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STIFFNESS CHANGES IN FROG SKELETAL MUSCLE DURING CONTRACTION RECORDED USING ULTRASONIC WAVES

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SUMMARY

1. A technique has been developed with which the stiffness changes in frog skeletal muscle can be continuously recorded by measuring the propagation velocity of ultrasonic waves (3–7 MHz) with negligibly small perturbations to the contractile system.

2. The resting muscle stiffness was $2{\cdot}256 \pm 0{\cdot}002 \times 10^9$ N/m^2 (S.D.) at 1–2 °C ($n = 10$) and $2{\cdot}480 \pm 0{\cdot}007 \times 10^9$ N/m^2 at 19–20 °C ($n = 12$) in the longitudinal direction, and $2{\cdot}223 \pm 0{\cdot}008 \times 10^9$ N/m^2 at 1–2 °C ($n = 8$) and $2{\cdot}437 \pm 0{\cdot}007 \times 10^9$ N/m^2 at 19–20 °C ($n = 9$) in the transverse direction.

3. The resting muscle stiffness measured with ultrasonic waves was virtually insensitive to the resting force development, i.e. the extension of the parallel elastic component.

4. The longitudinal muscle stiffness increased during isometric contraction at a rate faster than the force development. The amount of increase of the longitudinal stiffness in an isometric tetanus at 2·2 μm sarcomere length was $2{\cdot}4 \pm 0{\cdot}1 \times 10^7$ N/m^2 at 1–2 °C ($n = 10$) and $6{\cdot}5 \pm 1{\cdot}3 \times 10^7$ N/m^2 at 19–20 °C ($n = 12$).

5. On the other hand, the transverse muscle stiffness decreased during isometric contraction at a rate faster than the force development. The amount of decrease of the transverse stiffness in an isometric tetanus at 2·2 μm sarcomere length was $5{\cdot}6 \pm 0{\cdot}1 \times 10^7$ N/m^2 at 1–2 °C ($n = 8$) and $6{\cdot}4 \pm 0{\cdot}3 \times 10^7$ N/m^2 at 19–20 °C ($n = 9$).

6. The amount of both the longitudinal and the transverse stiffness changes during an isometric tetanus decreased linearly with increasing sarcomere length, indicating that the stiffness changes during contraction reflect the formation of cross-links between the myofilaments.

7. Both the longitudinal and the transverse stiffness increased when resting muscle was put into rigor state. The rigor muscle stiffness was insensitive to small stretches, i.e. the strain of the rigor cross-links.

8. These results are discussed in connection with the behaviour of cross-bridges during isometric contraction and in rigor.

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INTRODUCTION

Although it is generally accepted that the contraction in striated muscle results from cyclic formation of cross-links between the projections on the thick filaments, i.e. the cross-bridges, and the sites on the thin filaments (Huxley, 1957; Huxley, 1960), the mode of operation of the cross-bridges to produce force and motion still remains a matter for debate and speculation. In the contraction model of Huxley (1957), muscle stiffness is taken as a measure of the number of cross-bridges attached to the thin filaments at any one moment. Thus, to investigate the behaviour of the cross-bridges at various stages of muscle contraction, muscle stiffness changes have been studied by applying step or sinusoidal length changes and measuring the resulting force changes (e.g. Julian & Sollins, 1975; Ford, Huxley & Simmons, 1977) or measuring the propagation of longitudinal mechanical waves (Schoenberg, Wells & Podolsky, 1974; Truong, 1974).

To measure muscle stiffness changes during contraction with negligibly small perturbations to the contractile system and a high time resolution, we have developed a technique in which the stiffness changes can be continuously recorded by measuring the propagation velocity of ultrasonic waves applied to frog skeletal muscle in both the longitudinal and the transverse directions. Using this technique, we have found the unexpected phenomenon that, during isometric contraction of frog skeletal muscle, stiffness decreases in the transverse direction, though it increases in the longitudinal direction (Tamura, Hatta, Matsuda, Sugi & Tsuchiya, 1982). This paper is concerned with detailed features of the stiffness changes in frog skeletal muscle during isometric contraction and in rigor.

METHODS

Experimental arrangement. Figure 1 shows the experimental arrangement for measuring muscle stiffness in the longitudinal and the transverse directions. For the longitudinal stiffness measurement, the semitendinosus muscle (M) of bull-frog (*Rana catesbeiana*) was held between two stainless-steel cylinders (R, diameter 2·6 mm), and bent around the cylinders near both ends. One end of the muscle was firmly fixed to the lucite block (C), while the other end was connected to a strain gauge (F, Shinko type UT, compliance 0·1 mm/N, resonance frequency 980 Hz). A pair of ceramic piezoelectric transducers (P and P′), each of which was a circular plate (8 mm diameter) of $PbZrO_3$–$PbTiO_3$ (Berlincourt, Curran & Jaffe, 1964), were put in contact with the muscle at both bent end portions in such a way that their planes were perpendicular to the muscle long axis. The distance between the two transducers was 2–2·5 cm. One transducer was repetitively energized with brief trains of sinusoidal voltages (3–7 MHz) from a function generator (Wavetek, type 162) to transmit brief trains of ultrasonic waves to the muscle. The wave trains propagated longitudinally through the muscle to be sensed by another transducer (Fig. 1*A*).

For the transverse stiffness measurement, the sartorius muscle (M) of bull-frog was placed with its lower surface in contact with the bottom of a lucite chamber (G): its pelvic end was firmly fixed between the lucite block (C) and the chamber, while its tibial end was connected to the strain gauge (F). A ceramic transducer (P) was in contact with the upper muscle surface, so that its plane was in parallel with the bottom of the chamber, which served as a reflecting plane of ultrasonic waves. The distance between the transducer and the chamber was 1·5–2 mm. The ultrasonic wave trains from the transducer propagated across the muscle, and reflected by the chamber to be sensed by the same transducer as trains of echo waves (Fig. 1*B*).

In both cases, the muscle length was varied with a manipulator carrying the strain gauge. Since the sarcomere length as measured by light diffraction with He–Ne laser light was fairly uniform

along the length of resting muscle except for the regions close to the tendons, the sarcomere length around the middle of the muscle was normally taken as the representative value. The muscle was constantly soaked in Ringer solution, which had the following composition (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; pH adjusted to 7·2 by $NaHCO_3$. It was stimulated to contract isometrically with single or repetitive (20–50 Hz) 1 ms supramaximal current pulses given through six platinum wire electrodes connected as alternate anodes and cathodes. In our previous experiments (Tamura *et al.* 1982), the chamber was drained before the stiffness measurement. In the present experiments, the

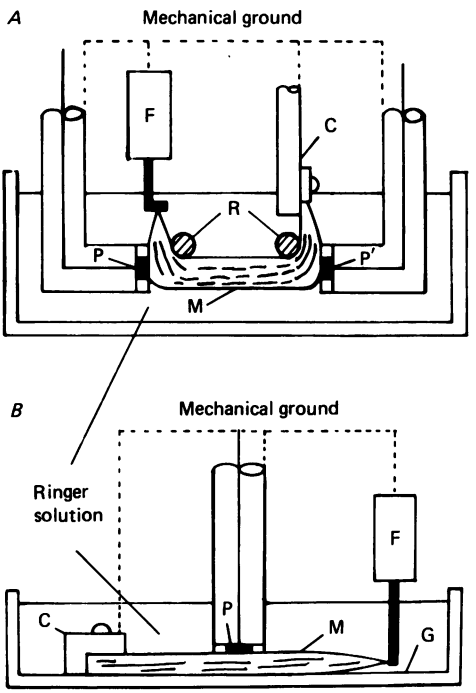

Fig. 1. Experimental arrangement for muscle stiffness measurement during isometric contraction. *A*, longitudinal stiffness measurement. *B*, transverse stiffness measurement. In both *A* and *B*, the muscle was soaked in Ringer solution, and was stimulated maximally with current pulses through six platinum wire electrodes (not shown). For further explanation, see text.

stiffness measurement was performed with muscles kept in Ringer solution. The internal shortening of the muscle during an isometric tetanus against the tendons and the recording system was less than 1·5% of the muscle length. The temperature of Ringer solution was controlled by an thermoelectric device (Yamato, Coolnics) with an accuracy of ±0·1 °C. After the experiments, the tendons were transected near the ends of the muscle fibres, and the muscle was blotted and weighed. The maximum isometric force per unit cross-sectional area was calculated as $P_0 l_0/W$, where P_0, l_0 and W are the maximum tetanic force, muscle length at a sarcomere length of 2·2 μm and muscle weight respectively. The values of $P_0 l_0/W$ ranged from 0·2 to 0·25 N/mm^2.

When the muscle was put into rigor state, it was first kept in Ringer solution containing 0·4 mM-iodoacetic acid for 90 min, and then stimulated to contract at 12 contractions/min until no mechanical responses were detectable (Mulvany, 1975).

Measurement of muscle stiffness. When mechanical waves with a constant frequency, f, propagate in muscle, their phase velocity, V_f, is determined by muscle stiffness, C_f, as:

$$V_f = (C_f/\rho)^{\frac{1}{2}}, \quad (1)$$

where ρ is the density of muscle. Assuming that muscle density remains unchanged during

contraction, muscle stiffness can be calculated from eqn (1), provided the measured velocities can be regarded as the phase velocities. To satisfy this condition, it is necessary to use the range of high enough frequencies where the frequency dependence of the propagation velocities is no longer obvious. As the propagation velocity did not change appreciably with waves of 3–7 MHz at a constant temperature (Hatta, Tamura, Matsuda, Sugi & Tsuchiya, 1984), the measured velocities in this frequency range are equal to the phase velocities. In the case of the longitudinal propagation velocities, the wavelength λ should also satisfy the condition, $a/\lambda > 2{\cdot}5$, where a is the muscle radius (Yu, Brennan & Sauer, 1955); otherwise, the waves propagate in muscle with mixed modes

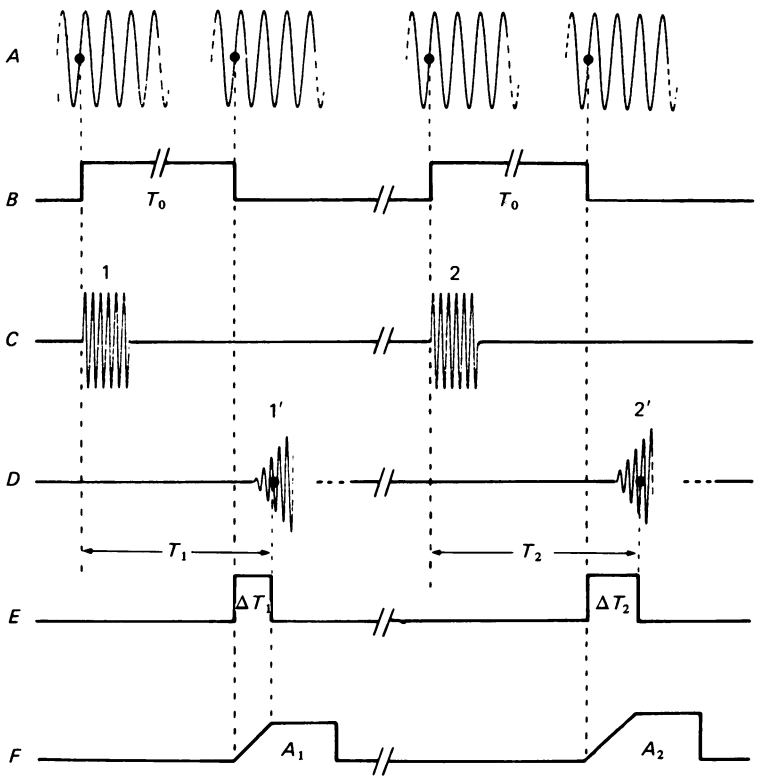

Fig. 2. Diagram illustrating the principle for recording the relative wave velocity changes. *A*, continuous 1 MHz sinusoidal waves from the synthesizer. *B*, rectangular T_0 pulses produced from the 1 MHz waves by a programmable divider. *C*, brief trains of original 3–7 MHz sinusoidal waves from the function generator. *D*, corresponding trains of transmitted waves. *E*, rectangular ΔT pulses generated by the flip-flop circuit. *F*, amplitude signals produced by integrating ΔT pulses. For further explanation, see text.

to make the propagation velocity complicated. In the present study, the semitendinosus muscle was nearly circular in cross-section with a radius of about 2·5 mm, while the wavelengths of 3–7 MHz waves were about 0·5–0·2 mm. Moreover, the conclusion of Yu *et al.* (1955) was obtained from the wave velocity measurement along metal rods in air. As the present wave velocity measurements were made with muscles kept in Ringer solution, the critical value of a/λ would actually be much lower than 2·5.

The method of recording the relative changes in the wave velocity was essentially the same as the time-to-amplitude converter method described by Odru, Riou, Vacher, Deterre, Peguin & Vanoni (1978) and Nakajima, Tanaka, Shimazaki, Yamanaka, Kinoshita & Wada (1979). The principle of the method is illustrated in Fig. 2. First, sinusoidal voltages of 1 MHz were

continuously generated by a synthesizer (Rockland, type 5100) in which the frequency of the output voltages could be varied over 9·5 figures (*A*). Then, rectangular pulses of a constant duration, T_0, were repetitively produced from the 1 MHz waves by a programmable divider at an appropriate interval (*B*). The T_0 pulse was around 15 and 1·5 μs for the longitudinal and the transverse stiffness measurements respectively. The accuracy of the T_0 pulse was checked by a frequency counter (Takeda-Riken, type TR5822), and was found to be less than 0·01 μs. At the beginning of each T_0 pulse, brief trains of sinusoidal waves (3–7 MHz) were generated by the function generator to transmit ultrasonic waves to the muscle through the ceramic transducer (*C*). The transmitted wave signals were sensed by the ceramic transducer, and fed to a voltage comparator after amplification with a video-amplifier with bandwidth capacity of DC to 40 MHz (*D*). The output of the comparator was further fed to a flip-flop circuit.

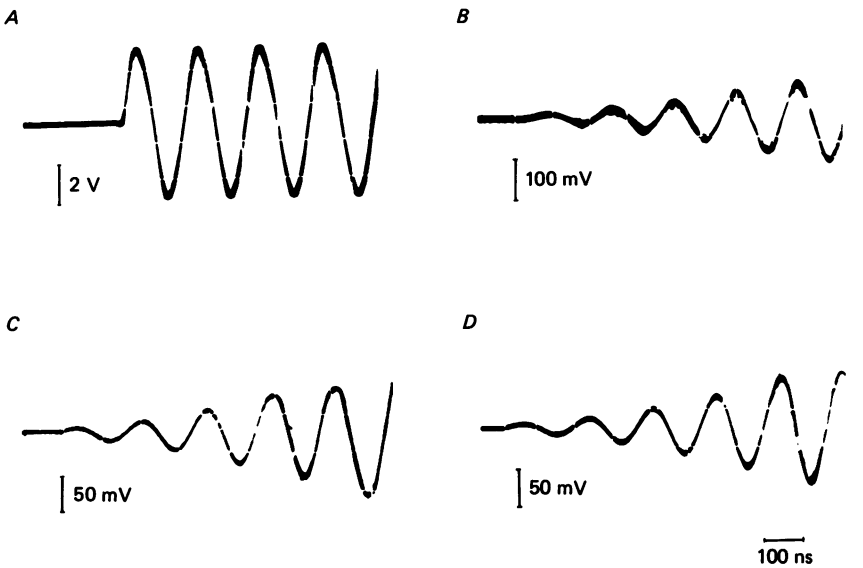

Fig. 3. Oscillographic records showing the early part of the 6 MHz sinusoidal waves from the function generator (*A*) and the transmitted waves in resting muscle (*B*), in isometrically tetanized muscle (*C*) and in pure water (*D*). The waves were transmitted in the muscle in the transverse direction. Temperature, 20 °C.

To measure the relative wave velocity changes, short rectangular pulses, ΔT, of less than 200 ns were generated by the flip-flop circuit (*E*); each ΔT pulse was initiated at the end of the T_0 pulse, and terminated not at the onset of the first wave of the transmitted wave train, but at the mid-point of the falling phase of the more distinct second or third wave (*D*). By the above method, it was possible to accurately measure the relative wave velocity changes independent of the changes in the degree of wave attenuation in the muscle during contraction. The overall limit of accuracy of the relative wave velocity change measurement was $\pm$0·05 m/s. The ΔT pulse was integrated by a time-to-amplitude converter to obtain an amplitude signal A proportional to the time ΔT (*F*). When the wave velocity changed with time to give values T_1, T_2..., the corresponding amplitude signals A_1, A_2... were successively recorded in a digital memoryscope (Hitachi, type VC 801), and displayed on a chart recorder together with the isometric force (see Fig. 6).

Figure 3 shows examples of oscilloscope records of the early part of the original sinusoidal wave train from the function generator (*A*) and the transmitted wave trains in resting muscle (*B*), in isometrically tetanized muscle (*C*) and in pure water (*D*). The amplitude of the transmitted waves was not uniform, but increased with time for the first several waves, reflecting the time course of resonance vibration of the ceramic transducer. The attenuation of the transmitted signals in resting muscle was roughly estimated by comparing the maximum amplitude of the transmitted wave

trains in the muscle with the corresponding value in pure water assuming that the attenuation of the waves is −0·1 dB/cm in pure water, and was found to be −10 dB/cm with 6 MHz wave at 20 °C. This value agrees well with that reported by Nassiri, Nicholas & Hill (1979), who measured the attenuation of megahertz waves in beef muscle. During an isometric tetanus, the attenuation of the transmitted signals in the muscle further increased by −3 dB/cm at 20 °C. The late part of the oscilloscope records consisted not only of the wave signals transmitted in the muscle, but also the delayed signals transmitted through the Ringer solution outside the muscle, though these delayed signals did not affect the wave velocity measurement.

As the method illustrated in Fig. 2 only measures the relative wave velocity changes, the absolute wave velocities were determined under various conditions by measuring the interval between the mid-point of the rising phase of the second or the third wave in the original wave train and the corresponding point in the transmitted wave train on a digital oscilloscope (Tektronix, type 2445A).

At the end of each experiment, the muscle was removed from the experimental chamber, and the value of T in pure water was measured with the ceramic transducers kept at the same positions. Thus, the distance of propagation of ultrasonic waves was calculated using the published values of the ultrasonic velocity in pure water (Greenspan & Tschiegg, 1957). The values obtained in this way agreed well with those by direct measurement with a micrometer, except that the accuracy was improved tenfold in the former. In the case of the transverse velocity measurement, the wave velocities measured were not significantly affected by small changes in the position of the ceramic transducer, altering the degree of compression of the muscle. Thus, the overall limit of accuracy of the absolute wave velocity measurement was ±2 m/s for the longitudinal direction and ±5 m/s for the transverse direction.

On the other hand, the time resolution of the wave velocity changes was determined by the frequency of repetition of ultrasonic wave trains given to the muscle, being 250 μs for the longitudinal velocity measurement and 30 μs for the transverse velocity measurement. The magnitude of perturbation produced in muscle by the applied ultrasonic waves was about 0·1 nm, being negligibly small compared to the perturbations by the ordinary step and sinusoidal length changes in the kilohertz region.

RESULTS

Resting muscle stiffness

Effect of temperature. Figure 4 shows a typical example of the effect of temperature on the ultrasonic wave velocity in resting muscles at their slack length (sarcomere length 2·0–2·2 μm). The wave velocity increased with increasing temperature from 0 to 25 °C in both the longitudinal and the transverse directions. The wave velocity in pure water also showed a temperature dependence similar to that in resting muscles, so that the wave velocity in resting muscles was higher than that in pure water by a constant amount (about 4 %) irrespective of temperatures examined. The longitudinal wave velocity in resting muscle was $1476{\cdot}4 \pm 0{\cdot}5$ m/s (S.D.) ($n = 10$) at 1–2 °C and $1548 \pm 2{\cdot}0$ m/s ($n = 12$) at 19–20 °C, while the transverse propagation velocity was $1465 \pm 2{\cdot}7$ m/s ($n = 8$) at 1–2 °C and $1534{\cdot}6 \pm 2{\cdot}0$ m/s ($n = 9$) at 19–20 °C. The values of resting muscle stiffness were calculated from eqn (1) assuming that ρ was 1·035 g/ml (Truong, 1974). The longitudinal stiffness was $2{\cdot}256 \pm 0{\cdot}002 \times 10^9$ N/m^2 (S.D.) 1–2 °C ($n = 10$) and $2{\cdot}480 \pm 0{\cdot}007 \times 10^9$ N/m^2 at 19–20 °C ($n = 12$), while the transverse stiffness was $2{\cdot}223 \pm 0{\cdot}008 \times 10^9$ N/m^2 at 1–2 °C ($n = 8$) and $2{\cdot}437 \pm 0{\cdot}007 \times 10^9$ N/m^2 at 19–20 °C ($n = 9$). Thus, the longitudinal stiffness was significantly larger than the transverse stiffness ($P < 0{\cdot}01$).

Effect of sarcomere length. Figure 5 illustrates a typical result of the experiments in which the ultrasonic wave velocity and the resting force were measured at various sarcomere lengths. It was found that, in spite of the resting force development as a

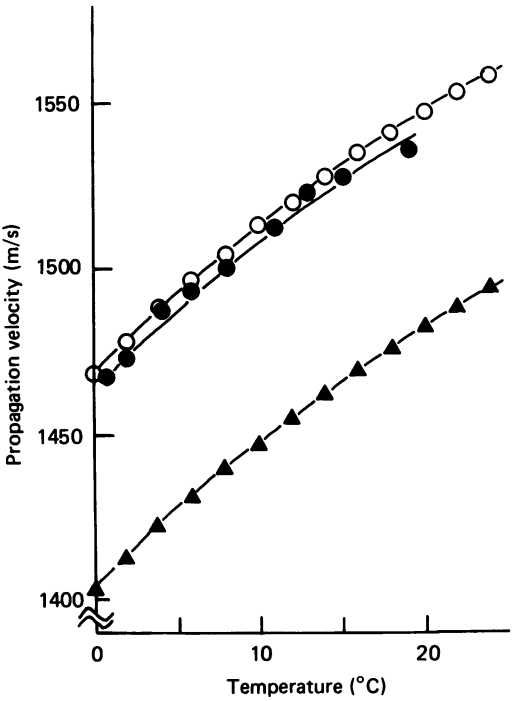

Fig. 4. Effect of temperature on the ultrasonic wave velocity in resting muscle in the longitudinal (○) and the transverse (●) directions. The semitendinous and the sartorius muscles were used for the longitudinal and the transverse wave velocity measurements respectively. The wave velocity in pure water (▲) is also shown.

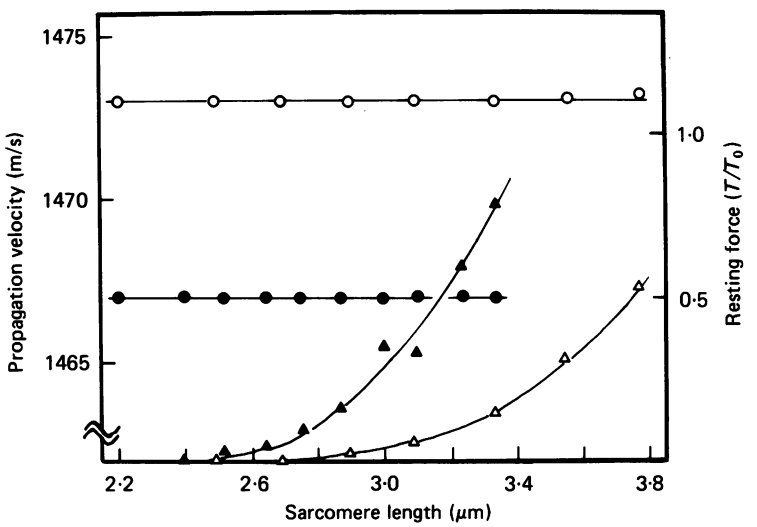

Fig. 5. Effect of sarcomere length on the propagation velocity of ultrasonic waves in resting muscle in the longitudinal (○) and the transverse (●) directions and on the resting force in the semitendinosus muscle (△) used for the longitudinal wave velocity measurement and in the sartorius muscle (▲) used for the transverse wave velocity measurement. The resting forces (T) are expressed relative to the maximum isometric tetanic force (T_0) at 2·2 μm sarcomere length. Temperature, 1 °C.

result of progressive stretching of resting muscles, the wave velocity did not change appreciably in both the longitudinal and the transverse directions in the range of sarcomere lengths from 2·2 to 3·4 μm. It was not possible to stretch the sartorius muscles beyond 3·4 μm sarcomere length because of the tendency of the tibial tendon to rupture. If, on the other hand, the semitendinosus muscles were stretched beyond 3·4 μm, the longitudinal wave velocity appeared to increase slightly with increasing resting force and stiffness; in Fig. 5, the longitudinal wave velocity at 3·8 μm sarcomere length is higher than that at sarcomere lengths below 3·4 μm by about 0·2 m/s, while the resting force at 3·8 μm sarcomere length is more than half of the maximum tetanic force T_0 with a slope of about $T_0/0{\cdot}8$ μm. This suggests that the longitudinal wave velocity reflects the resting muscle stiffness, though the effect is just barely detectable only at 3·8 μm sarcomere length, and was far smaller than the wave velocity changes during isometric contraction to be described later. Thus, these results indicate that the resting muscle stiffness measured with ultrasonic waves is virtually insensitive to the resting force development, i.e. the extension of the parallel elastic component.

Muscle stiffness changes during contraction

Time course of the stiffness changes. Since it is the bulk properties of muscle that are measured with ultrasonic waves, it is the stiffness changes during contraction that are relevant to the contractile events. Attention was therefore focused on the time course and the amount of the longitudinal and the transverse stiffness changes during isometric contraction. Typical records of the changes in the longitudinal wave velocity during an isometric twitch and a tetanus are shown in Fig. 6*A* and *B*. During a twitch, the wave velocity started to increase prior to the beginning of the isometric force development, and reached a maximum while the twitch force still continued to rise (*A*). During a tetanus, the wave velocity reached a steady maximum level which was maintained until the beginning of relaxation (*B*). The amount of increase of the longitudinal wave velocity above the resting value in a 1–1·5 s tetanus at 2·2 μm sarcomere length was $7{\cdot}8 \pm 0{\cdot}5$ m/s at 1–2 °C ($n = 10$) and $20{\cdot}0 \pm 4{\cdot}0$ m/s at 19–20 °C ($n = 12$). These values corresponded with an increase of the longitudinal stiffness of $2{\cdot}4 \pm 0{\cdot}1 \times 10^7$ N/m^2 at 1–2 °C ($n = 10$) and $6{\cdot}5 \pm 1{\cdot}3 \times 10^7$ N/m^2 at 19–20 °C ($n = 12$).

Figure 6*C* and *D* shows the records of the changes in the isometric force and the transverse wave velocity during an isometric twitch and a tetanus. In contrast with the longitudinal wave velocity, the transverse wave velocity started to decrease prior to the beginning of the isometric force development, and reached a minimum while the twitch force still continued to rise (*C*). During a tetanus, the wave velocity reached a steady minimum value until the beginning of relaxation (*D*). The amount of decrease of the transverse wave velocity below the resting value in a 1–1·5 s tetanus was $18{\cdot}6 \pm 0{\cdot}6$ m/s at 1–2 °C ($n = 8$) and $20{\cdot}3 \pm 1{\cdot}2$ m/s at 19–20 °C ($n = 9$). These values indicated a decrease of the transverse stiffness of $5{\cdot}6 \pm 0{\cdot}1 \times 10^7$ N/m^2 at 1–2 °C ($n = 8$) and $6{\cdot}4 \pm 0{\cdot}3 \times 10^7$ N/m^2 at 19–20 °C ($n = 9$).

Typical examples of relative stiffness and force changes during isometric contraction are shown in Figs 7 and 8. Both the longitudinal and the transverse stiffness started to change 10–20 ms before the beginning of the isometric force

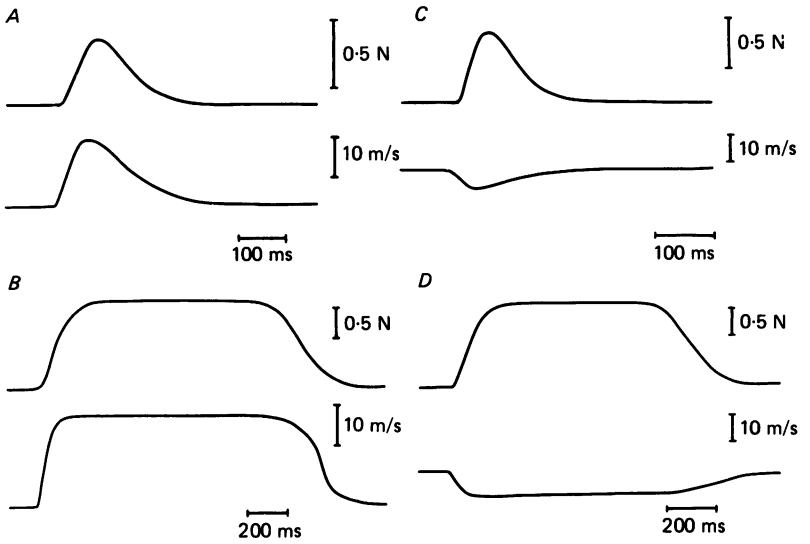

Fig. 6. Simultaneous recordings of the force (upper traces) and the ultrasonic wave velocity (lower traces) changes during contraction. The upward and downward deflections of the velocity trace indicate an increase and a decrease of the ultrasonic wave velocity respectively. *A* and *B*, changes in the longitudinal wave velocity during an isometric twitch (*A*) and an isometric tetanus (*B*). *C* and *D*, changes in the transverse wave velocity during an isometric twitch (*C*) and an isometric tetanus (*D*). Note that the transverse velocity decreases during the mechanical activity. Temperature, 20 °C.

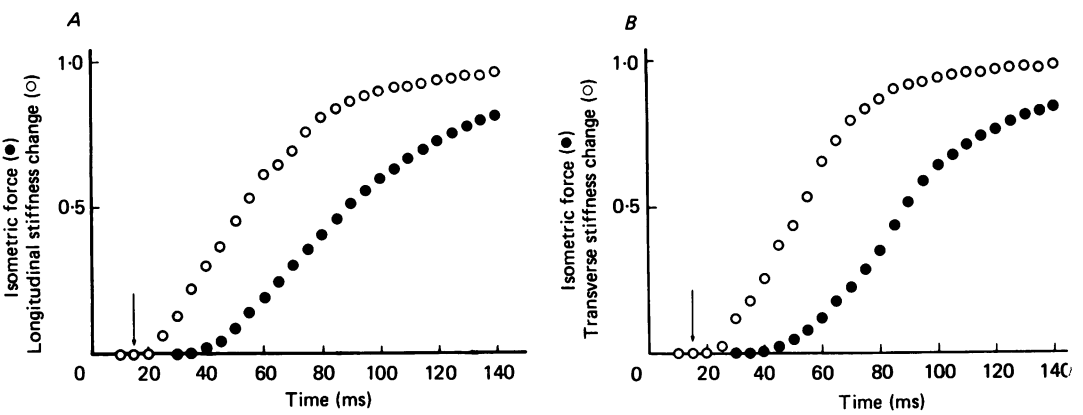

Fig. 7. Relative stiffness and force changes during the rising phase of isometric tetani. In Figs 7 and 8, the values of stiffness changes annd isometric force are expressed relative to their maximum values in a 1 s tetanus. *A*, the force (●) and the longitudinal stiffness (○) changes. *B*, the force (●) and the transverse stiffness (○) changes. Arrows indicate time of onset of stimulation. The data points are obtained from experiments different from those in Figs 6 and 8. Temperature, 20 °C.

development, and the half-maximum stiffness change in a tetanus was reached 25–40 ms ahead of the half-maximum isometric force at 19–20 °C (Fig. 7*A* and *B*). Although the force developed more quickly at the higher temperature, the interval between the half-rise-time of the stiffness and that of the isometric force development was not markedly affected by temperature, being 30–50 ms at 1–2 °C. As shown in

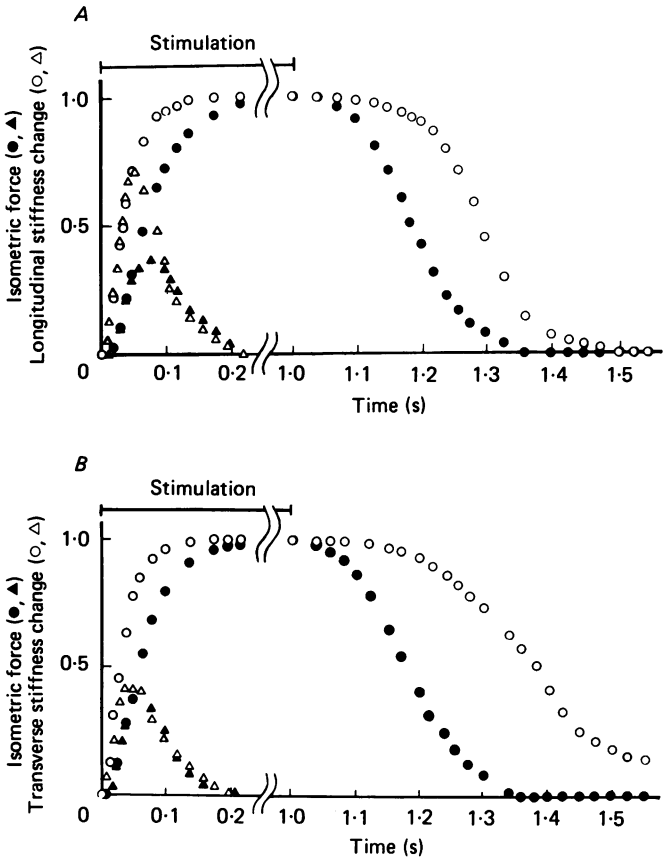

Fig. 8. Relative stiffness and force changes during isometric twitches and tetani. *A*, the force (●, ▲) and the longitudinal stiffness (○, △) changes. *B*, the force (●, ▲) and the transverse stiffness (○, △) changes. The data points are obtained from experiments different from those in Figs 6 and 7. Temperature, 20 °C.

Fig. 8*A*, the peak longitudinal stiffness change in a twitch at 19–20 °C amounted to more than 70% of the maximum stiffness change in a tetanus, and was appreciably larger than the corresponding twitch force relative to the tetanic force (40–50%). On the other hand, the peak transverse stiffness change in a twitch relative to the maximum change in a tetanus was almost equal to the peak twitch force relative to the tetanic force (Fig. 8*B*). During the relaxation phase of a tetanus, both the longitudinal and the transverse stiffness returned to the resting value at a rate much slower than the decay of isometric force (Fig. 8*A* and *B*).

Effect of sarcomere length. To ascertain whether the above longitudinal and trans-

verse stiffness changes during isometric contraction are relevant to the behaviour of the cross-bridges, the stiffness measurements during 1–1·5 s isometric tetani were performed at various sarcomere lengths from 2·2 to more than 3·6 μm in the semitendinosus muscles and from 2·2 to 3·4 μm in the sartorius muscles. In each preparation, the sarcomere length was varied in a random order. The results obtained are summarized in Figs 9 and 10. As the elongation of sarcomere length

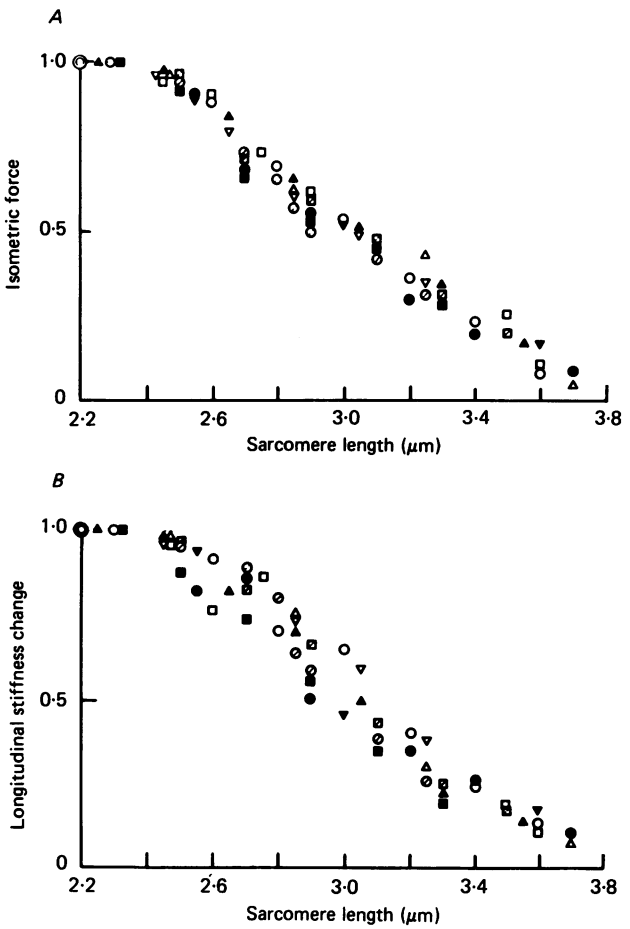

Fig. 9. Effect of sarcomere length on the isometric tetanic force and the amount of longitudinal stiffness change. Peak isometric forces in 1–1·5 s tetani and the absolute values of the corresponding stiffness changes are plotted against sarcomere lengths. Different symbols represent data points from different muscles. Temperature, 1–2 °C.

causes not only a decrease in the amount of overlap between the filaments, but also a decrease in the cross-sectional area of the muscle to result in an increase in the number of the cross-bridges per unit cross-sectional area, the values of the maximum stiffness change in an isometric tetanus were corrected by multiplying them by A/A_0, where A_0 is the cross-sectional area of the muscle at a sarcomere length of 2·2 μm and A is the cross-sectional area of the muscle at stretched sarcomere length. The A/A_0 values were calculated assuming a constant muscle volume. It was found that

both the isometric forces and the absolute values of the corresponding stiffness changes decreased linearly with increasing sarcomere lengths, approaching to zero at a sarcomere length of about 3·8 μm in the case of the longitudinal stiffness changes

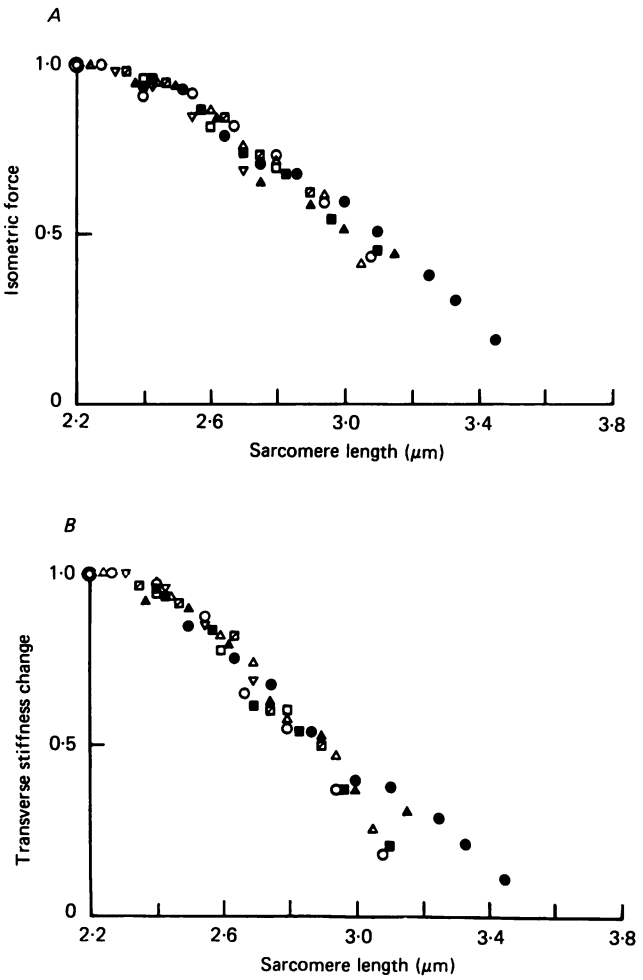

Fig. 10. Effect of sarcomere length on the isometric tetanic force and the absolute amount of transverse stiffness change. Peak isometric forces attained in 1–1·5 s tetani and the corresponding stiffness changes are plotted against sarcomere lengths. Both the isometric force and the amount of stiffness change are expressed relative to their maximum values at 2·2 μm sarcomere length. Different symbols represent data points obtained from different muscles. Temperature, 1–2 °C.

(Fig. 9) and about 3·6 μm in the case of the transverse stiffness changes (Fig. 10). This may be taken to indicate that both the increase of the longitudinal stiffness and the decrease of the transverse stiffness during isometric contraction reflect the formation of the cross-links between the myofilaments.

Rigor muscle stiffness

When the muscle was put into rigor state (sarcomere length 2·2–2·4 μm), both the longitudinal and the transverse propagation velocities increased above the resting value. The increase of the longitudinal wave velocity was $7{\cdot}0 \pm 1{\cdot}0$ m/s at 1–2 °C ($n = 3$) and $13{\cdot}0 \pm 4{\cdot}0$ m/s at 19–20 °C ($n = 10$), while the increase of the transverse wave velocity was $11{\cdot}3 \pm 1{\cdot}1$ m/s at 1–2 °C ($n = 4$) and $13{\cdot}0 \pm 4{\cdot}0$ m/s at 19–20 °C ($n = 7$). The increase of the longitudinal stiffness above the resting value was calculated to be $2{\cdot}2 \pm 0{\cdot}3 \times 10^7$ N/m^2 at 1–2 °C ($n = 3$) and $4{\cdot}2 \pm 1{\cdot}3 \times 10^7$ N/m^2 at 19–20 °C ($n = 10$), whereas the increase of the transverse stiffness was estimated to be $3{\cdot}5 \pm 0{\cdot}3 \times 10^7$ N/m^2 at 1–2 °C ($n = 4$) and $4{\cdot}2 \pm 1{\cdot}3 \times 10^7$ N/m^2 at 19–20 °C ($n = 7$).

The ultrasonic wave velocity in rigor muscles remained virtually unchanged when they were stretched by 1–2%, though the stretch produced a steady force as large as the maximum tetanic force. This seems to indicate that the muscle stiffness measured with ultrasonic waves is insensitive not only to the strain of the parallel elastic component, but also to the strain of the cross-links between the thick and thin filaments, as a small stretch of rigor muscles would be largely taken up by the strain of the rigor linkages.

DISCUSSION

Characteristic features of the muscle stiffness measurement with ultrasonic waves

In the ordinary muscle stiffness measurement with step or sinusoidal length changes in the kilohertz region, the resting muscle stiffness at the slack length is negligibly small compared to the stiffness during contraction, reflecting the fact that the thick and thin filaments readily slide past each other in resting muscle. On the other hand, the muscle stiffness measurement with 3–7 MHz ultrasonic waves in the present study is essentially the measurement of the wave velocity as based on eqn (1). Thus, the resting muscle stiffness measured in the present experiments depends principally on the bulk modulus of elasticity of the myoplasm, which is shown to be about 4% larger than that of pure water at 0–25 °C (Fig. 4). The resting muscle stiffness was significantly larger in the longitudinal direction than in the transverse direction, probably reflecting the longitudinal array of the myofilaments. The contribution of the resting force development to the longitudinal ultrasonic wave velocity was detectable only when the muscle was stretched to 3·8 μm sarcomere length (Fig. 5), so that the resting muscle stiffness was virtually insensitive to the extension of the parallel elastic component. In contrast with this, the resting muscle stiffness measured with kilohertz waves increases markedly with increasing resting force (Truong, 1974).

In the present study, we focused our attention on the amount of stiffness change taking place during contraction, which was obtained by subtracting the resting stiffness, i.e. the apparent bulk modulus of elasticity of the myoplasm, from the measured effective stiffness of contracting muscle. The validity of the above procedure in obtaining information about the contractile events seems to be proved by the linear relation between the amount of stiffness change during contraction and the amount of overlap between the thick and thin filaments (Figs 9 and 10) and also

by the agreement in the longitudinal stiffness values during contraction obtained by us and other investigators, as will be mentioned later.

General agreement in the longitudinal stiffness values during contraction obtained with different methods

The longitudinal stiffness of frog skeletal muscle or muscle fibre during an isometric tetanus has been measured by some investigators. With 300 Hz sinusoidal vibration, Mason (1978) reported a value of 2×10^7 N/m^2 (4 °C), and Hasan & Mason (1978) obtained a value of $3{\cdot}1 \times 10^7$ N/m^2 (5 °C) using propagation of 10 kHz waves. By measuring the propagation velocity of 500 Hz waves, Schoenberg *et al.* (1974) reported a value of 3×10^7 N/m^2 (6 °C). Also measuring the 3 kHz wave velocity, Truong (1974) obtained a value of 2×10^7 N/m^2 (25 °C). We also calculated a stiffness value of $6{\cdot}3 \times 10^7$ N/m^2 (2·5 °C) from the slope of the T_1 curve in the step length change experiments of Ford *et al.* (1977). These stiffness values are of the same order of magnitude as that of the increase of the longitudinal stiffness obtained in the present study ($2{\cdot}4 \times 10^7$ N/m^2 at 1–2 °C and $6{\cdot}5 \times 10^7$ N/m^2 at 19–20 °C). The above agreement in the longitudinal stiffness values in isometrically tetanized muscle or muscle fibre indicates that all the methods are measuring the same phenomenon taking place during contraction, and suggests that the cross-links between the thick and thin filaments behave as a pure elastic component exhibiting almost constant stiffness values over a wide frequency range from 300 Hz to 7 MHz.

In accordance with this view, the increase of the longitudinal stiffness leading to the isometric force development (Figs 6–8) has also been observed by use of perturbations in the kilohertz region (Mason & Hasan, 1980; Cecchi, Griffiths & Taylor, 1984; Ford, Huxley & Simmons, 1986). The change in the intensity ratio of the 1·0 and 1·1 equatorial reflections from frog skeletal muscle is generally taken to result from the radial movement of the cross-bridges towards the thin filaments, and is also known to lead the force development (Huxley, 1975; Matsubara & Yagi, 1978; Amemiya, Sugi & Hashizume, 1979). Recently, it has been shown that the increase in the intensity of the 5·9 and 17·9 nm actin layer lines precedes the change in the equatorial reflections in contracting frog skeletal muscle (Wakabayashi, Tanaka, Amemiya, Fujishima, Kobayashi, Hamanaka, Sugi & Mitsui, 1985; Kress, Huxley, Faruqi & Hendrix, 1986). These changes in the actin layer line intensities still take place in muscles stretched beyond myofilament overlap, indicating that they reflect structural changes when the thin filaments are activated by Ca^{2+}. Kress *et al.* (1986) have also examined time courses of the intensity changes in the actin layer lines and the equatorial reflections at various temperatures. Judging from their data, the changes in the equatorial reflection intensities seem to slightly precede the stiffness changes measured with kilohertz perturbations, in agreement with the view that the cross-bridges first move radially on stimulation and then rapidly attach to the thin filaments, though some tens of milliseconds are required for them to develop isometric force. On the other hand, the longitudinal and the transverse stiffness changes measured with ultrasonic waves in the present study appear to take place with a time course comparable to that of the actin layer line intensity changes at low temperatures (less than 6 °C), reaching their half-maximum changes about 40 ms ahead of the half-maximum isometric force. At high temperatures (around 20 °C),

the interval between the half-rise-time of the actin layer line intensity changes and that of the isometric force development is reduced markedly (by about 50%, Kress *et al.* 1986), while the corresponding value for the stiffness changes decreases only slightly, suggesting that the stiffness changes take place faster than the actin layer line changes at high temperatures. For a more accurate comparison, it is necessary to consider the temperature dependence of the rate of force development among the different experiments reported.

Possible mechanisms underlying the transverse stiffness changes during contraction

The most striking feature brought about by our muscle stiffness measurement with ultrasonic waves is that the transverse stiffness decreases during isometric contraction. As the transverse stiffness changes exhibit a time course similar to that of the longitudinal stiffness changes during the isometric force development (Figs 6–8), both changes are likely to reflect the same event leading to force generation in muscle. One possibility for the decrease of the transverse stiffness may be that the nature of the cross-links between the myofilaments is such that the strain in the transverse direction in muscle is largely taken up by the deformation of the cross-links resulting in a softening of muscle in the transverse direction. Another possibility is that the state of water molecules between the myofilaments changes during contraction to result in the muscle softening in the transverse direction. Using an acoustic microscope, Vinson, Eggleton & Meiss (1978) measured the propagation velocity of 100 MHz ultrasonic waves across the resting frog skeletal muscle in the transverse direction to be 1569–1587 m/s at 25 °C. These values agree with our value of the transverse wave velocity if the temperature effect (Fig. 4) is taken into consideration. They also noticed a decrease of the transverse wave velocity during isometric contraction, though they could not record its time course.

Still another possibility is that the decrease in the transverse stiffness is due to a decrease in the bulk modulus of elasticity of the myoplasm. If this is actually the case, the contribution of the formation of the cross-links between the filaments to the longitudinal stiffness should be estimated by subtracting the apparent transverse stiffness of contracting muscle from the corresponding apparent longitudinal stiffness. This procedure indicates that the increase in the longitudinal stiffness during contraction is about $8{\cdot}0 \times 10^7$ N/m^2 at 1–2 °C and about $12{\cdot}9 \times 10^7$ N/m^2 at 19–20 °C. These values are larger than the stiffness values estimated from the T_1 curve of Ford *et al.* (1977), being consistent with the expectation that the increase in the stiffness during the contraction measured with the megahertz waves should be larger than the corresponding value obtained with the kilohertz perturbation.

In rigor state, the cross-bridges attach permanently to the thin filaments. In the case of the static rigor linkages, both the longitudinal and the transverse stiffness increased, indicating a nearly isotropic nature of rigor muscle in contrast with the highly anisotropic nature of actively contracting muscle. It is widely held that the configuration of the cross-bridges in rigor state may correspond with that of the cross-bridges immediately after their power stroke during active contraction. The highly anisotropic nature of actively contracting muscle suggests, however, that the proportion of the cross-bridges with the rigor-like configuration, if any, is very small during contraction.

The muscle stiffness measurement with ultrasonic waves has proved to be very useful in obtaining interesting results which other methods cannot provide. We are currently using this method to measure muscle stiffness changes during quick and slow length changes, isotonic shortening and lengthening, etc. to give more detailed information about the contraction mechanism.

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