship in the presence of 1.6×10^{-2} *mole/lit.* $CaCl_2$ (circle) or $CaCl_2$ plus 2.5×10^{-5} *mole/lit.* $MgCl_2$ (triangle). Temperature at 11°. Glycine buffer at pH 9.3.

Youngburg's method is about four times as sensitive as Fiske-Subbarow's method employed usually.

are not satisfactorily exact but gave roughly a straight line when a reciprocal of initial velocity was plotted against the reciprocal of substrate concentration.

The Michaelis constants obtained in the presence of Ca^{2+} ion and Mg^{2+} ion are given in Table 1.

TABLE I

Michaelis Constant of ATPase

(10°, veronal-acetate buffer, [K] 1.8×10^{-1} *mole/lit.* [$CaCl_2$] 1×10^{-2} *mole/lit.* [$MgCl_2$] 1×10^{-4} *mole/lit.*)

pH	K_m (*mole/lit.*) in the presence of	
	Ca	Ca+Mg
9.0	$1.0\sim1.9\times10^{-4}$	$0.7\sim1.9\times10^{-4}$
6.3	$1.0\sim1.9\times10^{-4}$	$0.8\sim1.4\times10^{-4}$

These experiments suggest that the ATPase action may be formulated as follows;

$$M^* + S \underset{k_1^*}{\longrightarrow} M^*S \qquad (1^*)$$

$$M^*\ S \underset{k_2^*}{\longrightarrow} M^* + P \qquad (2^*)$$

and k_1^*/k_2^* is about 1.5×10^{-4} *mole/lit.* (#) where M and S represent respectively the enzymatic unit of actomyosin and an ATP molecule and then a symbol * affixed upon M stands for the deformated state in which actomyosin during the enzymatic reaction is present.

Now, the value of k_2^* referred to one mole myosin in the presence of Ca^{2+} ion, at pH 6.,3 and 21° is 44 *sec.*$^{-1}$, hence (by neglecting the temperature dependence of K_m) $k_1^*=30\times10^4$ *lit./mole.sec.* As one mole of myosin contains six units (##), the values per unit are calculated to be;

According to J.J. Blum (personal communication), a similar value of K_m was obtained by Quellet using Fisk-Sabbarow's method. The reverse reaction of the reaction (1*) is neglected by us on the basis of the analogy about the results of the light-scattering experiments.

The unit is 140,000 g. myosin or the quantity of actomyosin which contains 140,000 g. myosin. This quantity was chosen for such reason as will be reported in p. 45.

$$_{Ca}k_1^* = 5 \times 10^4 \ lit./mole.sec. \quad (\#),$$
$$_{Ca}k_2^* = 7 \ sec.^{-1}$$

It is further obvious from Table I that the addition of Mg^{2+} ion does not affect the K_m value. Most of experiments described below were conducted under such a condition that the ATP concentration is sufficiently large and so the reaction (2*) is the rate determining step.

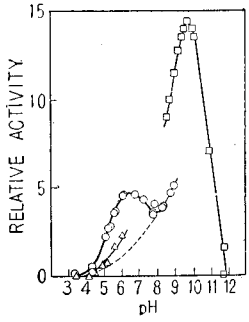


FIG. 4. Effect of pH on ATPase activity in the presence of Ca^{+2}. 10°, [ATP] 6×10^{-4} *mole/lit.*, $[CaCl_2] \times 10^{-2}$ *mole/lit.*

Effect of pH: As indicated in Fig. 4, the pH-activity curve shows two optima at around pH 6.3 and 9.7.

This fact may be interpreted as follows: The optimum at pH 9.7 is probably due to the fact that the acidic and basic components of the enzyme are inactive as in most enzymes. Then, the optimum at pH 6.4 may be due to such a condition that the complex of enzyme-ATP undissociated in its phosphate residue (M*S) is more reactive than that of enzyme-ATP dissociated (M^*S^-). That is, by assuming that the dissociation constant for the reaction $M^*S^- + H^+ \rightleftarrows M^*S$ is $10^{-6.5}$ (*9*) just as that for the reaction $S^- + H^+ \rightleftarrows S$ and that the velocity of M*S splitting is five times faster than that of M^*S^- splitting and that if not so the pH-activity curve should be the dotted line in Fig. 4, the pH-activity curve denoted by a big line which is in good agreement with the observed one, is obtainable.

The values of activation energy of the ATPase action at various pH are given in Table II.

Inhibition by Mg^{2+} Ion: At the concentration of K^+ ion used in our experiments (0.16 *M* or so and about 0.48 *M*), ATPase activity of purified myosin B is inhibited by Mg^{2+} ion even if used alone while it is activated by Ca^{2+} ion (Table III) (##). This activating effect of Ca^{2+} ion is competitive with the inhibiting effect of Mg^{2+} ion (Fig. 5).

$_{Ca}k_1$ is representative for the velocity constant of the reaction (1*) in the presence of Ca^{2+} ion and the same rule applies correspondingly to the following parts.

Further, $_{Ca}k_2^*$ at pH 7.0 (glycine buffer) and at 37° is about 42 $sec.^{-1}$

It has been reported that the behaviour of the glycerinized muscle-ATPase (10), unpurified myosin A-ATPase and unpurified myosin B-ATPase (42) towards

TABLE II

Activation Energy (ΔH^* Kcal) of ATPase Action
([K] 1.7×10^{-1} *mole/lit.*, [ATP] 1×10^{-5} *mole/lit.*)

Buffer	pH	$CaCl_2$	$MgCl_2$	ΔH^*
		mole per liter	*mole per liter*	*Kcal.*
Glycine	9.5	1.3×10^{-2}	0	27
Veronal-acetate	9.4	1.0×10^{-2}	0	25
,,	6.9	,,	1×10^{-4}	22
,,	8.0	,,	0	15
,,	7.1	,,	0	10
,,	6.8	,,	0	16
,,	6.8	,,	1×10^{-4}	9.3
,,	6.5	1.3×10^{-2}	0	19
,,	5.9	1.0×10^{-2}	0	21

TABLE III

Effect of Ca^{2+} aud Mg^{2+} on ATPase Activity
(10° glycine buffer (pH 9.2), [ATP] 1×10^{-3} *mole/lit.*, [K] 1.5×10^{-1} *mole/lit.*)

$CaCl_2$ (*mole/lit.*)	Relative Activity*	$MgCl_2$	Relative Activity*
		mole per liter	
4×10^{-2}	7.2[5]	20×10^{-4}	0.2
2×10^{-2}	9.5[5]	10×10^{-4}	0.2
1×10^{-2}	9.2[5]	5×10^{-4}	0.3
0.5×10^{-2}	7.0	1×10^{-4}	0.3
0.1×10^{-2}	4.1	0.5×10^{-4}	0.7[5]
		0.1×10^{-4}	1.0

$$\text{*Relativea ctivity} = \frac{\text{Initial velocity of ATPase action in the presence of } Ca^{2+} \text{ or } Mg^{2+}}{\text{Initial velocity of ATPase action in the absence of } Ca^{2+} \text{ or } Mg^{2+} \text{ (control)}}$$

Ca^{2+} and Mg^{2+} were different from that of the purified myosin-ATPase but this difference may come from the situation that Mg-activated ATPase, myokinase *etc.* are also present in these preparations.

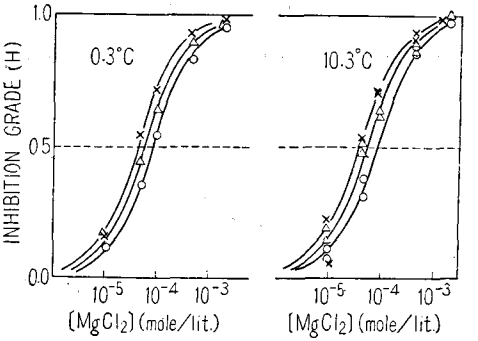


FIG. 5. Mg- inhibition of ATPase. glycine buffer pH 9.3, ATP 1×10^{-3} *mole/lit.* [$CaCl_2$] 2×10^{-2} *mole/lit.* (○), 1×10^{-2} *mole/lit.* (△) and 0.5×10^{-2} *mole/lit.* (×).

When inhibition grade H is defined as:

$$H = 1 - V_{Mg} / V_{Ca}$$

where V_{Ca} is the initial velocity in the presence of Ca^{2+} ion alone and V_{Mg} is that in the presence of both Ca^{2+} ion and Mg^{2+} ion, the inhibition curves form the first order sigmoid curves.

It was also stated in p. 32 that K_m values were not changed by Mg^{2+} ion, *i.e.*, Mg^{2+} ion had no effect on the binding of actomyosin with ATP. It may be taken, therefore, to indicate that the mechanism of the inhibition by Mg^{2+} ion is as follows:

$$M^* + Ca \underset{K_1}{\overset{Ca}{\rightleftarrows}} M^*, \quad M^* + Mg \underset{K_2}{\overset{Mg}{\rightleftarrows}} M^*$$

$$M^*S + Ca \underset{K_1}{\overset{Ca}{\rightleftarrows}} M^*S, \quad M^*S + Mg \underset{K_2}{\overset{Mg}{\rightleftarrows}} M^*$$

$$M^* + S \xrightarrow[k_1^*]{Ca} M^*S \xrightarrow[k_2^*]{Ca} M^* + P$$

where K_1, K_2 are the dissociation constants.

The inhibition by Mg^{2+} ion does not suffer any alterations even when the K^+ concentration is changed (Fig 6) but the competition values of Ca^{2+} to Mg^{2+} are slightly changed with the pH variations (Fig. 7): that is, about $10^{1.6}$ at sufficiently acidic ranges of pH, about $10^{2.0}$ at sufficiently basic ranges of pH and an about mean value between them at pH 6.5.

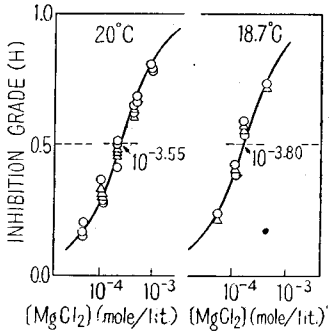


FIG. 6. Effect of the potassium concentration upon Mg-inhibition of ATPase. $[K^+]$ 0.5 *mole/lit.* (○), 0.16 *mole/lit.* (△); veronal-acetate buffer at pH 6.5; $[CaCl_2]$ 2×10^{-2} *mole/lit.* (left curve), 1×10^{-2} *mole/lit.* (right curve); [ATP] 1×10^{-3} *mole/lit.* [actomyosin] 0.445 mg. protein/ml.

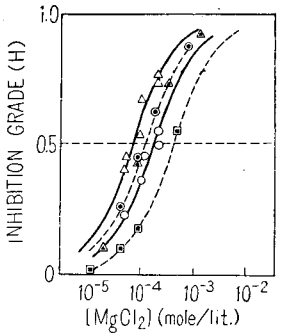
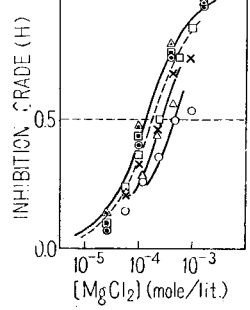


FIG. 7. Effect of pH upon Mg-inhibition of ATPase. Experiment I (full line) 19.7°, $[CaCl_2]$ 1×10^{-2} *mole/lit.* $[K^+]$ 0.48 *mole/lit.*, 0.22 mg. protein/ml., acetate buffer at pH 6.5 (○) and pH 9.1 (△). Experiment II (dotted line): 9.7°, $[CaCl_2]$ 1.6×10^{-2} *mole/lit.*, $[K^+]$ 0.16 *mole/lit.* ca. 0.3 mg. protein/ml. glycine buffer at pH 9.3 (⊙) and pH 8.0 (◬), veronal-acetate buffer at pH 5.5 (▣).

These results may be understandable simply by presuming that the ratio of the dissociation constant of Ca^{2+} to that of Mg^{2+} in M^*S^- is different from that in M^*S; that is, the ratio in M^*S^- is $10^{2.0}$ and $10^{1.6}$ in M^*S respectively.

It is further observed in Fig. 8 that the Mg^{2+} inhibition curves are shifted at the concentrations of enzyme protein over 0.22 mg per ml from such a fair Sigmoid curve that is obtainable below 0.22 mg. per ml. (#).

FIG. 8. Effect of enzyme concentration upon Mg-inhibition of ATPase. Experiment I; 8°, glycine buffer at pH 9.3, [K] 0.16 *mole/lit.*, ATP 4×10^{-4} *mole/lit.*, $[CaCl_2]$ 3.2×10^{-2} *mole/lit.* [actomyosin] 0.4 mg. protein per ml. (⊙), 0.27 mg. protein/ml. (▣), 0.2 mg. protein/ml. (◬). Experiment II: [K] 0.48 *mole/lit.*, $[CaCl_2]$ 1×10^{-2} *mole/lit.*, veronal-acetate buffer at pH 6.5, 15.5°, 0.22 mg. protein/ml. (□), at pH 6.1, 22.4°, 0.36 mg. protein/ml. (×), at pH 6.1, 21.3°, 0.72 mg. protein/ml. (△), at pH 6.5, 23°, 1.44 mg. protein/ml. (○).



This is probably due to the reason reported by Koga & Maruo (*12*).

In order to compare the case of ATPase action with that of the light-scattering, the ratio of Mg^{2+} concentration to Ca^{2+} concentration is calculated to be about 1/10 from these results, under such conditions that pH 6.3, temperature at 23.5°, actomyosin 1.6 mg per ml, [KCl] $=0.48$ *M* and the ratio of Mg- to Ca-actomyosinate is 42.5 per cent.

Inhibition by Inorganic Pyrophosphate: The ATPase activity is inhibited by inorganic pyrophosphate (Pr) and the inhibition is that of second order reaction and also the effect of Pr is competitive with ATP as may be seen in Fig. 9a-b.

These results seem to suggest such a mechanism as follows:

$$M^* + S \underset{k_1^*}{\longrightarrow} M^*S \underset{k_2^*}{\longrightarrow} M^* + P$$

$$M^* + 2Pr \underset{K}{\rightleftarrows} M^*(Pr)_2$$

According to the above reaction schema, the inhibition grade H can be represented as:

$$H = \frac{[Pr]^2}{\phi + [Pr]^2}, \qquad \phi = K\left(1 + \frac{k_2^*}{k_1^*} \cdot [S]\right).$$

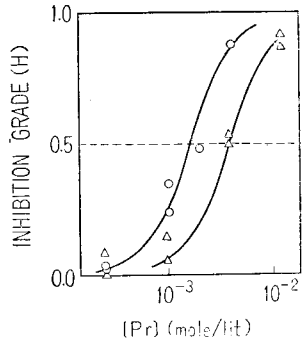


FIG. 9a. Inorganic pyrophosphate-inhibition of ATPase. 10° glycine huffer at pH 9.4, $[K^+]$ 0.18 *mole/lit.*, $[CaCl_2]$ 8.7×10^{-3} *mole/lit.*; [ATP] 2×10^{-4} *mole/lit.* (○) 4×10^{-4} *mole/lit.* (△).

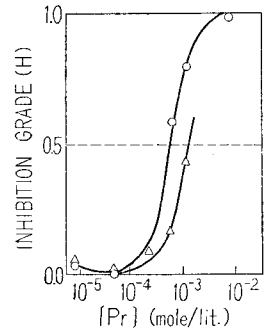


FIG. 9b. Inorganic pyrophosphate-inhibition of ATPase. 15°, veronal-acetate buffer at pH 6.5, $[K^+]$ 0.48 *mole/lit.*, 0.36 mg. protein/ml., $[CaCl_2]$ 2×10^{-2} *mole/lit.* [ATP] 2.5×10^{-4} *mole/lit.* (○), 1.3×10^{-3} *mole/lit.* (△).

This equation is in good agreement with the observed relation of H for [Pr] and [S]. It is found from our data by introducing $k_2^*/k_1^* = 1.5 \times 10^{-4}$ *mole/lit.* to the above equation that $K = 10^{-5.8}$ *mole/lit.*

THE CHANGE OF THE LIGHT-SCATTERING

Methods—The change of the intensity of the scattered light due to actomyosin solution upon the addition of ATP was traced with an electron multiplier-electromagnetic oscillograph (YEW, 3 elements, oscillator D-type) or μA-meter system. 0.5 ml. of ATP solution was breathed carefully into 14.5 ml. of the purified myosin B solution through a pipette whose tip was cut and the light-scattering of the protein solution as measured at an angle of 45° or 135° measured clockwise from the incident beam. Outline of the equipment for these measurements is shown in Fig. 9a. RCA-931A tube was used as an electron multiplier and its adjoining circuit is shown in Fig. 9b. The time for stirring is about 0.1 second and similar results were obtained at both angles, 45° and 135°. In so far as not mentioned specially all measurements were

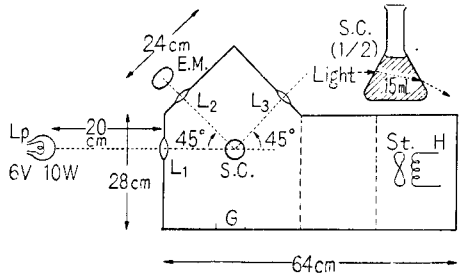


FIG. 10a. Apparatus for measurement of the light-scattering. E.M.: electron multiplier, S.C.: sample vessel, Lp: lamp, L_1, L_2, L_3: lens, G: window, St.: stirrer, H: heater.

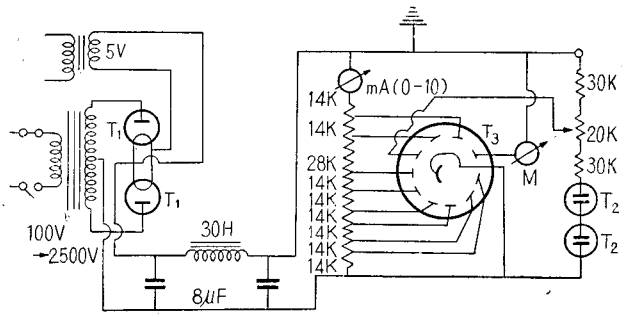


FIG. 10b. Adjoining circuit of an electron multiplier RCA-931-A. T_1: DC-762-A, T_2: VRB- 135/60, T_3: RCA-931-A, M: oscillograph or μA-meter.

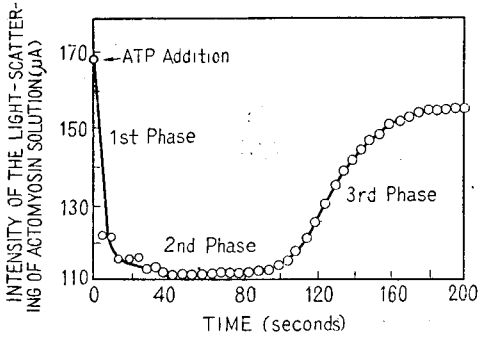


FIG. 11. Effect of ATP on the light scattering of actomyosin solution. 20°, pH 6.4, ATP 2.2×10^{-5} *mole/lit.*, $[MgCl_2]$ $(2/3)\times10^{-2}$ *mole/lit.*, [KCl] 0.48 *mole/lit.*

carried out under the condition that temperature at 20°—21°, at pH 6.4 and [KCl]=0.4—0.5 *M*.

Results and Discussion—The time course of the light-scattering change, an example of which is shown in Fig. 11, can be divided into three phases, being similar to that of the viscosity change. When ATP is added to a solution of actomyosin, there is a rapid decrease in the light-scattering intensity (First phase); following this is a period during which the reduced intensity remains constant (Second phase); finally, when ATP added is split to a certain extent, the light-acattering intensity rises slowly (to about original level) (Third phase).

(a). *First Phase:* This change is completed within a few seconds or slightly over ten at most, while it takes about 20—30 seconds for every one of the viscosimetric measurements which have been used hitherto to investigate the deformation of actomyosin. Therefore, it has not yet been possible to measure the velocity of this change.

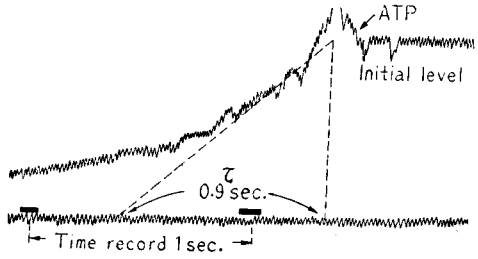


FIG. 12. An example of oscillogram of the light-scattering change of actomyosin solution. 20°, pH 6.4, $[MgCl_2]$ 1/100 *mole/lit.*, [ATP] $(1/16)\times1.8\times10^{-4}$ *mole/lit.* Arrow on upper right indicates the time when ATP is added and base line corresponds to the intensity of the light-scattering after the addition of sufficient amount of ATP.

Fig. 12 shows an example of our results obtained by means of oscillography. Here, τ is the time taken, at initial velocity, to complete the light-scaterring change, *i.e.*, to reach the minimum value of the intensity observed upon the addition of ATP sufficiently in quantity. The relationship between the reciprocal of τ and the amount of ATP in presence of various cations is shows in Figs. 13a and 13b.

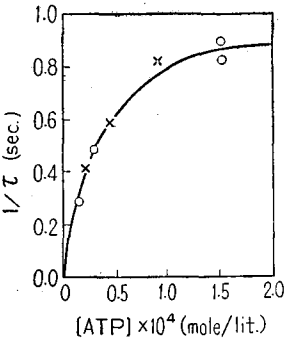


FIG. 13a. Relationship between initial velocity of the light-scattering drop (First Phase) and ATP concentration in the presence of K and Ca. 21°, pH 6.4, [K] 0.48 *mole/lit.* (○), [K] 0.48 *mole/lit.* plus [$CaCl_2$] 1/150 *mole/lit.* (×).

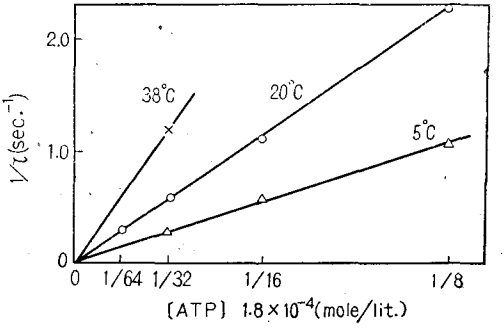


FIG. 13b. Relationship between initial velocity of First Phase of the light-scattering change and ATP concentration in the presence of 1×10^{-2} *mole/lit.* $MgCl_2$, pH 6.4, [KCl] 0.48 *mole/lit.*

When one takes into consideration that $1/\tau$ in the presence of K^+ ion and Ca^{2+} ion remains constant at higher ATP concentration and that the ATPase action occurs herewith, the following mechanism may be thought out:

$$M + S \underset{k_1}{\longrightarrow} MS \qquad (1)$$

$$MS \xrightarrow[k_2]{} M + P \quad (2)$$
$$MS \xrightarrow[k_3]{} M^*S \quad (2)$$

According to this reaction schema, the velocity of the light-scattering change (V) can be given by:

$$V = \frac{d[M^*S]}{dt} = k_3[MS].$$

At the stationary state,

$$\frac{d[MS]}{dt} = k_1[M][S] - (k_1 + k_2)\ [MS] = 0$$

Hence,

$$V_{initial} = \frac{k_3[M]}{1 + \frac{k_2 + k_3}{k_1[S]}}$$

i.e.,

$$\frac{1}{\tau} = \frac{k_3}{1 + \frac{k_2 + k_3}{k_1[S]}}$$

From the facts described in the previous chapter, it is deduced that reaction (2) does not proceed in the presence of Mg^{2+} and that Mg^{2+} as well as Ca^{2+} does not affect reaction (1).

Based on the above deduction, the velocity constants can be estimated from our results; i.e.,

$$_{K}k_1 = {}_{Ca}k_1 = {}_{Mg}k_1 = 10 \times 10^4 (lit/mole \cdot sec.)$$
$$_{K}k_2 = {}_{Ca}k_2 = 7/3\ (sec.^{-1}),\ {}_{Mg}k_2 \ll {}_{Mg}k_3$$
$$_{K}k_3 = {}_{Ca}k_2 = 1\ (sec.^{-1}),\ {}_{Mg}k_3 \gg 5\ (sec.^{-1})(\#)$$

Here, it is characteristic that Mg^{2+} ion accelerates reaction (3) remarkably. Ca^{2+} ion is competitive with Mg^{2+} ion. The values of τ upon the addition of 2×10^{-5} *mole/lit.* ATP at 23.5°, pH 6.3 are given in Table IV. Here, it can be interpreted that, when the ratio of the concentration of Mg^{2+} ion to that of Ca^{2+} ion added is 1 : 4, the ratio of Mg- to Ca-actomyosinate is 42.5 per cent.

Further, the activation energy of reactions (1) and (3) were estimated through the temperature dependence;

$$_{Mg}\Delta H_1^* = 7.5 \text{ Kcal. } (\#\#)$$
$$_{K}\Delta H_2^* = 7.5 \text{ Kcal.}$$

We symbolize the presence of K^+ ion or Mg^{2+} ion by the affix of K or Mg.

$_{Mg}\Delta H_1^*$ represents the activation energy ΔH^* for reaction (1) in the presence of Mg^{2+}.

TABLE IV

Effect of Ca^{++} and Mg^{++} on the Rate of the light-scattering change (First Phase)

(23.5° pH 6.3, [ATP] 2×10^{-3} *mole/lit.*, 1.6 mg. protein/ml.)

In the addition of	Time for the complete change with the initial velocity
$CaCl_2$ 1/400 *mole/lit.*	2.0 *second*
$MgCl_2$ 1/400 *mol/elit.*	0.42
$CaCl_2$ 1/100 *mole/lit.*	
$+MgCl_2$ 1/400 *mole/lit.*	0.77

(b). *Second Phase:* When ATP is present over a certain amount, a period appears during which the intensity of the light-scattering remains constant.

As pointed out by Csapó (*13*) in his viscosimetric work, the duration of this second phase is proportional to the amount of ATP added and is reversely propertional to the activity of ATPase; that is this phase corresponds with the reaction:

$$M^* + S \longrightarrow M^*S \longrightarrow M^* + P$$

(c). *Third Phase:* With the exception of the first few seconds in this phase when a small amount of ATP may still remain, the velocity of the recovery is independent of the initial concentration of ATP added and obeys the formula for a first order reaction and does not suffer much affect by the addition of Mg^{2+} or Ca^{2+}.

The velocity constant for the reaction:

$$M^* \longrightarrow M \quad (4)$$

can be estimated, that is:

$$_{K}k_4 = 1/40 \ (sec.^{-1})$$
$$_{Ca}k_4 = 1/30 \ (sec.^{-1})$$
$$_{Mg}k_4 = 1/30 \ (sec.^{-1})$$

The activation energy for this reaction is found to be:

$$_{Mg}\Delta H_4^* = 4.1 \text{ Kcal.}$$

The Change of Light-scattering upon the Addition of Inorganic Pyrophosphate: In the case of addition of ATP, the true equilibrium of the combination between ATP and actomyosin is not ascertainable because ATP is split. Inorganic pyrophosphate (Pr), on the other hand, is not split by actomyosin but gives rise to the light-scattering change of actomyosin solution only in the presence of Mg^{2+} ion.

Moreover, the deformation of actomyosin particles upon Pr-addition seems to be the same as upon the addition of ATP; *i.e.*, as in Table V, the degree of decrease in the light-scattering intensity upon the addition of the sufficient amount of Pr is the same as that of ATP.

TABLE V

The Light-scattering Change upon the Addition of ATP and Inorganic Pyrophosphate (Pr)

Angle ⟶	45°	135°
Pr	38	37
ATP	36	35

The numerical value $\frac{A-B}{A} \times 100$. A: Initial intensity of the light-scattering of actomyosin solution before the addition of ATP or Pr. B: Minimum intensity of the light-scattering of actomyosin solution after the addition of ATP or Pr.

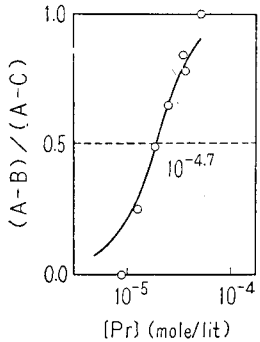


FIG. 14a. Effect of inorganic pyrophosphate (Pr) on the light-scattering of actomyosin solution (0.38 mg. pretein/ml.) in the presence of 1×10^{-2} *mole/lit.* $MgCl_2$. 12°, pH 6.4, [KCl] 0.48 *mole/lit.* A: Initial intensity of the light-scattering of actomyosin solution before the addition of ATP or Pr. B: The light-scattering intensity of actomyosin solution after Pr addition. C: The light-scattering intensity of actomyosin solution after the addition of the sufficient amount of ATP.

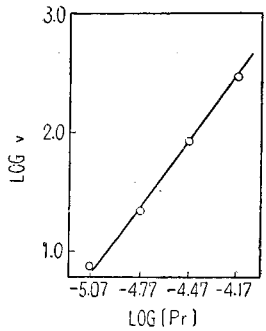


FIG. 14b. Relationship between initial velocity of the light-scattering drop and concentration of inorganic pyrophosphate (Pr) in the presence of 1×10^{-2} *mole/lit.* $MgCl_2$.

As may be seen in Fig. 14a, the relationship between the percentage of the light-scattering intensity after Pr-addition to the original intensity of the actomyosin solution and the concentration of Pr added is shown as a second order sigmoid curve.

Thus, the dissociation constant for the reaction:

$$M + 2Pr \rightleftarrows M^* (Pr)_2$$

is found to be $10^{-9.4}$ *mole/lit.*

Moreover, the initial velocity of deformation of actomyosin particles upon Pr-addition is proportinal to $[Pr]^2$ as may be seen in Fig. 14b.

THE SORT AND THE NUMBER OF ACTIVE CENTERS

Sort of Active Centers—How is the deformation of actomyosin particles related to the ATPase action? The question of whether ATP combines with the same action point (active center) of actomyosin molecule in both phenomena or with the different points of action is very important in order to ascertain the mechanism of these reactions.

In the mechanism described in the former chapter, it has been assumed tacitly that these two active centers are identical with each other. But even if we take the two active centers to be different, it may be possible to explain the experimental results although a more complicated mechanism must be thought out in this case.

However, the following results observed in both phenomena favour the view that the two active centers are identical:

(i) The reaction velocity is of the first order with respect to ATP concentration when [ATP] is low..

(ii) Mg^{2+} ion and Ca^{2+} ion are bound by actomyosin competitively with each other and noncompetitively with ATP and their binding is of the first order.

(iii) Pyrophosphate is bound competitively with ATP and its binding is of the second order.

As in Table VI, the velocity constants and the dissociation constants have the similar features in both cases and here, the slight variations of numerical values may come from the situation that the original actomyosin M takes the leading part in the light-scattering change, while the deformed actomyosin M* takes the leading part in the ATPase action.

Number of Active Centers per One Molecule of Myosin—The number of active centers per one molecule of myosin was estimated by means of the following procedure.

TABLE VI

Reaction	k or K	Reaction	k or K
$M+S \rightarrow MS$	$Kk = Cak = Mgk = 10 \times 10^4$ (*lit./mole sec.*)	$M^*+R \rightarrow M^*S$	$Kk = Cak = Mgk = 5 \times 10^4$ (*lit./mole sec.*)
$MS \rightarrow M+P$	$Cak = Kk = 7/3$ (1/*sec.*)	$M^*S \rightarrow M^*+P$	$Cak = 7$ (1/*sec.*)
$MS \rightarrow M^*S$	$Cak = Kk = 1$		
	$Mgk \gg 5$ (1/*sec.*)		
		$M^* \rightarrow M$	$Kk = 1/40$
			$Cak = Mgk = 1/30$ (1/*sec.*)
$M+Mg^{++} \rightleftarrows$ Ca $M+Ca^{++}$	$K \fallingdotseq 1/4$	$M^*+Mg^{++} \rightleftarrows$ Ca M^*+Ca^{++}	$K \fallingdotseq 1/10$
$M+2Pr \rightleftarrows M^*Pr_2$	$K = 10^{-9.4}$ (*mole/lit.*)^2	$M^*+2Pr \rightleftarrows M^*Pr_2$	$K = 10^{-5.9}$ (*mole/lit.*)^2

Plotting the quantity of ATP added in the presence of Mg^{2+} ion on the abscissa and the ratio of the minimum intensity to the original intensity of the light-scattering on the ordinate gives a broken line as shown in Fig. 15a.

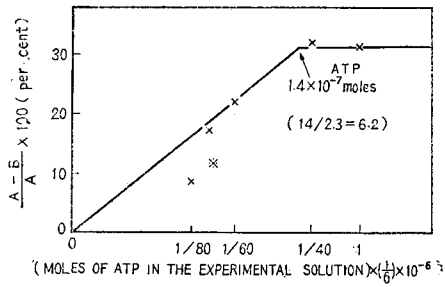


FIG. 15a. Actomyosin- ATP combination in the presence of 1/400 *mole/lit.* $MgCl_2$. 21°, pH 6.4, KCl 0.48 *mole/lit.* moles of myosin in the experimental solution $= \frac{25.9 \times 10^{-3} \times (3/4)}{8.4 \times 10^5} = 2.3 \times 10^{-8}$. A: initial light-scattering intensity of actomyosin solution before ATP addition. B: the light-scattering intensity of actomyosin solution of phase II.

(#) This lower drop is probably due to such a situation that the ATPase activity can not be neglected at the lower concentration of ATP.

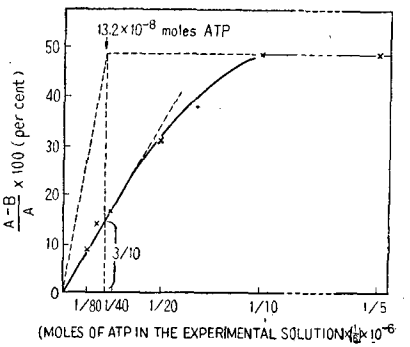


FIG. 15b. Actomyosin-ATP combination in the presence of Mg (dotted line) and in the absence of Mg (×). 21°, pH 6.4, [KCl] 0.48 *mole/lit.* Moles of myosin in the experimental solution $\frac{25\times 10^{-3}\times(3/4)}{8.3\times 10^{5}}$ $=2.2\times 10^{-6}$. A: Initial light-scattering intensity of actomyosin solution before ATP addition. B: Minimum light-scattering intensity of actomyosin solution after ATP addition.

Since ATPase activity in the presence of Mg^{2+} ion is negligible as proviously described, the above results may be interpreted as indicating that the reaction M+S→M*S proceeds in proportion to the concentration of ATP.(♯) Thus, the quantity of ATP at the breaking point should be equal to the quantity required for the complete deformation of all the myosin present to M*S.

Based on the above results, presuming that molecular weight of myosin is 840,000 (*14*) and that the myosin to actin ratio in myosin B used in our experiments 1:3, the number of ATP molecules required for the complete deformation of one molecule of myosin is calculated to be:

$$\frac{1.4\times 10^{-7}}{2.3\times 10^{-8}}=6.2\fallingdotseq 6,$$

that is, it comes to this, that the number of active centers, in other words, the number of units per one molecule of myosin, is six.

On the other hand, it is well known (*15*) that actomyosin is a stoichiometric complex which contains myosin (M) and actin (A) at the ratio of 2.5: 1 and that molecular weight of actin is about 70,000 (*16*); that is, it comes to this, that six molecules of actin combine with one

♯ From this fact, the reverse reaction of the reaction (1*) is neglected by us. See p. 31.

molecule of myosin, in other words, one molecule of actin with one unit of myosin.

Such being the case, we shall use the expression "AM" for the unit of actomyosin from now on.

Mommaerts (*17*), who carried out similar work using the viscosimeter, reported that one ATP combined with every 300,000 g. myosin. But his figure is not acceptable from the following view-points:

(i) In the ATP concentration in question, the viscosity drops to minimum level within several seconds after the ATP addition and then rises immediately. Therefore, the direct measurement of the minimum value by means of viscosimetry is impossible.

(ii) At 0°, at which his work was done, most of the actomyosin particles dissociate to actin and myosin (even in the absence of ATP) as will be mentioned later.

Then, Fig. 15b shows that upon the addition of ATP in quantity just to induce the complete deformation of actomyosin in the presence of Mg^{2+}, only three-tenths of the actomyosin deforms in the absence of Mg^{2+} ion. Considering the fact as described formerly that under these conditions, the two reactions:

$$MS \rightarrow M + P \quad (2)$$
$$MS \rightarrow M^*S \rightarrow M^* + P \quad (3)$$

occurred coincidently and the ratio of the velocity constant of the reaction (2) to reaction (3) was 7:3, this observation described above may be easily understandable.

MECHANISM OF MUSCULAR CONTRACTION

Based on the mechanims of ATP-actomyosin interactions as discussed in former chapters, we will discuss the mechanism of muscular contraction in this chapter.

What physical meaning has the deformation of actomyosin;

$$M \longrightarrow M^*$$

which is induced by the addition of ATP? Experimental answers to this question have already been sought by various methods; viscosimetry (*18*), ultra-centrifugation (*18*) and electron microscopy (*19*).

Generally speaking it has been hither-to emphasized that ATP addition causes a disaggregation of actomyosin into actin and myosin. But the enzymatic property of the myosin produced by this assumed disaggregation is different from that of the single myosin because it has

been reported that the behaviour of acotmyosin-ATPase towards Mg^{2+} ion and pH is different from that of myosin-ATPase (*20*). J.J. Blum (personal communication) states that the ATP addition causes a reversible shape change at constant molecular weight, indicating no depolymerization into " actin " and " myosin A." Therefore, the expressin AM⟶AM* instead of M→M* will be used hereafter.

It is further well known that in [KCl]+[NaCl]≒0.15 *M* this being the concentration observed in the muscle (*21*), ATP addition causes so-called " Superprecipitation " of actomyosin. It is also evident in the electron microscopic study by Astbury *et al.* (*19*) that this phenomenon involves at least two steps; once a deformation of actomyosin and then a new polymerization.

Myosin and actin in resting muscle are associated in the state of actomyosin (*22*). Muscle contains a considerable quantity of Mg^{2+} and Ca^{2+}, most of which are bound by actomyosin and other protein in muscle (*23*). Thus, the concentration of free ions must be very low and therefore even a small change of the binding force between these ions and actomyosin may have affect on the concentration of free ions.

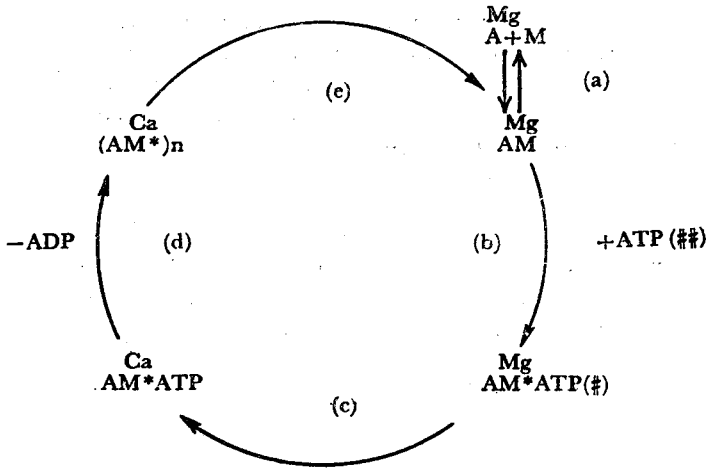
On the assumption that the ratio of the binding force of Mg^{2+} to Ca^{2+} by actomyosin is much larger in rest than in contraction, it may be possible that the ratio of free Mg^{2+} ion to free Ca^{2+} is more than 1/4 in rest and less than 1/10 in contraction. In such a case, actomyosin will be present as Ca-actomyosinate in contraction and as Mg-actomyosinate in rest. The facts that there are both the increase of free Ca^{2+} (*24*) and the decrease of free Mg^{2+} (25) with muscular activity may favour the above assumption.

Putting together the whole story of the above discussions, the interaction of actomyosin to ATP under such a condition as in muscle may be expressed as follows:

Sequence of Events in Muscular Contraction—It has been known for a long time that upon stimulating muscle there is a latent period for about 3—5 milliseconds and then contraction and finally relaxation. It takes about 1/10 seconds for one twitch.

Now, let us assume that when stimulated, a part of ATP bound by actomyosin and by the other proteins is set free and then follow such reactions as described in the former parts of this chapter. Thus, if the reaction (d) corresponds to contraction, then the reactions (b) and (c) are involved in latent period and the reaction (e) in relaxation.

The uniform concentration of ATP set free on that occasion may



be, of course, far less than the total ATP concentration as observed in striated muscle, that is, 5×10^{-3} *mole/lit.* However, the activity of ATP involved in the reaction (b) must be far more than that uniform concentration because the distribution of ATP is not uniform and is high in actomyosin surroundings. Therefore, it may be estimated to be about 5×10^{-3} *mole/lit.*

If this is the case, the velocity of the reaction (b) comes to about $10\times10^{4}\times5\times10^{-3}=5\times10^{2}$ *sec.*$^{-1}$ which is in good harmony with the fact that the latent period (the interval between stimulus and onset of contraction) is about 3—5 m sec. An already-known fact that on increasing temperature by 10°, there was a reduction by about 2/3 of the latent period (27) is also in accord with our already-described result that ΔH^* of the reaction (b) was 7.5 Kcal.

Further, the facts that the duration of shortening is 0.05 sec. and is reduced to a half by 10° increase are well corespondent to such results that the velocity constant is 42 *sec.*$^{-1}$ (glycine buffer, 0.16 KCl, 37°) and ΔH^* is about 12 Kcal in the ATPase action.

The existence of the complex AM*ATP is incompatible with the theory of Bailey and Perry (*26*) according to which it is one and the same SH-group which is responsible for splitting ATP and linking actin to myosin, the two functions competitive with one another.

In addition of one ATP per one unit of actomyosin, many ATP adsorbed on one actomysin are required for the contraction but they are unchanged in this contraction cycle. Therefore, they are left neglected in this paper.

It may be due to the difference between the interrelation of actomyosin particles to each other in the extracted actomyosin system and that in intact muscle that the relaxation in the extracted actomyosin system is very slow. The situation that relaxation depends delicately upon the structure of actomyosin in muscle is also made clear through the fact that even intact fibrers when made to shorten to an excess degree, do not relax any more (*28*).

Recently, A. V. Hill (*29*) reinvestigated the early heat production during an isotonic twitch. His results reveal the nature of the first event in heat production, the heat of activation. Such heat appears during the mechanical lag period and starts off at a maximum rate after a very short latency. It seems to be quite independent of the conditions of muscle contraction. Its value is 3 millicalories/g. muscle, that is 5.7 Kcal./140,000 g. muscle myosin per one twitch.

The heat produced during a twitch comprises the heat of activation and the heat of shortening. The latter is proportional to the shortening and primarily independent of the work alone. No heat is produced during relaxation if the load lifted by the muscle is detached before relaxation begins. If the load is left on, the equivalent of its potential energy is released during relaxation, but no more. These observations can be interpreted by the following postulate; heat of activation is attributed to heat content ΔH of the exothermic reaction (b) and heat of shortening to the exothermic reaction (d) whereas the fact that during relaxation no heat is produced is harmony with the results that ΔH^* for the reaction $M^* \longrightarrow M$ is very small.

D. K. Hill (*30*) stated that in a single twitch the contraction is accompanied by an increased transparency, roughly coincident with the latency relaxation, followed by an increased turbidity when contraction occurs. This, then, has to change back again in the opposite sence. These observations are interpreted by the previously mentioned correspondence of the changes of actomyosin to the phases of muscular contraction, that is, the light-scattering intensity decreases by the reaction (b), increases by the reaction (d) and changes back again by the reaction (e).

On the other hand, the change of the light-transparency of muscle in tetanus (*31*) may rise in a different mechanism from that in twitch.

ATP Consumption—

ATP Amount Involved in A Single Twitch: According to Lundsgaad (*32*), a striated muscle in iodoacetate poisoning performs 50 full twitches

anaerobically. Then, the contents of ATP and phosphocreatine (CP) known to be 5×10^{-6} and 20×10^{-6} *moles per g.* of muscle, respectively.

The followed three reactions are considered as those possible to occur in such an iodoacetate poisoning muscle;

(i) $ATP \longrightarrow ADP + P$ (Adenosine triphosphatase)
(ii) $CP + ADP \longrightarrow C + ATP$ (Creatine-ATP-phosphorase)
(iii) $2ADP \longrightarrow ATP + AMP$ (Myokinase)

When the twitches are repeated, the reactions (i) and (ii) proceed completely and the reaction (iii) proceeds only incompletely.

Thus, it comes to this, that the quantity of ATP split for one twitch is more than $5\times10^{-7}=(25\times10^{-6})/50$ moles per g. of muscle, that is $0.9=(5\times10^{-7}\times140{,}000)/0.075$ (#) moles per 140,000 g. of myosin and less than 1.8 moles per 140,000 g. of myosin.

Csapó (*33*) conducted a similar experiment with uterus and reported that uterine muscle in monoidoacetate poisoning performs seven full twitches (ten twitches) anaerobically and that the content of CP plus ATP in uterine muscle is 2.6×10^{-6} moles per g. of muscle, This result indicates the consumption of $0.7=(2.6\times10^{-6}\times140{,}000)/(7\times0.085)$—1.4 moles ATP per 140,000 g. of myosin for one full twitch.

Recently, Mommaerts (*34*) estimated the distribution of adenine nucleotides of frog muscle, which was fixed at the hight of contraction by means of putting the muscle in liquid air, and found that about one-fifth of the ATP content in the muscle, that is about $10^{-6.0}$ mole ATP per g. of muscle=1.8 moles ATP per 140,000 g. of myosin were split to ADP (AMP production is negligible during this one twitch).

On the other hand according to our theory, the minimum quantity of ATP involved in a single full twitch is one mole per 140,000 g. of myosin. It is, therefore, deduced that the ATP consumtion during muscular contraction in vivo is very economical and about 1.5 moles ATP per unit of myosin (though these figures vary a little (1—1.8 moles) according to the condition under which the twitch is induced) are consumed for one twitch(##).

Efficiency of Contracting Muscle: Varga (*35*) measured the reversible work of the glycerol-extracted musclus psoas of the rabbit and found that

It is assumed that the whole muscle contains 20 per cent of proteins composed of 50 per cent myosin B in which the ratio of action to myosin is 1:3.

This fact that ATP in amounts over one mole per unit myosin is consumed during one twitch in vivo may be due to the proceeding of the reaction (AMS→AM+P) to a few extensions or to the repeating of the reaction ($M^*+S\longrightarrow M^*S\longrightarrow M^*+P$).

it is about 9 Kcal per unit of myosin in the complete contraction(#). Similar values are also observed with rat muscle and frog muscle, that is about 9.0 Kcal with rat diaphragms and about 9.3 Kcal. with frog sartorious.

On the other hand, ΔF change for the ATP splitting is about 12 Kcal per mole. Therefore, the efficiency of muscular contraction comes to be $9/(12\times1.5)=50$ per cent; indicating a good agreement with the experimental results (*38*).

Breakdown of ATP in Active Muscle: For mammalian muscle at 37°, the rate of the breakdown of ATP in active muscle was estimated to be a value of the order of 10^{-3} mole per minute per gram of muscle (*18*). On the other hand, the velocity constant of ATP splitting per unit myosin by Ca-actomyosinate (##) was estimated to be about 42 *sec.*$^{-1}$ in the presence of glycine, at 37°, at pH 7.0. Further the number of the units of myosin per gram of muscle is estimated to be $(0.075\times6)/(8.4\times10^5)$. Therefore, the rate of the breakdown of ATP by Ca-actomyosinate comes to be $42\times60\times(0.075\times6)/(8.4\times10^5)=1.4\times10^{-3}$ moles per minute per g. of muscle; that is, it agrees approximately with the above results.

Temperatur Effect of Muscular Contraction—Laki *et al.* (*39*) reported at 26.5°, the larger part of myosin was combined with actin and at 4.9°, only a small amount of actomyosin was formed; that is, ΔH of the reaction (a) $A+M\rightleftarrows AM$ is very large (endothermic).

Therefore, on the assumption that when ATP is added to actomyosin, AM contracts and M does not, the contractibility of actomyosin thread and muscle upon the addition of ATP should be strikingly changeable with temporature-variations. In fact, this is established by Szent-Györgyi's scholars (*45*). They have regarded the temperature dependence of the contractibility as indicative of that of the equi librium reaction; AM relaxation$\rightleftarrows$AM contraction, but it should be, of course, interpreted as indicating that of the reaction (a).

Further, they concluded, through the comparison of the temperature dependence of the contractibility with that of the reversible work, that one mole of myosin is composed of 12 units on the assumption of 40

He seems to postulate according to Weber (*36*) that the total muscle protein contains about 40 per cent myosin B. Since this content is evidently too small (*37*) we re-calculated the work from Varga's results on the assumption that the content of myosin B is 50 per cent. The value of work per unit of myosin is only approximate because the measurements of the shape and the length of muscle used are considerably uncertain.

As mentioned previously, actomyosin is combined with Ca^{2+} in contraction.

per cent myosin B content in the total muscle protein, but it is also possible to explain their results even if it is assumed that 50% of the total muscle protein consists of myosin B and that one mole of myosin is composed of 6 units.

SUMMARY

With respect to the ATP splitting and the light-scattering change upon the addition of ATP to actomyosin solution, the relationship between the velocity of these two phenomena and the ATP concentration, in company with the function of inorganic pyrophosphate and some cations, were analysed kinetically and then the mechanism of the two phenomena was proposed.

It is also suggested that the ATP attacking points of actomyosin in the two phenomena are identical with each other and that the number of the active centers per one myosin molecule is six.

The mechanism proposed is able to illustrate the various facts observed about muscular contraction.

Finally, we are much indebted to Dr. Juro Horiuchi, the Head of the Research Institute for Catalysis, Prof. Hiroshi Tamiya, of Botanical Department, Faculty of Science (Tokyo University) and Prof. Naomoto Takasugi, of Chemical Department, Faculty of Science, for the facilities they have provided for this work and to Messrs Kinjiro Sukegawa and Junshiro Makino for valuable technical assistance.

REFERENCES

(*1*) Mommaerts, W.F.H.M., *J. Gen Physiol.*, **31**, 361 (1948).
(*2*) Needham, J. *et al.*, *Nature*, **150**, 46 (1942).
(*3*) Jordan W.K., and Oster G., *Science*, **108**, 188 (1948).
(*4*) Needham D.M. *et al.*, *J. Gen. Physiol.*, **27**, 355 (1944); *Nature*, **150**, 46 (1942).
(*5*) Kerr, S.E., *J. Biol. Chem.*, **139**, 121 (1941).
(*6*) Briggs, *J. Biol. Chem.*, **53**, 13 (1922); **59**, 255 (1924).
(*7*) Youngburg-Youngburg, *J. Lab. Clin. Med.*, **16**, 158 (1930).
(*8*) Müller, R.H., et al., *Experimental Electronics* (1942), Prentice-Hall Inc., New York.
(*9*) Albery, R.A., Smith, R.M., and Bock, R.M., *J. Biol. Chem.*, **193**, 425 (1951).
(*10*) Sarkar, N.K., Szent-Györgyi, A., and Varga, L., *Enzymologia*, **14**, 267 (1950).

(*11*) Klotz, C., *Cold Spring Habour Symp. Quant. Biol.*, **14**, 97 (1940).
(*12*) Koga, and Maruo, B., "*Kagakuno-Ryoiki*" (*Jap. J. Chem.*), April 1946, p. 180.
(*13*) Csapó, A., *Acta Physiol. Scand*, **19**, 100 (1949).
(*14*) Portyphl, H., and Weber, H.H., *Z. Natuforsch.* **56**, 2 (1950).
(*15*) Snellamann, O., and Erdös, T., *Biochim. et Biophys. Acta*, **3**, 50 (1949).
(*16*) Feuer, G., Straub, F.D. et., *Hungaria Acta Physiol.*, **1**, 50 (1948); Rozsa, G., Szent-Györgyi, A., and Wyckoff, R.W.G., *Biochim. et Biophys. Acta* **3**, 561 (1949).
(*17*) Mommaerts, W.F.H.M., *J. Gen. Physiol.*, **31**, (1948).
(*18*) Mommaerts, W.F.H.M., cited in *Muscular Contraction* (1950), Interscience Publishers, Inc., New York.
(*19*) Perry, S.V., Reed, R., Astbury, W.T., and Spark, L.C., *Biochim. et Biophys. Acta*, **2**, 674 (1948).
(*20*) Banga, I., and Szent-Györgyi, A., *Stud. Szeged*, **1**, 5 (1942); Mommaerts, W.F.H.M., and Seraidarian, K., *J. Gen. Physiol.*, **30**, 201 (1947).
(*21*) Dubuisson, M., *Arch. internatl. physiol.*, **52**, 439 (1942).
(*22*) Gerendas, M., Szarvas, P., and Maltoltsi, A.G., *Hungaria Physiol. Acta*, **1**, 121 (1948).
(*23*) Szyent-Györgyi, A., *Chemistry of Muscular Contraction*, 1st ed., (1947), Academic Press, New York; *Nature of life* (1948), Academic Press, New York.
(*24*) Honget, J., *Ann. de Physiol.*, **9**, 277 (1933).
(*25*) Hirschfelder, A.D., and Haury, V.G., *Proc. Exp. Biol. Med.*, **33**, 41 (1935).
(*26*) Bailey, K., and Perry, S.V., *Biochim. et Biophys Acta*, **1**, 506 (1947).
(*27*) Fulton, *J. Exp. Physiol.*, **18**, 16 (1928).
(*28*) Ramsey, R.W., and Street, S.F., *Biol. Symp.*, **3**, 9 (1941).
(*29*) Hill, A.V., *Proc. Roy. Soc.* (London), **136**, 195, 211, 228, 242 (1949).
(*30*) Hill, D.K., *J. Physiol.*, **108**, 292 (1949).
(*31*) Muralt, A., *Arch. d. gesamt. Ppysiol.*, **234**,653 (1934).
(*32*) Lundsgaard, E., *Biochem. Z.*, **217**, 162 (1930).
(*33*) Csapó, A., *Nature*, **166**, 1078 (1950).
(*34*) Mommaerts, W.F.H.M., and Rupp, J.C., *Nature*, **168**, 957 (1951).
(*35*) Varga, L., *Enzymologia*, **14**, 196 (1950).
(*36*) Weber, H.H., *Ergebn. Physiol.*, **36**, 109 (1934).

(*37*) Bate-Smith, *Rep. Food Invest. Board* (1938), p. 22. Great Britain.
(*38*) Hill, A.V., *Muscular Activity* (1924), Williams & Wilkins, Baltimore; *Proc. Roy. Soc., B*, **127**, 343 (1939).
(*39*) Mommaerts, W.F.H.M., *Muscular coutraction* (1950), p. 30. Interscience Publisher New Youk.
(*40*) Laki, K., Spicer S.S., and Carroll, W.R., *Nature*, **169**, 328 (1952).
(*41*) Szent-Györgyi, A., *Chemistry of Muscular Contration*, 1st ed. (1947), Academic Press New York, 2nd ed. (1951).
(*42*) Watanabe, S., and Sukegawa, K., "*Kagaku*" (*Science*, **22**, 471 (1952).

ADDENDUM

After this paper was written, we read Bozler's very interesting article "*Evidence for an ATP-Actomyosin Complex in Relaxed Muscle and Its Response to Calcium Ions*" (*Am J. Physiol.*, 168 760, 1952).

In his studies with the glyceroled muscle fibers, it was found 1) that in the relaxed condition the contractile elements are present in an aotivated state, possibly brought about by chemical combination of ATP with the contractile proteins. 2) that magnesium ions are essential for maintaining this state, and 3) that calcium ions in very low concentrations cause rapid contraction of this activated system, even in the absence of free ATP.

According to our mechanism (p. 47), when $k_d \ll k_e$ the muscle is in the state of relaxation and when $k_d \gg k_e$ it is present in the state of contraction.

As mentioned previously, the reaction (d) is inhibited by Mg^{2+} and is activated by Ca^{2+}. On the other hand, the reaction (e) is scarecely affected by these ions. Therefore, Bozler's results are illustrated by the following postulates: $Mgk_d \ll Mgk_e = Cak_e \ll Cak_d$.

The detailed discussion about the effects of divalent ions on glycerol-extracted muscle and Myosin B thread will be described in the next paper.