DEPENDENCE OF ENERGY TRANSDUCTION IN INTACT SKELETAL MUSCLES ON THE TIME IN TENSION

MASATAKA KAWAI, PHILIP W. BRANDT, AND MORTON ORENTLICHER,
Laboratory of Neurophysiology, Departments of Neurology and Anatomy, Columbia University, New York, New York 10032

ABSTRACT In intact single crayfish muscle fibers and frog semitendinosus muscles we have studied the tension response to sinusoidal length changes in the frequency range of 0.25–133 Hz. By this method we have resolved three processes in the interaction of myosin cross-bridges with actin in fully activated preparations. They are (A) a low-frequency phase advance, (B) a middle-frequency delay, and (C) a high-frequency advance. These processes can be used as probes to study the chemomechanical coupling of contractility. Process (B) represents net power output from the muscle preparation (oscillatory work). With maximal K or caffeine activation of crayfish muscle at 20°C, it decreases to zero in the initial 45 s of maintained tension. Similar results were obtained with frog semitendinosus whole muscles. We interpret this decrease of (B) with time as a gradual decrease in actomyosin ATP-hydrolysis rate.

INTRODUCTION

Physiologists have been attempting to probe the molecular mechanisms of muscle contraction by analysis of the time-course of the tension response to length changes. Significant advances were made by Hill (1953), who imposed step length changes on vertebrate skeletal muscles, and by Machin and Pringle (1959), who employed sinusoidal length changes in insect flight muscles. More recently Huxley and Simmons (1971) were able to study the fast component of the tension-time course in response to step-length changes in frog muscles. Their refined experimental technique allowed them to propose the existence of two attached states for cross-bridges generating tension.

Over the same period, biochemists, studying the interaction of the contractile proteins, have identified many intermediate steps in the hydrolysis of ATP by actomyosin. These are elements of a cyclic reaction sequence in which myosin alternately attaches to and detaches from actin filaments. Mechanical and biochemical approaches each have advantages and limitations: biochemical methods cannot detect work output, whereas physiological methods cannot discriminate among the numerous stages of the hydrolysis cycle. A major goal of muscle research is to integrate the mechanical and biochemical data into a unified explanation of contraction (cf. Pringle, 1975).

Since the biochemical schemes of the hydrolysis cycle proposed by Lymn and Taylor (1971) or by other workers (Tonomura et al., 1969; Eisenberg and Kielley, 1973)

are too complicated for direct application to mechanical studies, they must be simplified. We reduce the scheme to a cycle of three myosin cross-bridge states: (*a*) attached to actin in the energy transduction position, (*b*) attached to actin in the rigor position that follows the transduction, and (*c*) not attached to actin (cf. White, 1973).

We first studied rigor in skinned muscle fibers (Kawai and Brandt, 1976) to obtain basic data on the myofilament lattice when all cross-bridges are assumed to be attached to actin. In the current report we focus on cycling cross-bridges and determine their basic kinetic properties under their natural working conditions using intact muscle preparations. In future work we will return to skinned muscle preparations, in which we can control myoplasmic ionic constituents. The data reported here on intact preparations will be used as a standard, as we optimize experimental conditions in skinned fiber experiments.

We employ sinusoidal length changes, a technique used by insect muscle physiologists (for review, see Pringle, 1967; White and Thorson, 1973), because we believe

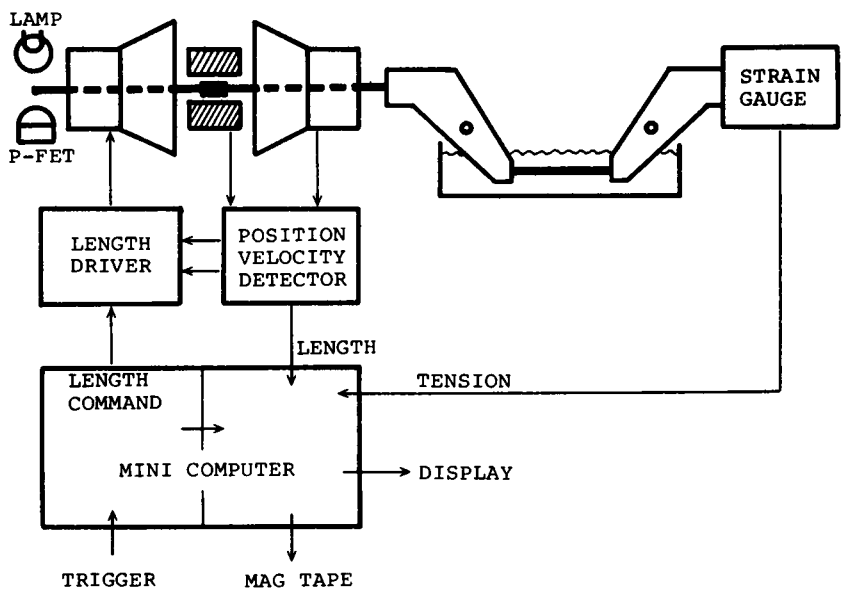

FIGURE 1 A sketch of the experimental apparatus. A single muscle fiber is held horizontally by two plastic clamps, one connected to a strain gauge for tension measurement, and the other to a length driver. The muscle preparation is bathed in the experimental saline (5 ml), which is constantly stirred while the temperature is regulated to ±0.5°C. The length driver consists of three parts: two loudspeakers (5″ in diameter; 40–1284, Tandy Corp., Fort Worth, Tex.) and an electromechanical position detector (200 DC-B, Shaevitz Engineering, Pennsauken, N. J.) of low frequency response (DC-250 Hz). Position and velocity are detected equally accurately up to 1.5 kHz by an appropriate hybridization of the position signal and the velocity signal from one speaker. These signals are fed back to the driving speaker, and the frequency response of the driver is DC-300 Hz. A lamp and a photo-field-effect transistor (FET) are occasionally used to calibrate the driver. Arrows show direction of information flow, and the several interfaces are abbreviated (e.g., A/D, D/A converters). Other details in text.

discrimination of cross-bridge transitions is optimal with this method. We expand the frequency range to cover nearly three orders of magnitude, in which many details of the cross-bridge cycle are characterized. A preliminary account of the present work was reported (Kawai and Brandt, 1975).

MATERIALS AND METHODS

Single muscle fibers were isolated from crayfish (genus *Orconectes;* purchased from Educational Products Div., The Mogul Corp., Oshkosh, Wis.) walking legs by the method used by Girardier et al. (1963) in crayfish control saline (200 mM NaCl, 5 mM KCl, 13.5 mM $CaCl_2$, 5 mM Tris buffer, pH 7.4), and the nontendon end attached to the shell membrane was dissected free of shell by peeling off the membrane. Frog (*Rana pipiens;* purchased from West Jersey Biological Supply, Trenton, N.J.) semitendinosus muscles were dissected in frog control saline (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 2 mM inorganic phosphate, pH 7.2), and either the ventral or dorsal head of the muscle was isolated and used for experiments. Chemicals used in the present report were purchased from Fisher Scientific Co. (Springfield, N.J.).

Preparations were immersed in a well-stirred, temperature-controlled bath (± 0.5°C, 5 ml). They were mounted between two plastic clamps (Fig. 1), and their lengths were adjusted to slightly over the slack length. This procedure resulted in slightly different sarcomere lengths from one experiment to another: crayfish 7.0–8.2 μm, frog 2.0–2.2 μm. The sarcomere length was measured by optical diffraction with a He-Ne laser (see Kawai and Kuntz, 1973). Muscle diameter and length (L_0) were measured with an ocular micrometer attached to a dissecting microscope (Nikon Inc., Instrument Div., Garden City, N. Y.).

One muscle clamp was connected to a length driver for length control, and the other to a strain gauge for tension measurement. The muscle length was under direct control of a minicomputer (Nova 1220, Data General Corp. Southboro, Mass.), programmed to produce sinusoidal length changes (ΔL = 0.2 – 0.3% L_0, peak-to-peak) sequentially in the range of 0.25–133 Hz (from high to low frequencies). The program accepts data on the muscle length and tension alternately and calculates "complex stiffness" (Y) defined as the ratio of two finite Fourier transformations of the tension and the length time-courses:

$$Y(f) = \frac{\int_{n\text{-cycles}} P(t) \exp(-2\pi f t i)\, dt}{\int_{n\text{-cycles}} L(t) \exp(-2\pi f t i)\, dt}$$

where f is frequency of the length oscillation (Hz), t is time(s), $P(t)$ is tension (dyn), $L(t)$ is length (cm), $i = \sqrt{-1}$, and the integration is over n integer cycles (n = 1, 2, 3, . . .) exceeding 0.4 s.

The complex stiffness is a frequency response function and represents mechanical properties of muscle such as elasticity, viscosity, mass, and negative viscosity (oscillatory work). For the present experiments the contribution of mass is insignificant (cf. Schoenberg et al. 1974; Truong, 1974). The complete frequency spectrum of complex stiffness describes the tension response to an arbitrary change in the length of the muscle (including steps) in the linear range.

The above method of complex-stiffness calculation, which includes signal averaging, rejects frequency components other than that of the experimental frequency. Consequently, the signal-to-noise ratio is high and the noise in the tension amplitude is 0.7 dyn (root mean square) for an integration over 0.4 s. Tension was measured with a strain guage (Bionix F-100, El

Cerrito, Calif.) of low compliance (4 nm/dyn) and amplified by a Clevite Brush carrier amplifier (Clevite Corporation, Cleveland, Ohio). The stiffness-detecting system was calibrated by using muscle fibers fixed with 2.5% glutaldehyde for 20 min as reference materials. This procedure enables us to determine the complex stiffness up to 133 Hz.

Measurements at 15 different frequencies in the range of 0.25–133 Hz required 21 s, and another 1 s was used to project amplitude (= $|Y|$) and phase (= arg(Y)) vs. log (frequency) plots to an oscilloscope screen, and to record the complex stiffness data on magnetic tape. Between each frequency the background tension level was measured so that errors due to a change in steady tension could be minimized. Before measurements at each frequency, a delay of 0.25 s was employed to ensure either a steady development of sinusoidal oscillation or steady level of tension. The experimental arrangements and data acquisition systems are depicted in Fig. 1.

RESULTS

In a typical experiment, a crayfish muscle fiber was initially soaked in a control saline in which Cl^- was the major anion and then this was replaced with a propionate-based saline. After the phasic tension due to the anion change (Reuben et al. 1967) had subsided, at least 30 min were allowed for equilibration. The muscle was subsequently activated with high K solution (200 mM K propionate, 5 mM imidazole, pH 7.0). Tension rose quickly to a plateau (Fig. 3 E) and during this plateau phase two records

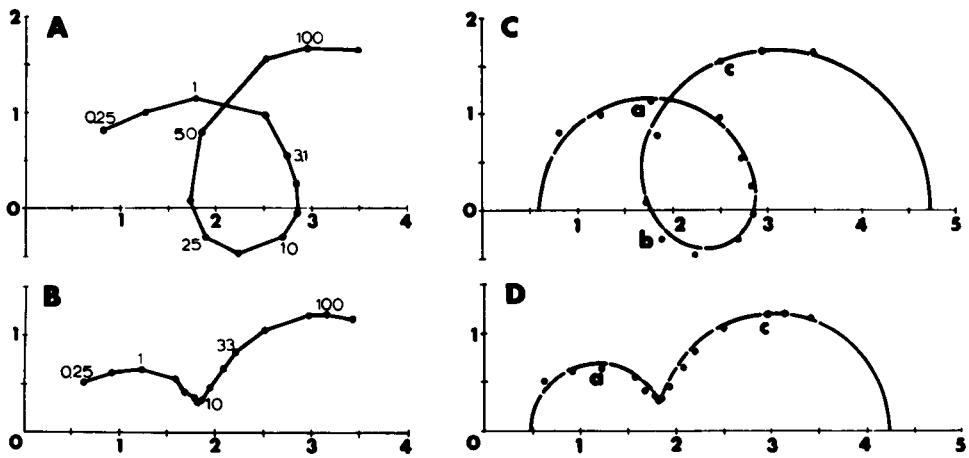

FIGURE 2 A, B: Nyquist plots of the complex stiffness $Y(f)$ in single crayfish muscle fiber activated by high K^+ at 20°C. The abscissa is the real part of the complex stiffness (elastic component), and the ordinate the imaginary (viscous component). Units are 10^5 dyn/cm for both axes. Points are (from left) 0.25, 0.5, 1, 2, 3.13, 5, 7.14, 10, 16.7, 33.3, 50, 80, 100, and 133 Hz. L_0 = 0.48 cm, diameter (approximate) = 350 μm, ΔL = 0.27% L_0. A, record obtained over the 12–33rd-s interval after activation (tension 2,014 ± 24 dyn). B, record obtained over the 47–68th-s interval of the same tension (1,994 ± 9 dyn). C, D: Result of fitting the data in A and B to Eq. 1. Points are experimental, while the continuous line is calculated (see Table I for fitted parameters together with 95% confidence limits). Breaks in the line represent the calculated values for frequencies corresponding to the experimental points. *a*, *b*, and *c* indicate frequencies corresponding to rate constants. C, 12–33rd s after activation. D, 47–68th s after activation.

of complex stiffness data were collected. The tension then declined and three more records were obtained in this phase.

Our initial data is presented in a plot of the real vs. imaginary parts (Nyquist plot) of the complex stiffness $Y(f)$, because each exponential rate process is represented by each hemi-circle in this plot (for review, see Machin, 1964). Fig. 2 A is a Nyquist plot of the complex stiffness record immediately after activation by high K solution (data collected during the 12th–33rd s after activation), and Fig. 2 B is that of later time (47th–68th s). The time and duration of the data collections are marked along the abscissa in Figs. 3 and 4. The tensions were nearly identical (Fig. 2 A, 2,014 ± 24 dyn; Fig. 2 B, 1,994 ± 9 dyn; N = 15, ± 1 SD) in both records.

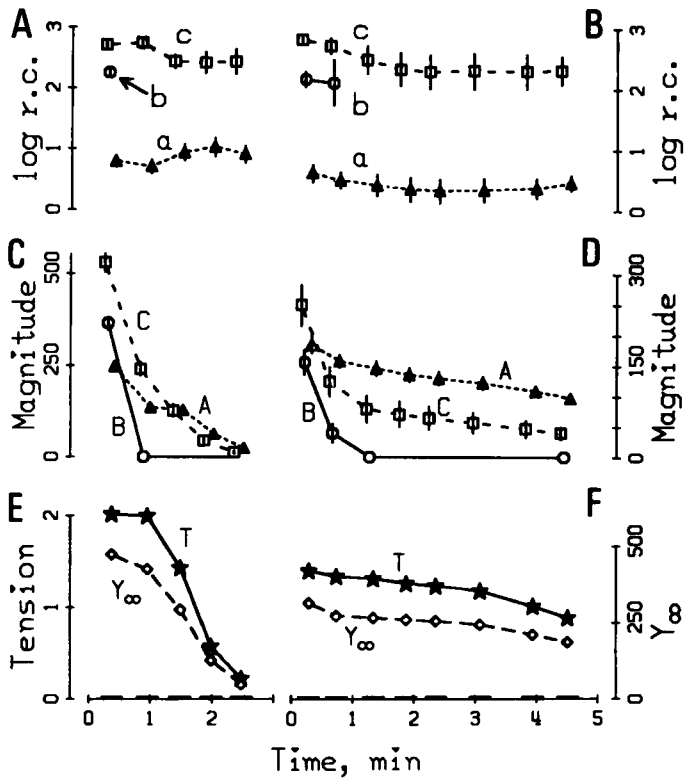

FIGURE 3 Results from a single muscle fiber of crayfish at 20°C. Time-courses of log rate constants (r.c.) $2\pi a$ (△), $2\pi b$ (○), $2\pi c$ (□) (in A and B), their corresponding magnitudes A (△), B (○), C (□) (in C and D), tension (T, ★) and extrapolated infinite-frequency stiffness (Y_∞, ◇) in E and F. Confidence limits (95%) are indicated by vertical bars. The frequency range is 0.25–133 Hz. The fiber was first activated with 200 mM K to product a phasic tension, with results shown in the figures on the left (A, C, and E). 12 min after relaxation, 15 mM of caffeine was introduced to the K saline, producing a prolonged tension (B, D, and F). Time was measured after activation, and tensions were zero at time 0. Each horizontal bar along the abscissa indicates a time interval of measurement of complex stiffness. Units are in log (s^{-1}) in A and B; 10^3 dyn/cm in C and D; 10^3 dyn for tension and 10^3 dyn/cm for Y_∞ in E and F. Note change of scale between C and D. First two points in A, C, and E, are obtained from Fig. 2 and Table I. See legend of Fig. 2 for other details.

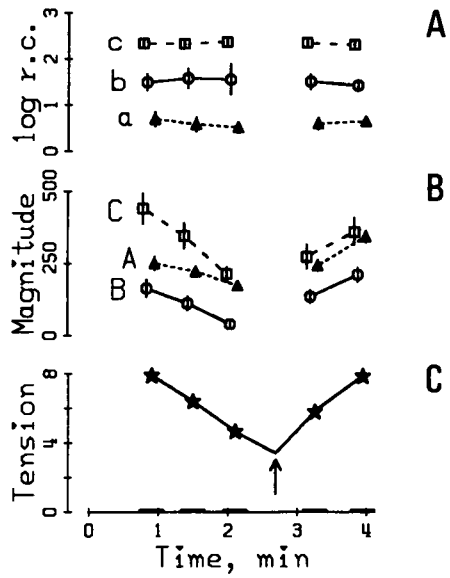

FIGURE 4 Results from one head of frog semitendinosus muscle at 8°C. Time-course of log rate constants (in A), their magnitudes (in B), and tension (in C) plotted with their confidence limits (vertical bars in A and B). Frequency range is 0.25–50 Hz. The muscle was activated by replacing 117 mM of Na with K in frog-control saline at time 0 (zero tension), and 20 mM caffeine was introduced to K saline at the arrow indicated in *C*. L_0 = 1.5 cm, diameter (approximate) = 0.14 cm, ΔL = 0.25% L_0. Units in plots are log (s^{-1}) in A, 10^3 dyn/cm in B, and 10^3 dyn in C. As in Fig. 3, horizontal lines along the abscissa indicate length of time required for each complex stiffness record.

By examination of Fig. 2 A one can identify at least three exponential rate processes and they are (A) an exponential lead (phase advance, see p. 437 of Machin, 1964, for terminology) at low frequencies, (B) an exponential lag (phase delay) at middle frequencies, and (C) another exponential lead at high frequencies. The same three components are observed in records from frog semitendinosus preparations activated by high K solution or by caffeine.

Component (B) is known as the oscillatory work or delayed tension (Pringle, 1967) in glycerinated insect muscles, and represents work production by the muscle on the oscillating driver. Fig. 2 A shows that the muscle produced net work on the forcing apparatus when the frequency of the length change was between 7–25 Hz, since the plot falls below the abscissa. This ability was lost with time (Fig. 2 B), although the muscle maintained the same tension and the other two components appear only moderately reduced in magnitude.

To demonstrate how well the complex stiffness could be described by three exponential rate processes, the data were fitted to a transfer function of the sum of three exponential terms (A, B, and C).

$$\underset{(A)}{} \qquad \underset{(B)}{} \qquad \underset{(C)}{}$$

$$Y(f) = Afi/(a + fi) - Bfi/(b + fi) + Cfi/(c + fi) + H \qquad (1)$$

TABLE I
FITTED PARAMETERS AND CONFIDENCE LIMITS

	Fig. 2 A, C	Fig. 2 B, D
Degrees of freedom	21	23
A	$246 \pm 12 \times 10^3$ dyn/cm	$135 \pm 8 \times 10^3$ dyn/cm
B	$365 \pm 18 \times 10^3$ dyn/cm	
C	$531 \pm 25 \times 10^3$ dyn/cm	$240 \pm 19 \times 10^3$ dyn/cm
H	$60 \pm 11 \times 10^3$ dyn/cm	$49 \pm 7 \times 10^3$ dyn/cm
$A - B + C + H$ ($= Y_\infty$)	$472 \pm 25 \times 10^3$ dyn/cm	$424 \pm 19 \times 10^3$ dyn/cm
$2\pi a$	$6.3 \pm 1.3\,s^{-1}$	$5.1 \pm 1.3\,s^{-1}$
$2\pi b$	$177 \pm 19\,s^{-1}$	
$2\pi c$	$503 \pm 38\,s^{-1}$	$555 \pm 60\,s^{-1}$

Parameters and their approximate 95% confidence limits are found after fitting data of Fig. 2 to Eq. 1.

where $2\pi a$, $2\pi b$, $2\pi c (a < b < c)$ are the apparent rate constants (hereafter these are referred to as the rate constants), A, B, C are the corresponding magnitudes, and H is a constant. The second term (B) is omitted in fitting the data of Fig. 2 B. Transfer functions were fit to complex stiffness data so as to minimize the sum of modulus-squared deviations in the complex plane, and calculated values of the complex stiffness are shown in Fig. 2 C and D with smooth lines. Fitted parameters and their 95% confidence limits (computed with Fisher's f-test) are summarized in Table I. The mean rate constants of intact crayfish muscle fibers are $2\pi a = 6.0 \pm 0.8$, $2\pi b = 137 \pm 16$, $2\pi c = 573 \pm 28$ (±SEM, N = 14, unit in s^{-1}) at 20°C. As seen in Fig. 2 C, D fits are generally good in the high and middle frequency ranges, and poor at low frequency. Apparently an exponential advance (first term in Eq. 1) only approximates component (A). Fig. 2 does not have experimental points above 133 Hz, but when measurements were done at lower temperatures (which shift the curve to lower frequencies), points fall along the portion of the curve extrapolated in Fig. 2 C and D.

The time courses of the rate constants, magnitudes, and tension are summarized in Fig. 3 (crayfish muscle fiber) and in Fig. 4 (frog whole muscle) together with their 95% confidence limits. Rate process (C) is observed slightly earlier than (B) and so forth, each point is plotted for the actual time of measurement. The muscles used to collect the data in these figures were activated with caffeine (15–20 mM) added to K saline to induce prolonged tension (Chiarandini et al., 1970; Orentlicher and Ornstein, 1971). As in the K activation, the oscillatory work (B) appeared early in the tension, and disappeared with time in the same tension. The disappearance of B was observed with activations in both Cl and propionate-based salines, and in tensions induced by Cl-to-propionate transients in crayfish muscle fibers. Replacing the Na of salines with the impermeant cation Tris made little difference in the experimental results. B decreased less rapidly, or not at all, when the crayfish muscle fibers were partially activated by low K concentrations.

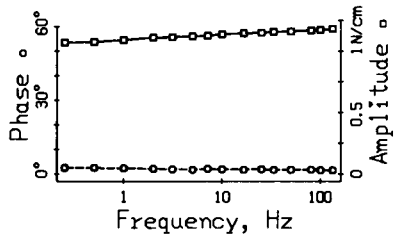

FIGURE 5 The complex stiffness $Y(f)$ of a skinned crayfish single muscle fiber in high-rigor condition is plotted against log (frequency). Phase is arg(Y), and amplitude is modulus (Y). The fiber was first placed in activating solution (3 mM $CaCl_2$, 9 mM ATP, 5 mM $MgSO_4$, 95 mM K propionate, 5 mM imidazole, pH 7.00), and then this was replaced with rigor solution (200 mM propionate, 5 mM imidazole, pH 7.00). Nine washes were made and 3 min were allowed for development of rigor before this record was obtained. Fiber dimension: L_0 = 0.36 cm, diameter (approximate) = 265 μm (measured after skinning), ΔL = 0.46% L_0. See text for further details.

Because stiffness extrapolated to infinite frequency ($Y_\infty = A - B + C + H$) paralleled tension (P) even when individual magnitudes were behaving differently with time, Y_∞ is plotted in Fig. 3 E and F. The ratio P/Y_∞ represents the instantaneous length release (in centimeters) required to bring the isometric tension to zero (from L_0 after extrapolation). The ratio was found to be 1.0 ± 0.2% L_0 (N = 7, ± 1 SD) in fully activated intact crayfish muscle fibers that developed a P_0 of 2.5 ± 0.4 × 10^6 dyn/cm^2 at 20°C. This ratio is in close agreement, within experimental error, with that of the "high rigor" of skinned crayfish muscle fibers (0.8 ± 0.1% L_0; Table II, of Kawai and Brandt, 1976). This result can be interpreted to signify that actively cycling cross-bridges in intact fibers can assume a geometrical arrangement similar to that of the high-rigor cross-bridges in skinned fibers.

To demonstrate directly that the three rate processes are not artifacts of the detecting apparatus, skinned crayfish fibers were placed in a high-rigor condition (for procedures, see Kawai and Brandt, 1976) and the complex stiffness was detected. As shown in Fig. 5, those exponential processes present in active muscles are undetectable in rigor muscles, which behave essentially as elastic bodies over the frequency range studied. This conclusion is expected since all the myosin cross-bridges are believed to be attached to actin and no head rotation of these attached bridges takes place in rigor (Dos Remidios et al., 1972) when the length is perturbed. The data in Fig. 5 are consistent with that of Heinl et al. (1974) and Kawai and Brandt (1976).

DISCUSSION

In the present work the technique of sinusoidal analysis is applied over a wide range of frequencies (0.25–133 Hz) to fully activated intact muscle preparations. We demonstrate that three exponential rate processes can describe the complex stiffness of the active myofilament system. Since these processes are not detected in resting or rigor muscles, we conclude that they are associated with the active interaction of myosin cross-bridges with actin which leads to chemomechanical energy conversion.

Our work is a significant advance over the earlier studies (e.g. Buchthal and Kaiser, 1951; Rack, 1966) that applied sinusoidal length oscillations to intact muscle preparations. Because of limitations in instrumentation, among other factors, the earlier reports could not identify rate processes as we do in our present work.

Of three apparent rate processes, (A) and (C) are exponential leads in which the muscle absorbs work from the length oscillator. (B) is the sole exponential lag in which the muscle produces work on the oscillator. The rate process (B) can serve as a marker to identify other rate processes from various muscle preparations, because there is only one exponential lag observed in the tension response of muscle to length changes. (B) is distinctive in that it has a negative polarity in Eq. 1. This negative polarity predicts a delayed tension rise (decay) on step length increase (decrease).[1] (A) can be readily identified since there is only one exponential lead which is slower than the process (B). Similarly the component (C) can be identified relative to (B) as the next faster rate process. In this way the assignment of (C) is unambiguous even if there were another exponential lead at the higher frequency range where we did not make observations (cf. Abbott, 1972).

From these considerations we conclude that the component (A) corresponds to the process studied by Hill (1953) with quick length release experiments, which he interpreted as internal rearrangement of the length segments (sarcomeres). Also, we conclude that the component (C) corresponds to the fast rate process "2" of Huxley and Simmons (1971), which they assigned to head rotation and resulting energy transduction, whereas Thorson and White (1969) assigned the component to distributed viscous interaction between thick and thin filaments.[2]

Component (B) is known as oscillatory work in glycerinated insect muscle systems (for review see Pringle, 1967; White and Thorson, 1973). We demonstrate in this report that oscillatory work is also present in intact skeletal muscles of crayfish and frog (cf. Armstrong et al., 1966). It is found in other muscle types, such as glycerinated or freeze-dried rabbit heart (Steiger, 1971), and glycerinated frog sartorius (Heinl, 1972). Thus it seems that oscillatory work is a universal property of striated muscles rather than a unique property of insect muscles.

Because of the earlier demonstrations that ATP hydrolysis rate is linearly related to power output associated with the oscillatory work in glycerinated insect muscle (Rüegg and Tregear, 1966; Steiger and Rüegg, 1969; Pybus and Tregear, 1973), one can assume that the magnitude B is linearly correlated with the rate of ATP hydrolysis. From this assumption we infer that the decline of the oscillatory work is accompanied by a decline in ATP-hydrolysis rate, while the muscle is maintained in full tension. There are three general mechanisms which could cause this phenomenon: (I) Some of the transitions in the actomyosin ATP-hydrolysis cycle (Tonomura et al. 1969; Lymn and

[1] Tension shows a plateau when magnitude B is small.

[2] We do not agree, however, with either of these interpretations of this component. We have evidence that component (C) represents MgATP binding to the myosin heads, and subsequent head dissociation, as reported by Kawai and Brandt (1975) and by Kawai and Orentlicher (1976).

Taylor, 1971; Eisenberg and Kielley, 1973) are load-sensitive, and establishment of steady tension therefore may minimize hydrolysis. Experimental evidence of such load control was reported by Kawai and Brandt (1976). (II) A myosin-hydrolysis product complex (Lymn and Taylor, 1971) ready to react with actin and abundant in resting muscle (Marston and Tregear, 1972; Marston, 1973) may become depleted with time in tension. According to Eisenberg and Kielley (1973), the formation of this product is the slowest of all the reactions in the ATP hydrolysis cycle of actomyosin, and it is possible that its supply becomes rate limiting. (III) Phosphoarginine (crayfish) or phosphocreatine (frog) may become depleted and ATP concentration drop. This might mimic the "high-tension state" described by Jewell and Rüegg (1966) and by Pringle (1967) in insect muscles. Note, however, that tension is constant or lower when *B* diminishes in our experiments (Fig. 3 and first activation of Fig. 4).

To discriminate between the above mechanisms, the following evidence is available at the present time: (*i*) If the muscle is partially relaxed in a K-induced contracture, a second activation with caffeine restores *B* as tension increases in frog muscle (Fig. 4 B). (*ii*) The rate constants *a* and *c* are invariant while *B* diminishes (Table I and first two points in Fig. 3 A; first three points in Figs. 3 B and 4 A). *b* appears to be constant (Figs. 3 B, 4 A), although its confidence limit becomes larger as the relative magnitude of *B* diminishes. We have reported that both *b* and *c* are functions of MgATP concentration (1–20 mM) in skinned crayfish (Kawai and Brandt, 1975) and rabbit psoas (Kawai and Orentlicher, 1976) muscle preparations. (*iii*) Component (B) is lost soon after K-induced contracture reaches a steady plateau, and with time the fiber completely relaxes without *B* reappearing (Fig. 3 C, E). (*iv*) The infinite frequency stiffness(Y_∞) does not increase significantly (Fig. 3 E, F) as *B* diminishes; thus we do not detect an increase in the attached cross-bridge population. (*v*) A similar decrease of *B* with time in tension is observed in the presence of an ATP-regenerating system (17mM phosphocreatine, 64 U/ml phosphocreatine kinase; Sigma Chemical Co., St. Louis, Mo.) in skinned crayfish muscle fibers with Ca activation (Kawai, unpublished observation).

No clear discrimination between mechanisms (I) and (II) follows from these observations, but there is no support for (III). Although decrease in the MgATP supply thus seems unlikely as the cause of the decrease in *B*, further experimentation is required for a definitive conclusion. These experiments could be carried out on skinned muscle fiber preparations (Natori, 1954; Reuben et al., 1971) where one can control such factors in the millieu of the myofilaments as the Ca, MgATP, inorganic phosphate, and so forth, or perhaps by the technique of quick freeze and analysis for phosphate compounds (Gilbert et al., 1971).

The decrease in oscillatory work magnitude *B* with time may correlate with other time-dependent changes in active muscle, such as the decrease in shortening heat with closely spaced test activations (Dickinson and Woledge, 1974). It, like the Fenn effect (Fenn, 1923), may result in energy conservation although its mechanism must differ since it varies with time at a constant force. The Fenn effect depends only on the

fractional load (Brandt and Orentlicher, 1972), at least for relatively short times in tension.

Thus an interesting question yet to be resolved is why oscillatory work is transient in the intact crayfish and frog muscles. Perhaps crayfish and frog muscles are not adapted to uninterrupted oscillatory work and the label "phasic fibers" may have an added significance.

We thank Professors John P. Reuben and Harry Grundfest for many helpful discussions and constant encouragement of the present work, and Mr. Sidney Steinberg and Dr. George M. Katz for their assistance in electronics.

This work is supported in part by grants from the National Science Foundation (PCM76-00441); National Institutes of Health (NS 11766, NS 05910, and HL 16082); and the Muscular Dystrophy Associations of America Inc., to the H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related Diseases.

Received for publication 22 September 1976 and in revised form 21 December 1976.

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