

Excitation-Contraction Coupling in a Barnacle Muscle Fiber As Examined with Voltage Clamp Technique

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ABSTRACT Relations between the membrane potential and the tension associated with changes in membrane potential were analyzed in barnacle giant muscle fibers by using voltage clamp techniques. With a step change in membrane potential the tension reaches its final level with a time course which is expressed by the difference of two exponential functions. The time constants τ_1 (0.2–0.4 sec at 23°C) and τ_2 (0.07–0.12 sec at 23°C) are independent of the new membrane potential at least for a relatively small membrane potential change while the final level of tension is a function of the potential. Decreasing the temperature increases both τ_1 and τ_2 ($Q_{10} = -2$ to -3) and the increase of the tonicity of the external medium increases τ_1 but not τ_2 . The final level of tension is related by an S-shaped curve to the membrane potential. The slope of the final tension–membrane potential curve increases with increasing external Ca concentration and is reduced when a small amount of transition metal ions is added to the medium. This suggests that the influx of Ca ions through the membrane is an important factor in the development of tension.

The relation between muscle tension and membrane potential has been studied in frog muscle fibers (Hodgkin and Horowicz, 1960), in cardiac muscle fibers (Niedergerke, 1956 *b*), and in muscle fibers of a barnacle, *Balanus nubilus* Darwin (Hoyle and Smyth, 1963) by observing potassium contractures. Similar studies have also been done by altering the membrane potential with polarizing currents in crayfish muscle fibers (Orkland, 1962 *a*) and in barnacle muscle fibers (Edwards, Chichibu, and Hagiwara, 1964). Considerable information has been accumulated from these studies on the relation between the amplitude of muscle tension and the membrane potential. However, very little has been done to analyze how the time course of tension development is determined by changes of the membrane potential.

For this type of analysis it is necessary to control the membrane potential of

the muscle fiber, and this can be done by means of a space clamp situation. The muscle fibers of a barnacle are large enough to permit the insertion of wire electrodes, thereby permitting the space clamp condition over the whole length of the fiber which contributes to the tension development (Edwards et al., 1964). The present paper deals with the analysis of the time course of tension as a function of the membrane potential change under various experimental conditions.

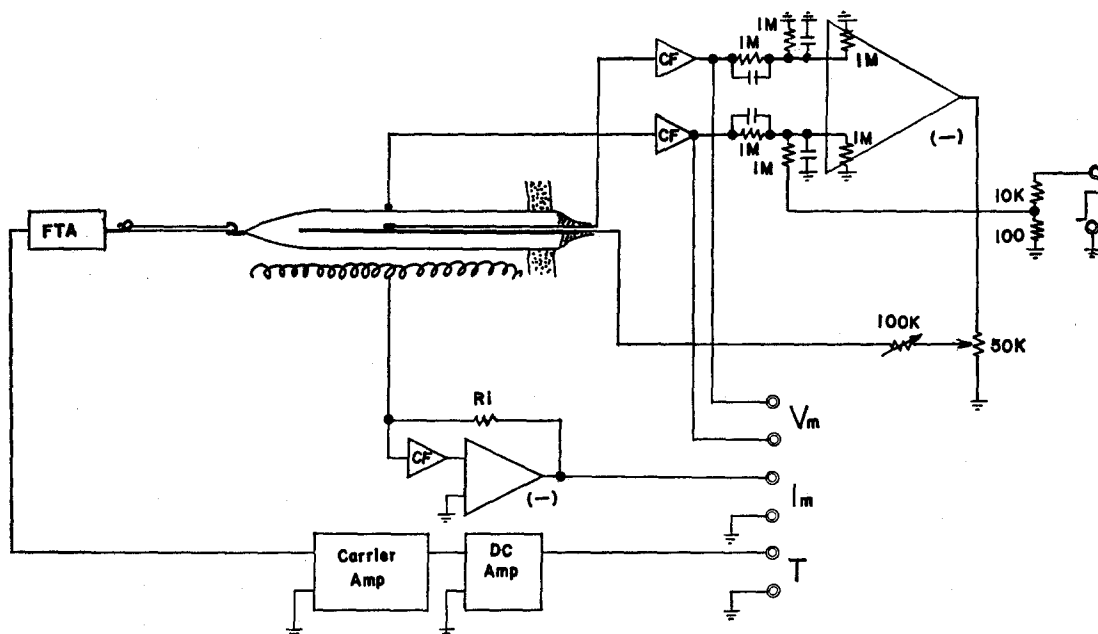


FIGURE 1. General arrangement of voltage clamp experiment. See text.

MATERIALS AND METHODS

Muscle fibers of a barnacle, *Balanus nubilus* Darwin, were used. The specimens were collected off the Pacific coast of Northern California. Fibers had diameters of 1–2 mm and lengths of 4–5 cm.

The experimental arrangement is shown in Fig. 1. Single muscle fibers were prepared as described previously (Hagiwara and Naka, 1964) and placed on a glass platform. The tendon end of the fiber was tied to a fine glass rod of about 1 cm in length and this was connected to the stylus of the transducer for recording tension. The cut end of the muscle fiber was tied with a string which was fixed to the base of the platform. Through this end of the muscle fiber a double wire electrode was inserted, longitudinally. Usually, preliminary insertion was made with a glass pipette of 0.3 mm diameter. This was simply to make a track in the myoplasm for the flexible metal electrode to follow. The wire electrode was inserted until its tip reached about 0.5 cm from the tendinous end of the fiber. The first 0.5 cm from the cut end was kept out

of the saline until this portion of the fiber was completely dry and became adhesive to the electrode which was firmly connected to the micromanipulator. This gave a good fixation of this end of the fiber. The next 0.5 cm of the fiber was covered with vaseline to prevent the cut end effect from spreading to the rest (3–4 cm) of the fiber covered with saline.

The metal electrode consisted of two wires: one was a platinized platinum wire of 200 μ diameter and the other was a silver-silver chloride wire of 60 μ . The former served as the current electrode while the latter served as the potential electrode. The platinized platinum wire was found to be best suited for the current electrode because of its low interphase resistance (Moore and Cole, 1963). The platinum electrode was uninsulated over the whole length of the fiber while the silver electrode was uninsulated over a 3 mm portion about 1.5 cm from the tip of the electrode.

The tension was recorded with a Sanborn myographic force transducer model FTA100-1, Sanborn, Co., Waltham, Mass. in conjunction with a carrier amplifier (Sanborn Model 350-3000B). The natural frequency of the transducer was 390 cps, and the frequency response of the transducer plus glass connecting rod was flat below the maximum frequency component of the responses observed (about 20 cps). The filter contained in the carrier amplifier introduced a 7° phase shift at 20 cps, and correspondingly less phase shift at lower frequencies. The compliance of the transducer was 0.3 mm per 100 g. Since the recorded tension was below 20 g and since the cut end of the fiber was firmly connected to the electrode, the recorded tension was considered to be isometric. The initial length of the fiber was adjusted so as to be slightly longer than the resting length. It was not possible to record tension larger than 20 g since the portion just next to the dried fiber gave a *locus minoris resistentiae* at which the fiber broke for a large stretch.

The resting potential of the fiber was frequently checked with a 3 M KCl-filled glass micropipette. Changes of the membrane potential from the resting level were observed between the silver wire electrode and the external silver-silver chloride electrode placed in the saline close to the fiber at the region where the uninsulated portion of the internal potential electrode was located. The potential difference was fed to one beam of the oscilloscope as well as to the inputs of an operational amplifier for the voltage clamp. The internal and external potentials were fed to the inputs out- and in-phase with the output of the amplifier respectively. A commanding voltage was fed to the in-phase input. The output was connected to the current electrode through two variable resistors. At first the two resistors were set at a maximum and then decreased until the membrane potential began to follow the commanding voltage satisfactorily (see Fig. 1).

The membrane current was often recorded as an IR drop across the feedback resistor between the ground and the indifferent electrode in the bathing solution by using a feedback circuit with a Burr-Brown 1305 operational amplifier (Burr-Brown Research Corp., Tucson, Ariz.). The indifferent electrode consisted of two groups of numerous fine chlorided silver wires, one on each side of the muscle fiber. The reason for using many fine wires was to increase the surface area of the electrode, to permit passing the rather large currents required for voltage clamping (up to 5×10^{-5} amps).

The normal barnacle saline had the following composition (Hoyle and Smith,

1963); NaCl, 466 mM; KCl, 8 mM; CaCl₂, 12 mM; Tris-maleate-NaOH buffer, 10 mM. The composition of Ca (or Mg) saline was CaCl₂ (or MgCl₂), 342 mM; KCl, 8 mM; Tris-maleate-NaOH buffer, 10 mM. The saline with desired concentrations of Ca and Mg was obtained by mixing appropriate amounts of the Ca and/or Mg salines with Ca-Mg-free Saline (NaCl, 514 mM; KCl, 8 mM; Tris-maleate-NaOH buffer, 10 mM). The pH of the solution was always 7.8. Cobalt-containing salines were obtained by adding an appropriate amount of CoCl₂ to the saline. This made the solution slightly hypertonic but the increase in tonicity was usually negligible since the Co concentration did not exceed 20 mM.

The temperature of the saline surrounding the fiber was kept constant by circulating water of a given temperature through a chamber beneath the preparation platform. The temperature of the saline was continuously monitored by a thermistor with a small heat capacity. Most experiments were performed at room temperature (22–23°C).

RESULTS

1. Time Course of Tension Development during Voltage Clamp

The tension development of the muscle fiber was recorded when the membrane potential was changed from the resting potential level to various other voltage clamped levels (Fig. 2 A). The membrane potential, V , refers to the internal potential measured from the potential level of the external saline. An increase of the membrane potential indicates a positive going change of V ; i.e., either a decreasing absolute amplitude of negative membrane potential or an increasing amplitude of positive potential. After the membrane potential was changed the tension approached a final steady amplitude (T_{∞}), which depended on the new membrane potential (V). The time course of the tension change $T(t)$ was similar at different membrane potentials, and to a first approximation followed the equation:

$$T(t) = T_{\infty} - Ae^{-(t/\tau_1)} \quad (1)$$

For a given Δt

$$T(t + \Delta t) = e^{-(\Delta t/\tau_1)}T(t) + T_{\infty}(1 - e^{-(\Delta t/\tau_1)}) \quad (2)$$

Equation (2) indicates the linearity between $T(t + \Delta t)$ and $T(t)$ for a fixed Δt . A plot of $T(t + 0.2 \text{ sec})$ against $T(t)$ for the three cases shown in Fig. 2 A is given in Fig. 2 B. The plots show a good fit to a straight line for the late phase of tension development, but a significant deviation was found for the early phase. The intersection between the extension of each straight line and the one passing through the origin with an angle of 45° to the X-axis gave T_{∞} ; since at $t = \infty$, $T(t + \Delta t)$ should be equal to $T(t)$. T_{∞} estimated by the above method usually showed a good agreement with the observed final steady

amplitude of the tension. However, a small discrepancy was occasionally found and in such cases the estimated T_∞ was used for the analysis.

When T_∞ had been found, $T_\infty - T(t)$ was plotted on a logarithmic scale against t for the three cases (Fig. 2 C). The plots gave a straight line for the late phase of tension development as expected from the preceding analysis. The slope of this straight line was equal to $1/\tau_1$, where τ_1 was the time con-

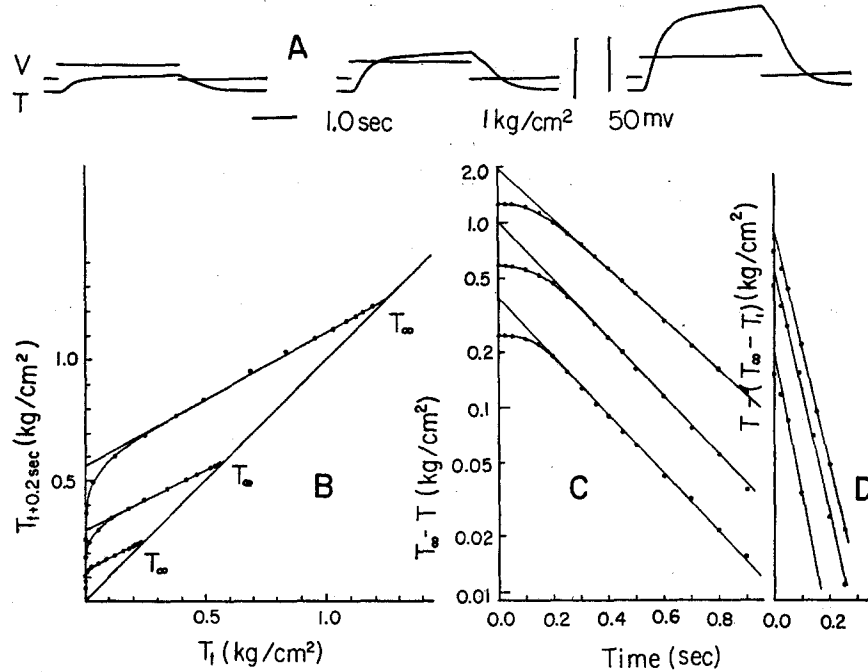


FIGURE 2. A, tension development associated with a rectangular membrane potential change. The upper trace of each record shows the membrane potential change from the resting potential level (-60 mv) and the lower trace, tension. At 23°C . B, C, and D, see text.

stant in equation (1). This late exponentially decaying component was denoted as $T_1(t)$. Time constants for different membrane potentials and hence different final steady tensions were estimated in several different fibers and some of the results are shown in Table I. The time constant, τ_1 , was fairly constant and independent of V or T_∞ . In these experiments, however, the membrane potential V was not increased much above the threshold for tension development. This was simply due to a technical difficulty; for higher V 's the final tension, T_∞ , became too large to permit the recording without rupture of the muscle fiber at the cut end. Therefore the present results do not guarantee the same constancy of the time constant, τ_1 , for a wide range of V or T_∞ , but it is at least certain that the change of τ_1 with T_∞ is much less than would be found were it directly or inversely proportional to T_∞ .

$A = T_1(0)$ can be found from the intercept on the ordinate of the straight line in Fig. 2 C. Then the late phase of $T(t)$ will be given by:

$$T(t) = T_\infty - T_1(t) = T_\infty - Ae^{-(t/\tau_1)} \quad (3)$$

However, in the early phase of tension the actual tension deviates significantly from this relationship. If $T_2(t)$ represents this difference, $T_2(t)$ is given by:

$$T_2(t) = T(t) - \{T_\infty - T_1(t)\} \quad (4)$$

TABLE I
TIME CONSTANTS AT DIFFERENT V OR T_∞

	V	T_∞	τ_1	τ_2
	<i>mv</i>	<i>g</i>	<i>sec</i>	<i>sec</i>
Fiber 1 (23°C)	-41	1.4	0.17	0.10
	-39	2.7	0.19	0.09
	-36	4.3	0.18	0.08
Fiber 2 (22.5°C)	-47	1.6	0.27	0.06
	-45	3.8	0.27	0.06
	-42	8.1	0.31	0.07

By plotting $T_2(t)$ in logarithmic scale against t (Fig. 2 D), it was found that $T_2(t)$ was also an exponential function but with a time constant, τ_2 , which was much smaller than τ_1 . As shown in Table I the second time constant, τ_2 , is also found to be constant and independent of the membrane potential or the final tension T_∞ in the present experimental range. $B = T_2(0)$ can be found from the intersection of the extension of the straight line and the ordinate in Fig. 2 D. Then $T(t)$ finally becomes:

$$T(t) = T_\infty - T_1(t) + T_2(t) = T_\infty - Ae^{-(t/\tau_1)} + Be^{-(t/\tau_2)} \quad (5)$$

As seen in Fig. 2 D the observed $T_2(0)$ is always slightly smaller than B obtained from the extrapolation of the exponential time course. This discrepancy suggests the existence of a systematic deviation between the observed tension and that described by equation (5) for t close to 0. The initial 100 msec of the tension curves are illustrated in Fig. 3 A for five different membrane potentials. The figure next to each trace indicates the membrane potential measured from the resting potential level (-60 mv). The lowermost trace illustrated by a solid line corresponds to the initial part of the tension shown to the right of the three records in Fig. 2 A. In this case the tension increased only after a certain latent period d which lasted about 45 msec. The broken

line indicates the tension calculated from equation (5). The observed and calculated tensions show good agreement for t longer than about 45 msec. For the first 45 msec nonzero tension can be calculated from equation (5), although no tension is observed during this period. This result suggests that a latent period d has to be taken into consideration for equation (5) to describe the tension satisfactorily. The result in Fig. 3 A suggests that d becomes

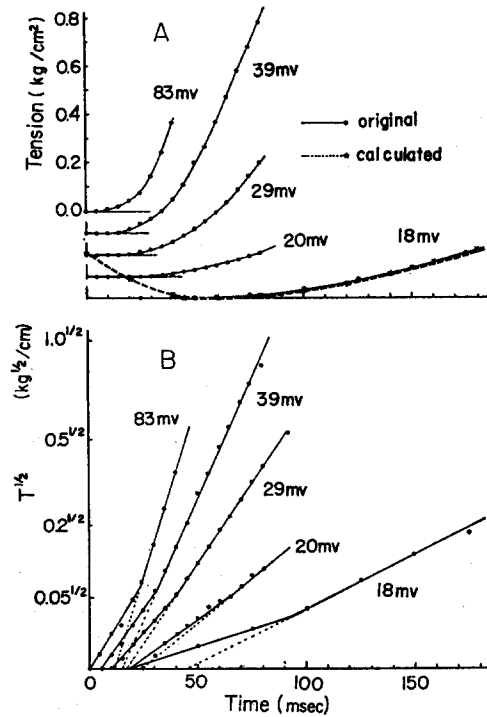


FIGURE 3. A, initial portion of tension produced by a rectangular potential change. Figure near each curve indicates the membrane potential level measured from the resting potential level (-60 mv). The calculated trace (illustrated by a broken line) was obtained from equation (5). B, square root of tension plotted against time. Each trace in B corresponds to one of those in A. Obtained at 23°C .

smaller as V increases. In other words d is voltage-dependent. Thus equation (5) is rewritten:

$$T(t) = T_{\infty} - T_1(d)e^{-(t-d)/\tau_1} + T_2(d)e^{-(t-d)/\tau_2} \quad \text{for } t \geq d \quad (6)$$

$$T(t) = 0 \quad \text{for } t < d$$

At

$$t = d \quad T(d) = T_{\infty} - T_1(d) + T_2(d) = 0$$

Fig. 3 A indicates that dT/dt is also zero at $t = d$

$$\left\{ \frac{dT(t)}{dt} \right\}_{t=d} = \frac{T_1(d)}{\tau_1} - \frac{T_2(d)}{\tau_2} = 0$$

When these conditions are introduced, equation (6) becomes:

$$T(t) = \frac{T_{\infty}}{\tau_1 - \tau_2} \left\{ \tau_1 (1 - e^{-(t-d)/\tau_1}) - \tau_2 (1 - e^{-(t-d)/\tau_2}) \right\} \quad \text{for } t \geq d \quad (7)$$

$$T(t) = 0 \quad \text{for } t < d$$

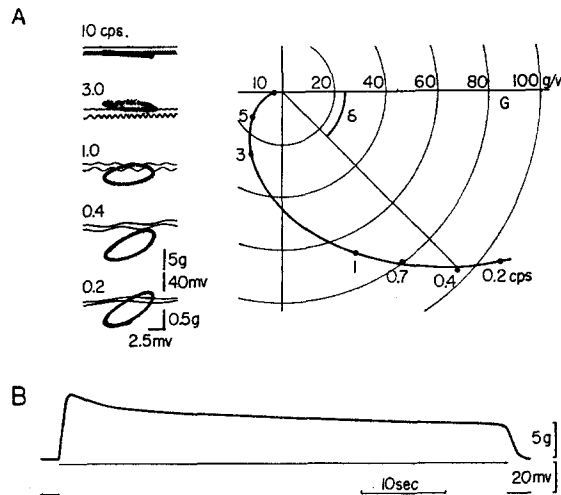


FIGURE 4. A, tension changes associated with alternating potential changes superposed on a steady depolarization. Each group of records shows tension (upper trace), potential (lower trace), Lissajou figure (tension, vertical and potential, horizontal). Nyquist diagram obtained from this experiment is shown in the middle of the figure where the magnitude of the gain (G) is plotted against phase lag δ . B, tension associated with a prolonged depolarization. The membrane potential was depolarized by 20 mv for about 1 min. At 23°C.

When equation (7) is expanded in terms of $(t - d)$ and higher terms of $(t - d)$ are neglected for $(t - d)$ smaller than τ_2 ,

$$T(t) = \frac{T_{\infty}}{2\tau_1 \tau_2} (t - d)^2$$

This indicates that the square root of $T(t)$ is proportional to $(t - d)$ for this range of t . Thus d can be determined from the intercept on the t -axis of the straight line in the $T^{1/2}$ vs. t curve. Fig. 3 B shows plots for the range of t smaller than 100 msec. Each trace in the figure corresponds to one of those in Fig. 3 A. The plots gave a good fit to a straight line for tension above a certain limit (in this case about 0.05 kg/cm²). The intercept on the t -axis of the extrapolation of the straight line (illustrated by broken lines) gave the

delay, d in equation (7). The result shows that the delay decreases with increasing membrane potential.

The discrepancy of the plots from the straight line found for smaller tensions indicates that equation (7) does not describe the tension of this very early phase and also that the latent period thus obtained is only approximate.

2. Tension for Sinusoidal Membrane Potential

As described above, the tension reaches the final steady level during maintained depolarization. When the voltage clamp was much more prolonged as

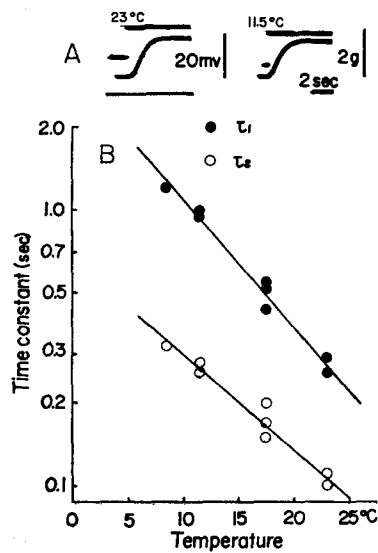


FIGURE 5. A, effect of temperature upon the time course of tension (lower trace) associated with a rectangular potential change (upper trace). Two records were obtained from the same muscle fiber at different temperatures. Notice the difference in time calibrations. B, temperature dependence of time constants, τ_1 and τ_2 , obtained from the same fiber.

shown by Fig. 4 B, the tension did not stay at the steady level indefinitely but started to decline. The rate of decline was relatively fast at first but this was followed by a very slow rate. Thereafter the tension stayed at almost the same level for a considerable period. The tension in this period was observed when sinusoidal potentials of a small amplitude (3–8 mv) were superposed on the maintained depolarization. In the experiment shown in Fig. 4 A the frequency of the sinusoidal potential was altered without altering the amplitude at an average membrane potential of -45 mv. In each pair of traces the upper trace shows the change of tension produced by the change of the potential illustrated in the lower trace. Lissajou diagrams were obtained oscillographically by feeding tension and potential to the vertical and horizontal inputs respectively. Nyquist diagrams such as that illustrated in Fig. 4 A were obtained from the Lissajou diagrams. The magnitude of the gain, G , was plotted against phase lag δ . The common features are the following: (a) The gain went to zero

as the frequency increased. (b) The phase lag approached 180 degrees as the frequency became larger but never exceeded 180 degrees.

The transfer function, $Z_v(\omega)$, at a membrane potential V , was calculated from equation (7) as:

$$Z_v(\omega) = \frac{K_v e^{-jd_v}}{(j\omega \tau_1 + 1)(j\omega \tau_2 + 1)} \quad (8)$$

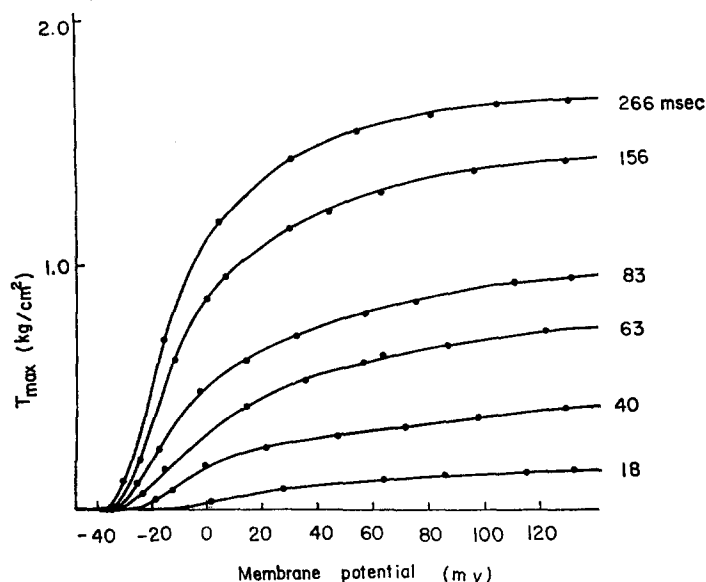


FIGURE 6. Relations between the maximum tension and the membrane potential obtained with various pulse durations. At 17°C.

Here K_v is a function of V . Since change in T_∞ is linearly related to change in V for small voltage changes, K_v can be expressed as $(dT_\infty/dV)_v$. The latency d_v in equation (7) was in the range between 30 to 50 msec for V 's used in the present experiments. In other words, d_v was not negligible compared with τ_2 which was 70 to 100 msec. Under this condition equation (8) predicts that the phase lag should significantly exceed 180 degrees at high frequency. This fact suggests that the latency-like phenomenon occurs only when the contraction is initiated in the resting muscle fiber and that the tension-membrane potential relation of the active fiber can be described without introducing a delay. This was found to be the case as shown below.

If the delay is neglected, the transfer function $Z_v(\omega)$ becomes

$$Z_v(\omega) = \frac{K_v}{(j\omega \tau_1 + 1)(j\omega \tau_2 + 1)} \quad (9)$$

This equation predicts the two features of the experimental results described above. The two time constants estimated from the Nyquist diagram were always close to those obtained with the square pulse method.

3. *Temperature Dependence of τ_1 and τ_2*

Although the two time constants, τ_1 and τ_2 , were largely independent of the membrane potential, V , they were dependent on temperature. The two records in Fig. 5 A were obtained from the same muscle fiber at 23° and 11.5°C bath temperature. The time course became much slower with decrease of temperature (see time calibration), and this was associated with an increase in the magnitude of both time constants. In Fig. 5 B, τ_1 and τ_2 of the same muscle fiber, obtained at several different temperatures, are plotted on a logarithmic scale against the temperature. The plots indicate that both τ_1 and τ_2 increase as the temperature is decreased. If it is assumed that the velocities of the reactions controlled by the two time constants are inversely related to the time constants, then the Q_{10} 's are -2.9 for τ_1 and -2.2 for τ_2 .

4. *T_∞ As a Function of the Membrane Potential*

As described above, the final steady tension, T_∞ , is a function of the membrane potential, V . The relation, however, could not be obtained for the range of large V , simply because the tension became too large for the present recording conditions as described previously. If a short pulse was used instead of a maintained voltage, the tension gave a peak after the termination of the pulse. Fig. 6 shows the relationship between the peak tension, T_{max} , and the membrane potential, V , obtained with pulses of 266, 186, 85, 63, 40, and 18 msec respectively. T_{max} begins to saturate at a membrane potential of about +20 mv and their shapes are very similar except for the range of V 's close to the threshold of contraction. This finding suggests that T_∞ should also show a similar saturation with the membrane potential. This agrees with the report of S-shaped T_∞ - V curves by others in various preparations (Hodgkin and Horowicz, 1960; Niedergerke, 1956 *b*; Hoyle and Smyth, 1963; Orkland, 1962 *a*; Edwards et al., 1964).

5. *The Effect of Conditioning Hyperpolarization upon the T-V Relation*

The two records in Fig. 7 A show tension recordings of the same muscle fiber taken at different base line membrane potentials obtained by conditioning hyperpolarization. In records 1 and 2 the membrane potential was shifted to +5 mv for 80 msec from the base line membrane potentials of -60 and -110 mv, respectively. The amplitude and the time course of the tension records were identical. The results indicate that the tension depends on the absolute value of the membrane potential and is independent of the base line membrane potential if it is below the threshold for tension development. Fig. 7 B

shows the T_{\max} - V relation obtained with 80 msec voltage pulse at three different base line membrane potentials (-60 , -110 , and -137 mv). The relations are identical for the three cases.

Membrane currents associated with a rectangular depolarization were similar to those described previously (Hagiwara, Nakajima, and Takahashi,

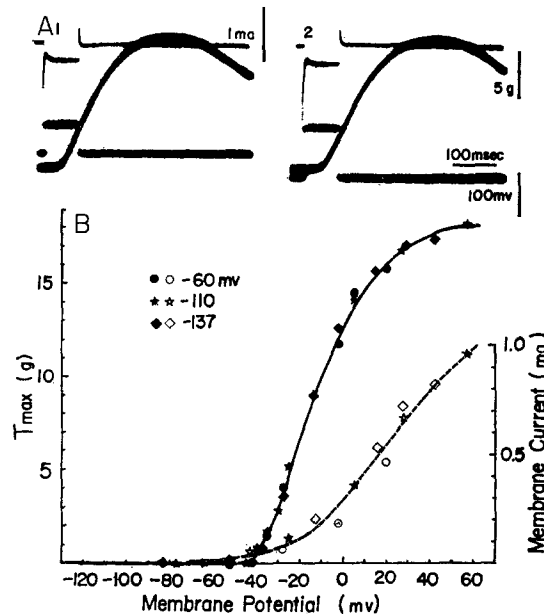


FIGURE 7. Effect of conditioning hyperpolarization upon the relation between the maximum tension and the membrane potential. In each record in A the upper trace indicates the membrane current, the middle trace, membrane potential, and the lower trace, tension. Downward deflection of the current trace corresponds to the outward direction of the membrane current. The membrane potential level during the pulse was the same in A 1 and A 2. A 1 was obtained at the resting potential level (-60 mv) and A 2 after hyperpolarizing the membrane potential to -110 mv. For B, relations between the maximum tension and the membrane potential during the pulse are shown by continuous lines and those between the maximum outward membrane current and the membrane potential are shown by broken lines. At 22°C .

unpublished; Hagiwara, 1965) except that no appreciable initial inward current was found in the present case. This was simply a result of the lack of all or none spike potentials in the normal barnacle muscle fiber. In the previous experiments the membrane was made capable of producing all or none spikes by reducing the internal Ca ion concentration with EGTA (ethyleneglycol bis (β -aminoethylether)- N,N' -tetraacetic acid). The delayed rectifying current found in the EGTA-treated muscle fiber was also found in the normal muscle fiber. The rectifying current was observed at the end of

the 80 msec pulse in the experiment shown in Fig. 7 and plotted against V in Fig. 7 B. The rectifying current is also unaffected by hyperpolarization. The membrane potential at which this current starts to develop coincides with that for tension development. Further relations between tension and membrane current, however, will not be discussed in this paper.

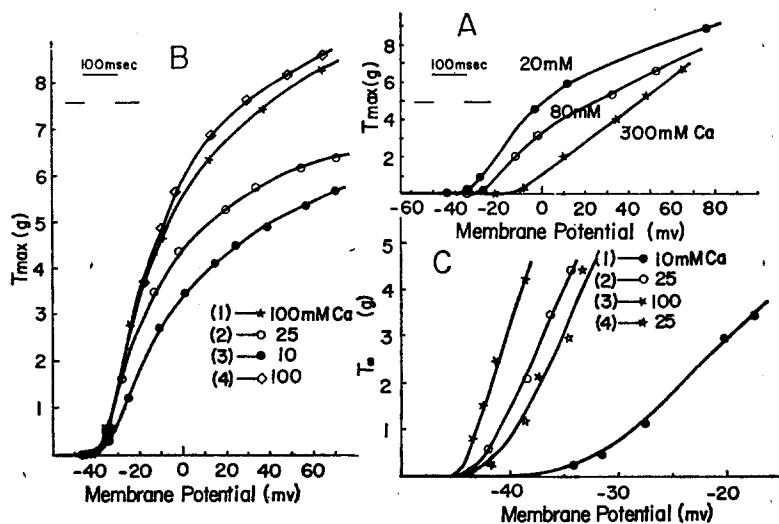


FIGURE 8. Effect of external Ca concentration upon the tension-membrane potential relation. A, relations between the maximum tension associated with a 100 msec pulse and the membrane potential, obtained at three different Ca concentrations. The solution did not contain Mg. At 16°C. B, similar relations obtained in media containing 100 mM Mg. At 13°C. C, relations between the final steady tension (T_{∞}) and the membrane potential obtained in media containing 100 mM Mg. At 23°C. (1), (2), (3), and (4) indicate the order of measurements performed.

6. Effect of External Ca on Muscle Tension

T_{max} - V relations were obtained with the same muscle fiber at three different external Ca concentrations. The Ca concentrations were 20, 80, and 300 mM respectively and the solutions did not contain Mg. The pulse duration was fixed at 100 msec throughout. The result is shown in Fig. 8 A. The major effect of increasing Ca concentration is the shift of the T_{max} - V curve along the V -axis in the positive direction. A similar phenomenon has been found for frog twitch muscle fibers (Lüttgau, 1963) and also for fibers of a crayfish muscle (Orkand, 1962 *b*). The phenomenon is probably based on a mechanism similar to that for the stabilizing action of Ca ions on the current-voltage relation of the axonal membrane (Frankenhaeuser and Hodgkin, 1957). Because of the shift, T_{max} for a given V decreased with increasing external Ca concentration at least for a certain range of V 's. It is desirable to examine

the effect of Ca concentration on contraction independently of the stabilizing action. The threshold membrane potential for the Ca spike of the barnacle muscle fiber shifts in the positive direction with an increasing external Ca concentration (Hagiwara and Naka, 1964). However, the threshold became

TABLE II
TIME CONSTANTS OF THE SAME FIBER AT DIFFERENT
EXTERNAL Ca CONCENTRATIONS (AT 23°C)

Ca concentration	V	T_{∞}	τ_1	τ_2
<i>mM</i>	<i>mv</i>	<i>g</i>	<i>sec</i>	<i>sec</i>
10	-32	0.4	0.39	0.13
	-28	1.1	0.33	0.13
	-21	3.0	0.38	0.12
25	-42	0.56	0.32	0.12
	-37	3.5	0.36	0.11
	-35	4.5	0.35	0.12
100	-44	0.80	0.40	0.14
	-41	2.5	0.36	0.13
	-38	4.2	0.38	0.12

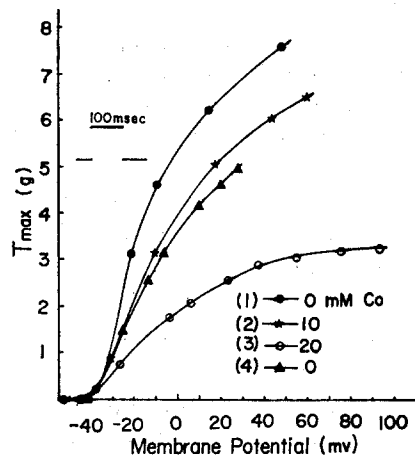


FIGURE 9. Effect of Co ions upon the T_{\max} - V relations. The Ca concentration was 25 mM and the Mg concentration, 100 mM. (1)-(4) indicate the order of measurements. At 10°C.

constant and independent of the external Ca concentration in the range between 10 and 100 mM Ca if the external solution contained 100 mM MgCl_2 (Hagiwara and Takahashi, 1967). Under this condition it was possible to examine whether or not Ca ions enhance the tension development. Fig. 8 C shows the T_{∞} - V relation obtained with the same fiber at different Ca concentrations in the presence of 100 mM Mg in the solution. Fig. 8 B shows the result of an experiment in which the T_{\max} - V relation was obtained with a 100 msec pulse. In either case the threshold membrane potential was nearly

constant among recordings in different external Ca concentrations. The tension for a given membrane potential increased as the Ca concentration was raised and this effect was reversible (Fig. 8 B and C). The conclusion is straightforward for the T_{∞} - V relation since it is not based on the assumption that the time course of the tension development is independent of the external Ca concentration. However, the observed T_{\max} - V relation covered only the range of small V 's and gave no information about the range of large V 's where the tension tends to saturate. This can be seen in the T_{\max} - V relation

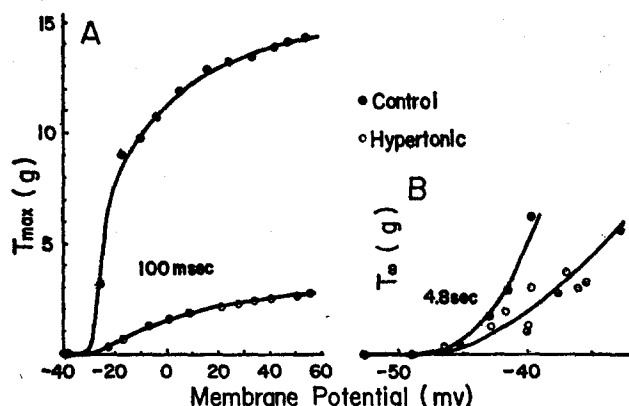


FIGURE 10. Effect of hypertonic solution (1 mole of urea in 1 liter of the normal saline) upon the tension development. T_{\max} - V relations in A (100 msec pulse) and T_{∞} - V relations (4.8 sec pulse) in B were obtained with the same muscle fiber before (closed circles) and 10 min after immersion in the hypertonic saline (open circles). At 23°C.

obtained with a short pulse. The relation is meaningful only if the time course of the tension development is independent of Ca concentration. In a few cases τ_1 and τ_2 were estimated in the same fiber at different external Ca concentrations and some of the results are shown in Table II. They show that neither τ_1 nor τ_2 depends on Ca concentration. In other words, the result in Fig. 8 B suggests that the T_{∞} - V relation probably shows a similar change for the alteration of the external Ca; i.e., the saturation level of the tension at large V 's increases with an increasing external Ca concentration. From the above result it may be concluded safely that the tension development is enhanced by external Ca ions.

The Ca spike is suppressed in the presence of transition metal ions such as Co^{++} (Hagiwara and Takahashi, 1967). The suppression is apparently due to the competitive binding of Co ions to the membrane sites which are normally occupied by Ca ions, thereby decreasing the surface density of Ca ions. The effect of Co ions on tension development was examined. The T_{\max} - V relations obtained from a muscle fiber in three different concentrations of Co ions (0, 10, and 20 mM) are shown in Fig. 9. The solutions con-

tained 25 mM Ca and 100 mM Mg throughout. The tension for a given membrane potential was reduced as the external Co concentration was increased. The recovery from the suppression was often incomplete but some recovery was always seen as shown in Fig. 9. The time course of tension was not altered by Co ions. Orkand (1962 *b*) observed a similar suppression of contraction by Mn^{++} .

7. Effect of Hypertonic External Solution upon the Tension Development

In the frog muscle fiber the amplitude of the twitch is greatly reduced if the fiber is immersed in a saline solution made hypertonic by adding extra NaCl or urea (Hodgkin and Horowicz, 1957). The effect of hypertonicity was examined in the barnacle muscle fiber by adding 1 mole of urea to 1 liter of the normal barnacle saline. A and B of Fig. 10 show the T_{max} - V relations

TABLE III
EFFECTS OF TONICITY ON TIME CONSTANTS

	V	T_{∞}	τ_1	τ_2
	<i>mv</i>	<i>g</i>	<i>sec</i>	<i>sec</i>
Control	-42	4.9	0.24	0.081
Hypertonic	-36	3.3	0.47	0.082

and the τ_{∞} - V relations of the same fiber obtained with voltage pulses of 100 msec and 4.8 sec duration, before and 10 min after immersion in the hypertonic solution, respectively. The initial slope of the T_{max} - V curve was reduced by a factor of 6.5, while the slope of the τ_{∞} - V curve was reduced by a factor of 1.7. The greater attenuation for a short voltage pulse was found to be due to the slower time course of the tension development in the hypertonic solution. The time constants, τ_1 and τ_2 , were estimated in a few fibers before and after the treatment and the results are summarized in Table III. The larger time constant, τ_1 , always showed a considerable increase while τ_2 remained unchanged. The increase of τ_1 may be due to an increased viscosity caused by the loss of internal water. The above change was as a rule reversible.

DISCUSSION

The time course of the development of the tension is approximately described by the difference between two terms of the form $1 - \exp(-t/\tau)$. The time constant (τ_1) of the first (positive) term is increased as the temperature is decreased or as the tonicity of the bathing solution is increased but is independent of the external Ca^{++} concentration. A similar description of the time course of isometric tension has been reported in the frog muscle (Matsumoto 1967). It is consistent with the assumption that muscle is a two com-

ponent system consisting of a series elastic element and a contractile component (Hill, 1938).

The time constant (τ_2) of the second, or negative, term is increased as the temperature is decreased, but is independent of hypertonicity or external Ca^{++} concentration. The negative term is necessary because initially the measured tension increased less quickly than predicted by the factor containing τ_1 . This deviation decreased with time. At first sight this deviation might be attributed to instrumental deficiencies. However, the frequency response of the recording system was found to be linear up to 20 cps. Furthermore, the temperature sensitivity of this component would seem to preclude a mechanical basis.

The effect of external Ca^{++} concentration upon the tension development of the muscle fiber has been investigated in several different preparations and the effects are somewhat different among them. The major effect of an increase in external Ca ions on frog twitch muscle fiber (Lüttgau, 1963) and on one kind of crayfish muscle fiber (Orkand, 1962 *b*) is a shift of the tension-membrane potential relation along the potential axis in the positive direction. The effect has a common basis with the Ca stabilizing action for the action potential in the axonal membrane (Frankenhaeuser and Hodgkin, 1957). Because of this shift, the tension for a given membrane potential decreases with increasing Ca^{++} concentration at least in a certain range of membrane potentials. In contrast to the above effect, for certain cardiac muscle fibers (Niedergerke, 1956 *b*) and frog slow muscle fibers (Lüttgau, 1963) Ca does not show any appreciable stabilizing effect and the major effect of Ca^{++} is the enhancement of tension for a given membrane potential. The present experimental result shows that the stabilizing action of Ca^{++} is found for the tension development in the barnacle muscle fiber and therefore, in this sense, the fiber resembles frog twitch fiber. If the effect of stabilization is eliminated, however, an appreciable enhancement of tension for a given membrane potential is found in the barnacle muscle fiber for an increase in the external Ca^{++} concentration. These results suggest that the inward movement of Ca^{++} through the membrane, during the membrane potential change, plays a significant role in the initiation of contraction. In this respect, the barnacle muscle fiber resembles cardiac muscle fibers. Other evidence consistent with this idea has been reported by Edwards and Lorkovic (1967, personal communication). It has been shown that the Ca spike in the barnacle fiber is competitively suppressed by transition metal ions such as Co ions. A similar suppression by Co ions is found for tension development. This suppression can be interpreted as due to the competitive occupation of Ca^{++} membrane sites by Co^{++} , indicating that the important Ca ions for contraction are those at the muscle membrane surface. This conclusion agrees with that proposed by Niedergerke (1956 *a*) for cardiac muscle fibers. The above argument,

however, does not mean that the contraction is initiated exclusively by the inward movement of Ca from outside the fiber.

It has been confirmed in various muscle preparations that the twitch is greatly reduced in hypertonic media even when the action potential is not much altered (Hodgkin and Horowicz, 1957). Howarth (1958) showed that this apparent uncoupling of electrical and mechanical events is due mainly to the slowing of mechanical events because of the increase of viscosity, and that the active state-membrane potential relation is still operative. The present analysis in barnacle muscle fibers shows that the reduction of tension in hypertonic solution is exclusively due to the increase of time constant, τ_1 , and that the time constant, τ_2 , which may be related to the onset of the active state, is unaffected. This finding agrees with the result obtained by Howarth (1958) for frog twitch muscle fibers.

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