The Effect of Bathing Solution Tonicity on Resting Tension in Frog Muscle Fibers

J. LÄNNERGREN and J. NOTH

From the Nobel Institute for Neurophysiology, Karolinska Institutet, S-104 01 Stockholm 60, Sweden. Dr. Lännergren's present address is Department of Physiology, University College, London W. C. 1, England. Dr. Noth's present address is Physiologisches Institut II, Universität Düsseldorf, D-4000 Düsseldorf, Germany.

ABSTRACT Resting tension and short-range elastic properties of isolated twitch muscle fibers of the frog have been studied while bathed by solutions of different tonicities. Resting tension in isotonic solution at 2.3-μm sarcomere spacing averaged 0.46 $mN \cdot mm^{-2}$ and was proportional to the fiber cross-section area. Hypertonic solutions, containing 0.1–0.5 mM tetracaine to block contracture tension, caused a small sustained tension increase, which was proportional to the fiber cross-section area and which reached 0.9 $mN \cdot mm^{-2}$ at two times normal tonicity (2T). Further increases in tonicity caused little increase in tension. Hypotonic solutions decreased tension. Thus, tension at 2.3 μm is a continuous, direct function of tonicity. The dependence of tension on tonicity lessened at greater sarcomere lengths. At 3.2 μm either a very small rise or, in some fibers, a fall in tension resulted from an increase in tonicity. Hypertonic solutions also decreased the tension of extended sarcolemma preparations. In constant-speed stretch experiments the elastic modulus, calculated from the initial part of the stretch response, rose steeply with tonicity over the whole range investigated (1–2.5T). The results show that tension and stiffness of the short-range elastic component do not increase in parallel in hypertonic solutions.

INTRODUCTION

An isolated, unrestrained muscle fiber bathed in Ringer's solution, acquires a length corresponding to a sarcomere spacing of 2.0 μm (Simmons, 1971). The sarcomere spacing of fibers in situ is about 2.3 μm (Mittenthal and Carlson, 1971); the fibers therefore display some tension. In the present study we have chosen a reference sarcomere length of 2.3 μm and term the tension exerted by a fiber at this length "resting tension." The resting tension may either be passively or actively generated or both. Passive tension may either represent tension in stretched structural elements such as the sarco-

lemma (Ramsey and Street, 1940), the sarcoplasmic reticulum (Sandow, 1966), or the myofilaments (A. F. Huxley, 1957), or it may be due to electrostatic repulsion forces between the filaments (H. E. Huxley, 1960). Resting tension may also be actively generated by cross bridges operating at a low rate as suggested by Hill (1968).

Hill (1968) investigated the effect of hypertonic solutions on the resting tension of whole sartorius muscles at lengths close to the in situ length and found that a change from Ringer's solution to a hypertonic medium caused a small, long-lasting tension increase and ascribed this to enhanced cross-bridge activity. In small bundles of muscle fibers (Gordon and Godt, 1970) and in isolated fibers (Lännergren, 1971) a change to hypertonic solution was found to be associated with relatively large, transient tension development. It has recently been demonstrated (Lännergren and Noth, 1973), also in isolated fibers, that the tension response in hypertonic solution can be resolved into two components, a large, transient tension which may reach about 35% of the maximal tetanic tension, and a small, maintained tension increase, detectable after the former is blocked by tetracaine.

The contracture-inhibiting effect of tetracaine has been used in the present work to study the lasting tension change associated with changes in tonicity of the bathing solution. The experiments were performed on isolated fibers in order to obtain short diffusion times after solution changes and to get information about the relation between the tension and fiber cross-section area. A preliminary report has been given elsewhere (Lännergren and Noth, 1972).

METHODS

Preparation and Mounting

The experiments were carried out on frogs (*Rana temporaria*) kept at 4–7°C up to the moment of use. Single twitch fibers were dissected from the dorsal head of the semitendinosus muscle in an acrylic chamber containing Ringer's solution. Adherent strands of connective tissue were carefully removed from the fiber. A small hook was tied to each tendon with nylon thread. The fiber was then suspended in a horizontal channel in the chamber between a fixed steel hook, located near the inlet to the channel, and the movable plate of a force transducer. In experiments in which small length changes were performed, the fixed hook was replaced by a horizontal lever connected to an electromechanical transducer. Details about the mounting and the stretching device have been given in a preceding paper (Lännergren, 1971).

During the experiment Ringer's solution was flowing continuously through the channel at a slow rate. A stopcock system allowed quick changes to be made between different solutions. Shortly before introducing a test solution the rate of flow was increased to about 12 $mm \cdot s^{-1}$. The rate of flow of the test solution was carefully adjusted to the same value in order to minimize artifacts from differences in fluid

drag forces. The time from turning the stopcock arm until the test solution reached the distal end of the fiber was approximately 2 s.

Force Transducers

In most experiments force was measured with a capacitance transducer with a natural frequency of about 100 Hz and a compliance of 0.07 $m \cdot N^{-1}$. This compliance was corrected for in the stretch experiments. The entire system had a linear response of 6 $mV \cdot \mu N^{-1}$ up to 0.5 mN. Drift was usually less than 0.5 $\mu N \cdot min^{-1}$. In some of the stretch experiments, in which larger forces were encountered, a variable resistance transducer element was used (AE 801, Akers Electronics, Horten, Norway) incorporated into a Wheatstone bridge. A 5 mm-long, thin glass tube was glued to the silicon beam containing the resistive material. The compliance at the point of attachment of the preparation was 0.013 $m \cdot N^{-1}$ with a natural frequency ca. 2 kHz. Changes in force as small as 5 μN could be detected and the system was linear for forces up to at least 6 mN. Drift was less than 1 $\mu N \cdot min^{-1}$. In a few experiments both transducers were used simultaneously. Each was then connected to one end of the fiber.

Measurements of Mechanical Properties

The resting tension of a small muscle fiber at 2.3-μm sarcomere length was about 2 μN. In order to measure such small fibers accurately it was necessary to correct for instrumental drift. Zero force was recorded for each measurement of resting tension by relaxing the fiber against a small adjustable stop positioned in the channel. The flow of solution was stopped when the measurements were made.

The elastic properties of the muscle fibers at the beginning of a constant-speed length change were evaluated according to Hill (1968). The slope of the initial part of the tension curve was measured and the stiffness expressed in the form of Young's elastic modulus (E), calculated as $E = \Delta P \cdot L_i / \Delta L \cdot A$, where ΔP is the tension change, ΔL the length change, L_i the initial length of the fiber, and A its cross-section area.

Microscopy

The fiber was held between movable clamps during the dissection and could be rotated around its long axis. The largest and smallest diameter were determined at three different sites using a dissection microscope fitted with an ocular micrometer. A (the cross-section area) was estimated by the product $\frac{1}{4} \cdot \pi \cdot a \cdot b$, where a is the largest, b the smallest diameter. The mean diameter ($\bar{d}$) was calculated from the equation $\bar{d} = 2 \cdot (\bar{A}/\pi)^{\frac{1}{2}}$, where $\bar{A}$ is the mean of the three values of A (cf. Blinks, 1965).

The fiber was held at a length corresponding to 2.3 μm-sarcomere spacing, as determined by a high-power microscope ($\times$1000 magnification). In eight additional experiments measurements were performed at sarcomere spacings of 2.6, 2.9, and 3.2 μm.

Solutions

The standard Ringer's solution had the composition (millimoles/liter): NaCl 115; KCl 2.5; $CaCl_2$ 2.0; Na_2HPO_4 2.15; NaH_2PO_4 0.85.

Hypertonic solutions were prepared by adding sucrose to the Ringer's solution and hypotonic solutions were obtained by omitting the appropriate amount of NaCl from Ringer's solution. The tonicity (T) is referred to that of Ringer's solution. In solutions with higher potassium concentrations than Ringer's, NaCl was substituted with an equivalent amount of KCl. The [K] × [Cl] product of the extracellular fluid was thus not constant, but this was probably of little importance because the elevation of [K] was small, the exposure time to the high potassium solution was short, and the internal [K] × [Cl] product rises when the fiber shrinks in hypertonic solution.

Tetracaine chloride was added as stock solution (2%) or, in some experiments, in powder form. Double-distilled water from a quartz distiller and analytic grade chemicals were used except for tetracaine. The pH of all solutions was between 7.1 and 7.2. The experiments were performed at room temperature (20–24°C).

Experimental Procedure

All fibers were stimulated electrically (100 impulses·s^{-1}; 0.5-s train duration) at the beginning of the experiments. Only those fibers responding with a fused tetanus were used. The response to tetanic stimulation was also checked periodically during the experiment, and the experiment was discontinued when the fiber failed to give a fused tetanic tension.

Full recovery after application of hypertonic solutions requires about 30 min (Lännergren and Noth, 1973). However, in the present experiments, tetracaine, applied before each test, prevented development of contracture tension. In this case case recovery times of 15 min were found to be sufficient. Several fibers were exposed over 20 times to hypertonic solutions without any changes in maximum tetanic tension or resting tension.

The following tetracaine concentrations (millimoles/liter) were used and were found to be sufficient to block completely contracture tension when applied 1 min before the test solution: 0.05 at 1.25T; 0.1 at 1.5T; 0.2 at 1.75T; 0.3 at 2T; 0.4 at 2.5T; 0.5 at 3T.

RESULTS

Relation between Fiber Size and Resting Tension

The resting tension was measured in 20 fibers and the results are given in Fig. 1 in which tension is plotted against mean fiber diameter with a logarithmic scale for both axes. The slope of the relationship is approximately two, indicating that resting tension is nearly proportional to the fiber cross-section area. This was also found for the increase in tension, seen in hypertonic solution (see below) when a similar plot was made. Hence, in the following paragraphs all tension values will be given relative to the cross-section area.

The mean resting tension was 0.46 mN·mm^{-2} ± 0.09 (mean ±SD) a value smaller than previously obtained at 2.2 μm (0.77 mN·mm^{-2} ± 0.23, n = 7, Lännergren, 1971). The difference may be due to a seasonal variation

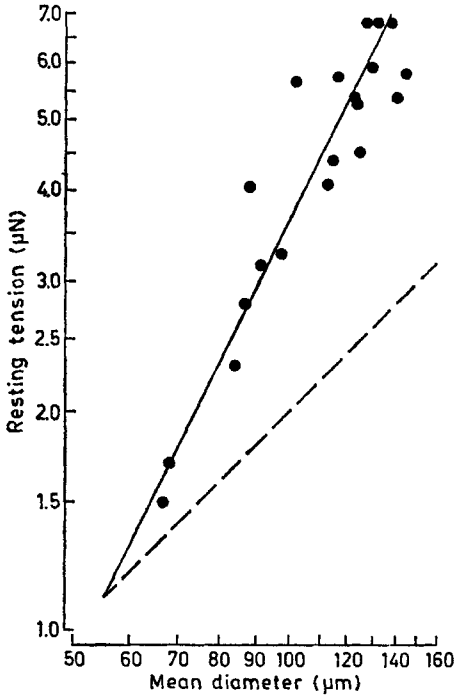

FIGURE. 1. Relation between resting tension and mean fiber diameter at 2.3-μm sarcomere spacing. Line drawn in full has a slope = 2; for interrupted line slope = 1.

but methodological differences related to the zero force measurement may also be important.

Effect of a Change in Tonicity on Steady-State Tension

The records in Fig. 2 show the effect of changing the tonicity of the bathing solution on the tension of a resting fiber held at 2.3-μm sarcomere length. About 2 s after the change from Ringer's fluid to a hypertonic solution, tension started to rise and reached a steady level in about 45 s. Returning to Ringer's fluid caused tension to fall after a short delay and to return to the original level with a time-course similar to that of the tension rise. The delay is probably explained by the time required for exchange of solution in the channel.

Longer test periods than those depicted in Fig. 2 were used in some cases. Four fibers were exposed to 1.75T solution for 5 min. In two of these no decline in the steady-state tension could be detected, in the other two, tension decreased by less than 5%. At the highest tonicity tested (3T), however, a slow tension fall was often seen after the initial tension rise. This transient nature of the response is probably not due to an incomplete blocking of contracture tension, which is very large at this tonicity, because the time-

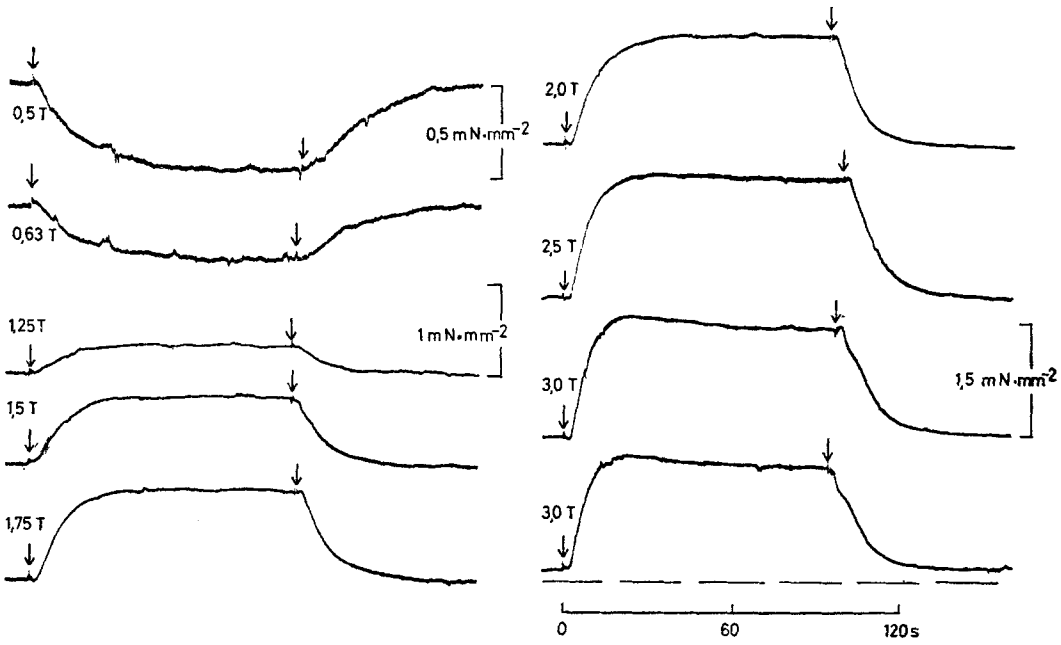

FIGURE 2. Time-course of isometric tension change at different tonicities. Change from Ringer's solution to test solution at first arrow, change back at second arrow. Tonicity given relative to that of Ringer's solution. Tetracaine added to Ringer's solution 1 min before change to hypertonic solution. Tetracaine concentrations were those given in Methods except for lower 3.0T record, for which concentration was 0.8 mM. This by itself caused a slight tension increase, indicated by the position of the base line above the interrupted line, which represents resting tension in Ringer's solution. Records in hypotonic solution without tetracaine. All records from same fiber, mean diameter 133 μm, sarcomere spacing 2.3 μm, resting tension at beginning of experiment 0.46 $mN \cdot mm^{-2}$.

course of tension development was completely unaffected by a change in tetracaine concentration from 0.5 mM (upper record) to 0.8 mM (lower record). At 0.8 mM concentration tetracaine itself caused a slowly rising tension, indicated by the position of the base line above the interrupted line, the resting tension level before applying tetracaine.

Hypotonic solutions were also applied to the same fiber and were found to decrease tension (Fig. 2 left-hand side, uppermost records). This agrees with the observation of Blinks (1965) that fibers, which are initially stretched beyond their slack length, become slack when they are exposed to hypotonic solutions.

Relation between Steady-State Tension in Hypertonic Solution and Fiber Size

The sustained tension increase caused by 1.75T solution is plotted against cross-section area for 16 fibers in Fig. 3 *a*. In spite of some scatter of the data

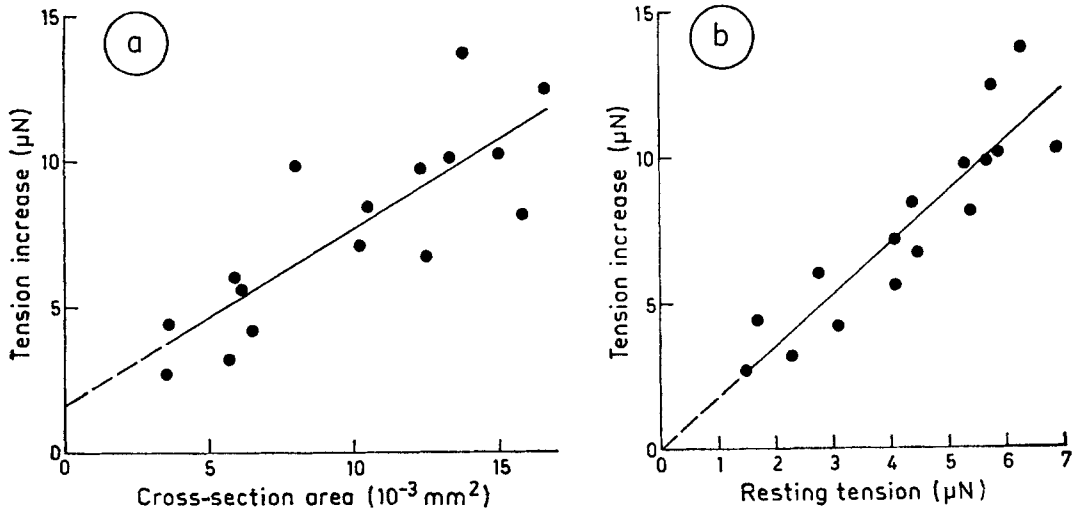

FIGURE 3. (*a*) Relation between tension increase and fiber cross-section area. Tension measured 90 s after change to 1.75T solution in the presence of 0.2 mM tetracaine. Sarcomere spacing 2.3 μm. Regression line fitted to experimental values by method of least squares, $r = 0.82$. (*b*) Relation between tension increase in 1.75T solution and resting tension. Values from the same fibers as in *a*. Regression line fitted by method of least squares, $r = 0.91$.

the two variables appear to be well correlated ($r = 0.82$). In Fig. 3 *b*, tension increase is plotted against resting tension for the same fibers. The scatter is clearly smaller in this case, which suggests that part of the scatter in Fig. 3 *a* is due to errors in the determinations of fiber diameter.

Relation between Steady-State Tension and Tonicity

The records in Fig. 2 suggest that the steady-state tension changes continuously with a change in tonicity. The results presented in Fig. 4 support this idea. Tension change values from 15 different fibers are plotted against the tonicity of the test solution. Only those records in which the tension was steady were measured. With this criterion values at 3.0T were difficult to obtain and only two fibers could be used. At this tonicity there was often a gradual decline in tension, such that the tension measured at 90 s was often lower than that at 2.5T or even 2.0T.

The form of the left-hand part of the curve is somewhat uncertain but zero tension probably lies somewhere between 0.5 and 0.63T; all the fibers tested were still taut at 0.63T whereas they became slack, as judged by their appearance in the microscope, at 0.5T.

Steady-State Tension Increase at Different Sarcomere Lengths

It is well known that the maximum tetanic tension of a single muscle fiber decreases as the amount of overlap of thick and thin filaments is reduced

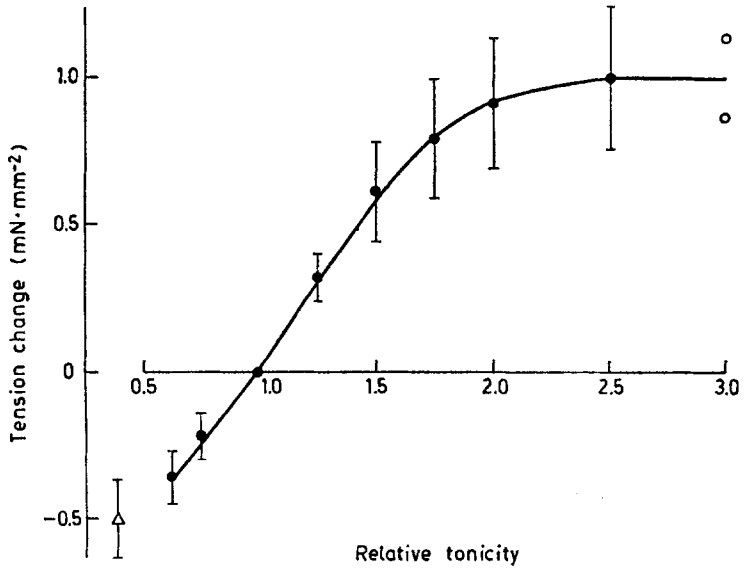

FIGURE 4. Relation between tension change and relative tonicity. Tension measurements made 90 s after tonicity change. Resting tension set to zero on the ordinate. Filled circles represent mean values of measurements on 6–13 different fibers, ±SD. Open circles, single observations. Open triangle indicates total loss of resting tension at 0.5T; all six fibers studied became slack at this tonicity. Tetracaine present only in hypertonic solutions (concentrations given in Methods).

by stretching the fiber (Ramsey and Street, 1940; Gordon et al., 1966). A similar reduction would be expected for the tension increase in hypertonic solution if some form of interaction between the filaments is responsible.

The tension change in hypertonic solution at different sarcomere lengths was determined in a total of eight fibers. A first group of experiments was performed in late spring on four fibers and mean value for the resting tension at four sarcomere spacings for this group is shown by the open circles in Fig. 5. Original records of the tension change after application of 1.75T solution in one of these fibers are presented in Fig. 6 *a*. The rise in tension became smaller with increasing fiber length, and the time-course of the tension rise and especially tension fall was affected. The collected data from all four fibers are given by the open circles in Fig. 7. Up to 2.9-μm sarcomere length the reduction in the steady-state tension increase was similar to the fall in tetanus tension observed when a fiber is held at increasingly greater lengths (interrupted line, taken from Fig. 5 of Ramsey and Street, 1940; cf. Gordon, et al., 1964). At 3.2 μm, however, the reduction was greater than expected, the mean value at this length being only 13% of that at 2.3 μm, instead of about 50% as obtained by Ramsey and Street.

A second set of experiments was performed in late autumn on four other

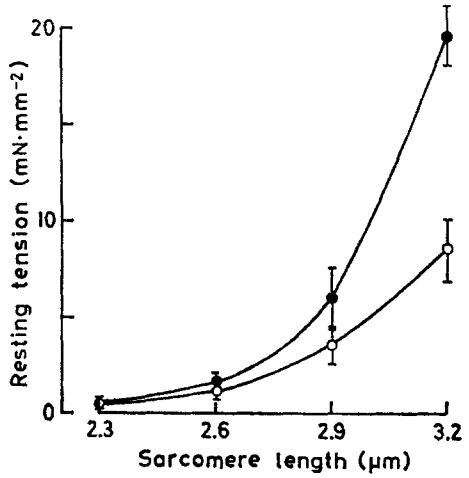

FIGURE 5. Relation between sarcomere length and resting tension. Measurements performed on fibers from two different batches of frogs. Four fibers used from each batch, mean values (±SD) from each group represented by different symbols. The measurements were taken 3 min after setting the length (open symbols), and 3–10 min after the length change (filled symbols).

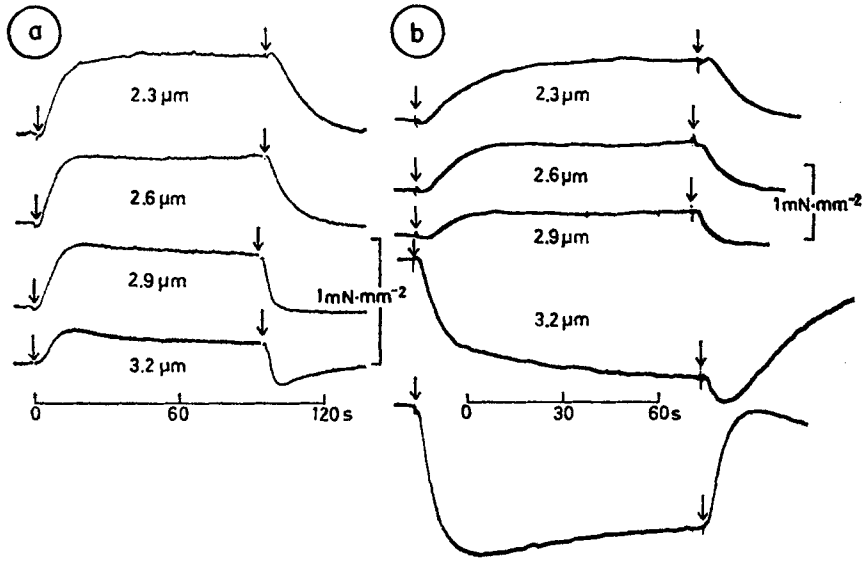

FIGURE 6. (*a*) Tension increase in 1.75T solution in the presence of tetracaine at four different sarcomere lengths. Change to test solution at first arrow, change back to Ringer's solution at second arrow. All records from same fiber, mean diameter 138 μm, resting tension at 3.2 μm 9.9 mN·mm^{-2}. (*b*) Same type of experiment on a different fiber. Resting tension at 3.2 μm 22.4 mN·mm^{-2}, mean diameter 136 μm. Lowermost record shows response of the same fiber about 70 min after destroying it with 10 mM caffeine ("sarcolemma preparation"). Preparation stretched (after damage) to give a tension of about 24 mN·mm^{-2} (about 150% stretch from reference length, 2.3 μm).

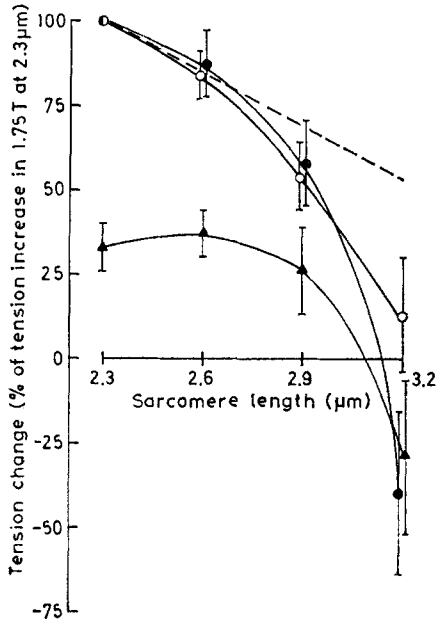

FIGURE 7. Tension change at different sarcomere lengths. Collected data from eight fibers. Circles represent means of measurements (±SD) performed 90 s after change to 1.75T solution. Values obtained from the same eight fibers, whose resting tension values were given in Fig. 5. Filled and unfilled circles, respectively, refer to same fiber groups as in Fig. 5. The four fibers represented by filled circles were also used for measurements in 1.25T solution (filled triangles). Tetracaine (0.2 mM) was present in all experiments. Interrupted line, taken from Ramsey and Street (1940, Fig. 5) indicates relation between tetanic tension in isotonic solution and sarcomere length.

fibers from a different batch of frogs. These fibers had a higher resting tension at corresponding sarcomere lengths than the previous group (Fig. 5, filled circles). The response to 1.75T solution for these fibers was similar up to 2.9-μm sarcomere length; at 3.2 μm however, all fibers responded with a tension *fall* instead of a slight tension rise. Original records from one of these fibers are shown in Fig. 6 *b*, and the mean values from this latter group of fibers are given by the filled circles in Fig. 7.

That those fibers which had a relatively low resting tension at 3.2 μm responded with a tension increase at this length, and that the fibers with a higher resting tension reacted with a tension decrease, suggests that the mechanical properties of the sarcolemma might influence the response at greater sarcomere lengths. There is evidence that the elasticity of the sarcolemma starts to contribute to the resting tension at about 3 μm (Casella, 1951; Podolsky, 1964; Fields and Faber, 1970) and it is possible that the tension in the sarcolemma itself decreases when the fiber shrinks in hypertonic solution.

In order to test this possibility the contractile substance in two fibers was

destroyed (after recordings had been made in hypertonic solution). This was done with the fibers held at 2.3-μm sarcomere length, by applying Ringer's solution containing 10 mM caffeine, which caused a large irreversible contracture. After 5–10 min in the caffeine contraction clots started to form inside the fiber with pieces of empty sarcolemma tube between the clots. During the disintegration process the tension of the preparation fell and eventually became negligible. After about 1 h, when no further change was seen, the preparation was stretched to give a tension similar to that of the intact fiber at 3.2 μm. This required an extension corresponding to a sarcomere spacing of about 3.5 μm in the intact fiber. 10 min later, when the tension was very nearly steady, 1.75T solution was applied. In both fibers this caused a rapid fall in tension of approximately the same amplitude as in the intact fiber, and a rapid rise when isotonic solution was reapplied (Fig. 6 *b*, bottom record). This result indicates that the fall in tension at greater sarcomere lengths is due to an effect on the sarcolemma.

In the second group of fibers, experiments were also performed with 1.25T solution (before destroying the fibers with caffeine). The mean values of the tension change at different sarcomere lengths are given by the filled triangles in Fig. 7. In contrast to the results with 1.75T solution, no clear reduction in the tension increase was seen in the range 2.3–2.9 μm and the mean value at 2.6 μm was in fact somewhat higher than at 2.3 μm. At 3.2-μm sarcomere length, however, the general effect was a tension fall, just as in 1.75T solution.

Tension Changes Caused by Constant-Speed Length Changes in Hypertonic Solutions

Hill (1968) showed that stretching a resting sartorius muscle at constant speed reveals an intial elastic resistance, followed after about 0.2% elongation ("elastic limit") by a frictional resistance. The short-range elasticity was ascribed to spring-like properties of a small portion of attached cross bridges thought to form long-lived contacts between the two sets of filaments in the resting muscle. Hypertonic solutions were found to have two effects: one was to increase the permanent tension of the muscle, the second was to increase the elastic resistance. Both effects were interpreted as being due to an increased "efficiency" of the cross-linking process in hypertonic solution.

Determinations of stiffness at higher tonicities may be difficult to interpret since the tension response to stretch is markedly changed by activation (Lännergren, 1971), which seems to occur above 1.7T (Lännergren and Noth, 1973). This difficulty may be avoided by the use of tetracaine as shown in Fig. 8. The upper record of the figure shows the tension response to a 33 $\mu m \cdot s^{-1}$ extension at 1.75T in the absence (upper curve) and in the presence of 0.2 mM tetracaine (lower curve). Without tetracaine there was a

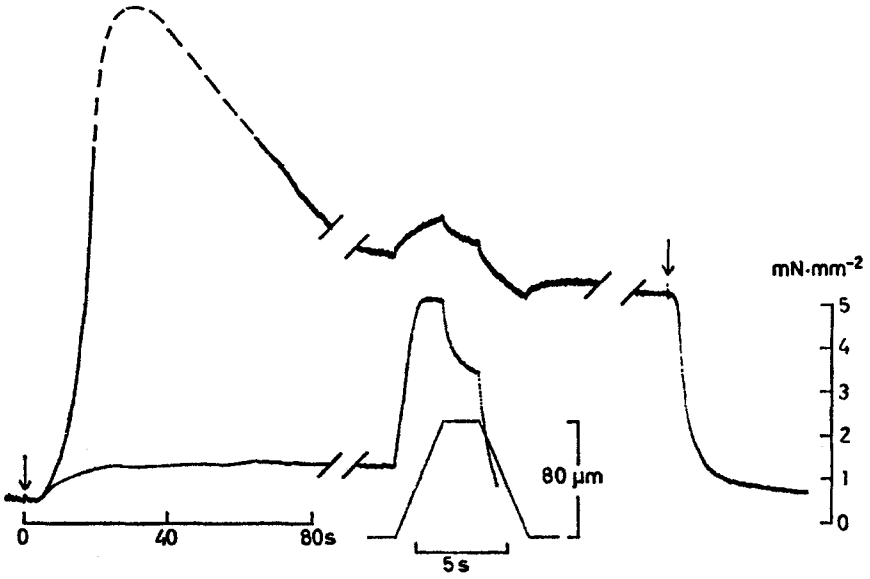

FIGURE 8. Tension change associated with constant-speed extension in 1.75T solution in the presence of 0.2 mM tetracaine (lower record) and in the absence of the drug (upper record). With tetracaine there was a small, maintained tension rise in the hypertonic solution (replotted from a record taken at two times higher amplification); without tetracaine the test solution caused a large, transient tension (not recorded in full because the pen was off scale). Mean fiber diameter 91 µm. Note that paper speed was five times higher during the length change.

large, transient tension rise (not shown in full) and a relatively small stretch response without a clear elastic limit. With tetracaine present the usual small tension rise was seen and the stretch response had a steep initial rising phase, a very clear elastic limit at about 50-µm extension and a distinct plateau during the later part of the length change.

Tetracaine itself did not influence the stretch response. This was tested both in Ringer's solution and in 1.25T solution, which was subthreshold for initiating contracture tension. In both instances 0.4 mM tetracaine, applied for 3 min, neither affected the amplitude nor the form of the tension response.

The diagram of Fig. 9 summarizes measurements of the initial stiffness of eight different fibers at various tonicities. Tetracaine was used at all tonicities above 1.5T. The stiffness is expressed in terms of Young's elastic modulus (E), calculated as described in the Methods. The E values are given on a logarithmic scale since they span a large range. The elastic modulus increased rapidly with tonicity over the whole range investigated, and very high values were obtained at 2.5T. This behaviour contrasts with the steady-state tension which showed a moderate rise up to 2T (2.8 times the isotonic value; see Fig. 4), and increased very little further at higher tonicities.

In order to test whether or not there was a velocity-dependent component of the stretch response, one fiber was subjected to length changes of widely

different speeds at 2T. The results are presented in Table I. The results show that the speed of the length change did not have a large effect on the length-tension relationship and indicates that viscous forces are relatively unimportant.

Data were also collected showing the variation of the elastic limit with tonicity and these are presented in Fig. 10. As was the case for elastic modulus an increase with tonicity was seen, but this was much less prominent.

Effects of Increased External Potassium Concentration

Some experiments were performed in which the external potassium concentration was changed. Tetracaine was not used in these cases. In two

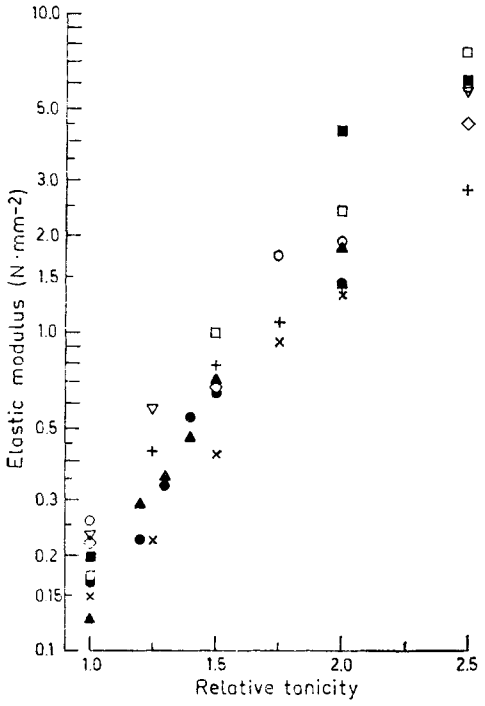

FIGURE 9

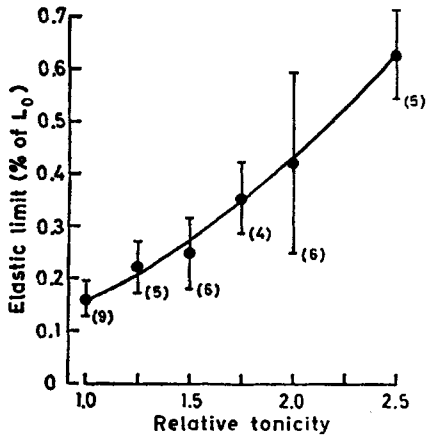

FIGURE 10

FIGURE 9. Relation between elastic modulus (logarithmic scale) and relative tonicity. Elastic modulus calculated from slope of initial part of tension rise at beginning of constant-speed stretch (about 25 $\mu m \cdot s^{-1}$) performed 2 min after tonicity change. All solutions from 1.5T and upwards contained tetracaine in concentrations given in Methods. Data from eight fibers, each denoted by a different symbol.

FIGURE 10. Relation between elastic limit and relative tonicity. Elastic limit measured as length change required to cause a sharp fall of the length-tension curve recorded during constant-speed extension. Symbols represent means of values from four to nine fibers, number of fibers at each tonicity given by numbers in brackets. Vertical bars indicate ±SD. Fiber lengths at 2.3 μm sarcomere spacing ranged between 12.0 and 14.0 mm.

experiments isotonic (95 mM) K_2SO_4 solution was applied which elicited a normal, short-lasting potassium contracture. 2 min after this had subsided and while the fiber was still in high $[K]_o$, a hypertonic (1.5T; 95 mM K_2SO_4 + sucrose) was applied. This caused a maintained tension rise of the same time course and amplitude as a standard 1.5T hypertonic solution (Ringer's + sucrose). This shows that a normal resting potential is not a prerequisite for the maintained tension rise.

In further experiments $[K]_o$ was increased from 2.5 to 15 mM before raising the tonicity, i.e., to a value close to the mechanical threshold. This has been shown before to reduce both the elastic modulus and the elastic limit in isotonic solution (Lännergren, 1971). Two of these experiments are depicted in Figs. 11 and 12, respectively. From the records of Fig. 11 it can

TABLE I
EFFECTS OF DIFFERENT STRETCH VELOCITIES ON THE MECHANICAL RESPONSE OF AN ISOLATED FIBER (DIAMETER 93.5 μm) BATHED BY 2.0T SOLUTION

Stretch velocity	Elastic modulus	Plateau tension ("frictional" resistance)
($\mu m \cdot s^{-1}$)	($N \cdot mm^{-2}$)	($mN \cdot mm^{-2}$)
1.7	2.1	3.8
17.0	2.0	6.1
151.5	2.3	8.7
1,540	2.8	14.7

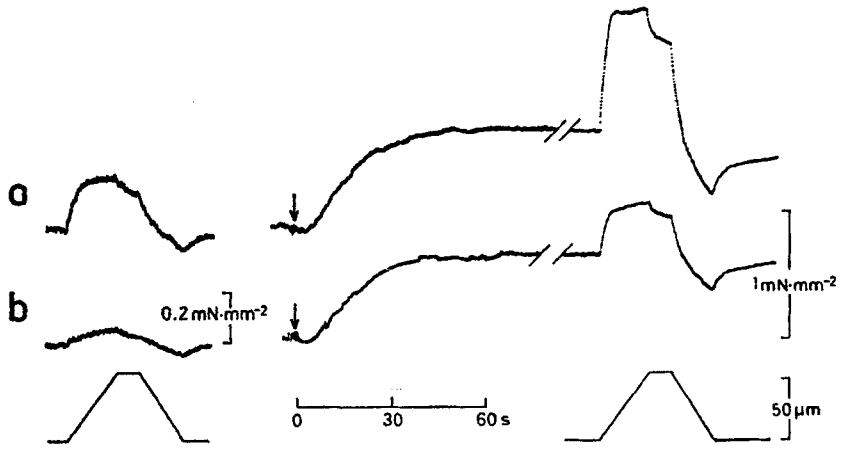

FIGURE 11. Effect of raised $[K]_o$ on tension rise and stretch-response in hypertonic- (1.5T) solution. (*a*) Left-hand record (at high amplification) is stretch-response in Ringer's solution. 1.5T solution was then applied (arrow) which caused a maintained tension increase. The stretch-response was recorded 2 min after the solution change. (*b*) Same as in *a* but $[K]_o$ was increased from 2.5 to 15 mM 3 min before the left-hand record was taken and maintained at this level throughout. Mean fiber diameter 118 μm. Time scale refers to tension rise in 1.5T solution; paper speed during length changes was 5 times higher.

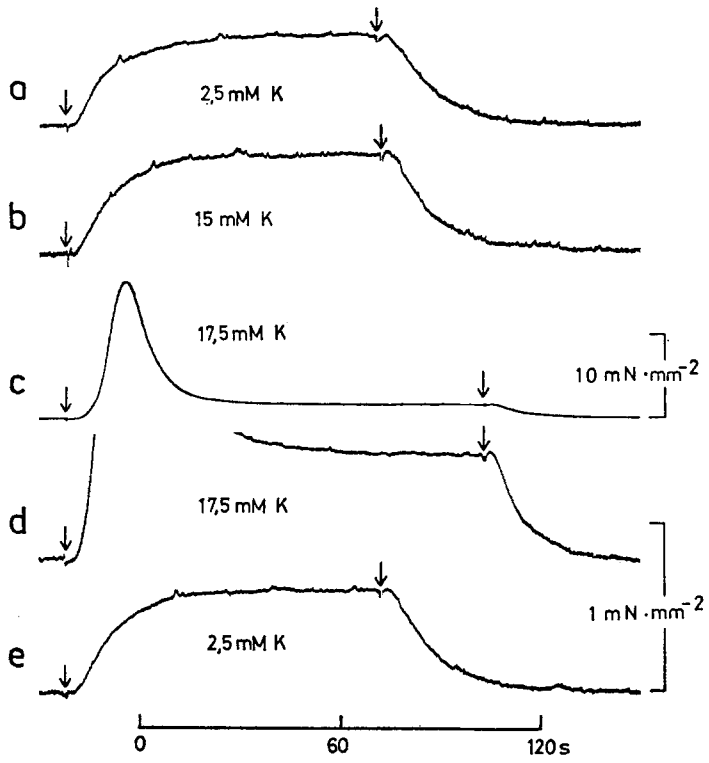

FIGURE 12. Effect of raised $[K]_o$ on tension rise in 1.5T solution. Change from isotonic to hypertonic solution at first arrow, change back at second arrow. *a* and *e* are controls at beginning and end of experiment, other records taken at $[K]_o$ raised to values indicated 2 min before tonicity change. Note different tension calibration in *c*, otherwise identical from top to bottom. Mean fiber diameter 138 μm.

be seen that although the stretch response was markedly diminished at a $[K]_o$ of 15 mM the hypertonicity-induced tension increase was not affected. In this case both E and the elastic limit were lower in the 15 mM-K hypertonic solution (*b*) than in the standard hypertonic medium (*a*). In other fibers the effect on E was small. The elastic limit, however, was always lower by 20–30%.

Records *a*, *b*, and *e* of Fig. 12 also show that 15 mM K had no effect on the maintained tension rise. The stretch response was not tested in this case but it is quite clear that the fiber was close to the mechanical threshold, since at 17.5 mM K, application of hypertonic (17.5 mM K) solution elicited a relatively large, transient contracture (*c* and *d*). When this subsided, tension settled to a level very close to that attained in 1.5T solution with normal (2.5 mM) K concentration. Exactly the same result was obtained in another fiber.

From these results it would seem that the normal activation process does

not interfere with the mechanism responsible for the maintained tension rise.

DISCUSSION

The purpose of the present investigation was to obtain information about the mechanism underlying the resting tension in muscle. The interest was aimed at the role played by the muscle fibers, hence isolated fibers were used in order to avoid tension contribution by stretched connective tissue strands and other structures in parallel with the fibers. The steady-state tension was changed by changes in the tonicity of the bathing solution. Such changes have long-lasting effects on the tension of unstimulated whole muscle (Hill, 1968). In a preceding investigation on isolated fibers (Lännergren and Noth, 1973) it was shown that increase in tonicity in fact has a composite effect on fiber tension: in the range up to 1.7 times normal tonicity only a small maintained tension increase is caused, above 1.7T a transient tension begins to be superimposed on the maintained tension. The development of transient tension, which can exceed 100 $mN \cdot mm^{-2}$ at about 2.5T, is reversibly blocked by tetracaine. In the present investigation tetracaine was used throughout to block transient tension development, since this was interpreted as being due to an activation of the normal tension-generating mechanism.

The resting tension itself was found to be proportional to cross-section area of the fiber. Hypotonic solutions decreased resting tension, hypertonic solutions increased it in a continuous way. At our standard sarcomere length (2.3 μm) doubling the tonicity caused the steady-state tension to increase about threefold. Higher tonicities caused very little further increase.

It now seems pertinent to discuss two questions, first: which structure(s) sustains the tension at this standard length, and second: what is the possible mechanism for the tension rise in hypertonic solution. Regarding structure there are three main possibilities. Both the sarcolemma and the sarcoplasmic reticulum, together with the central elements of the triads, form continuous elements running from one end of the fiber to the other. The actin and myosin filaments are arranged in discrete sets and thus do not form a continuous structure. It is possible, however, that the ends of the actin filaments are joined by other (ultrathin) filaments (Hanson and Huxley, 1955) or that stable bonds exist between the actin and myosin filaments (Hill, 1968).

Several reports indicate that the sarcolemma does not contribute to resting tension at normal fiber length (Casella, 1951; Podolsky, 1964; Fields and Faber, 1970; Rapoport, 1973). Our finding that the resting tension was proportional to cross-section area rather than to diameter also suggested that the material responsible for the tension is located inside the fiber. Furthermore, at greater lengths where the sarcolemma does contribute, the effect of hypertonicity was to cause a *fall* in fiber tension (Fig. 6 *b*). The sarcolemma

preparation reacted in the same way. The fact that the sarcolemma responded in a converse way makes the interpretation of results obtained at fiber lengths in this range complicated and means that a diminished response cannot be attributed to reduced filamentary overlap only.

The idea that part of the resting tension is borne by the sarcoplasmic reticulum has been suggested by Sandow (1966). He also proposed that latency relaxation is due to a reduction in its tension via an osmotic effect when calcium is released from the reticulum as a result of stimulation. The cross-section area of the longitudinal elements of the reticulum seems to *increase* in hypertonic solution (Birks and Davey, 1969). According to Sandow's hypothesis this would manifest itself as a tension increase. However, recent experiments on isolated, skinned crayfish muscle fibers (Kawai and Brandt, 1973), in which destroying the internal membrane system was found not to affect resting tension, do not support the idea that the reticulum acts as a load-bearing element.

For the actin filaments to be under tension in the resting fiber requires that they be in series with other structures. Hanson and Huxley (1955) have shown in glycerol-extracted myofibrils that even after dissolving away the myosin the fibrils returned to their original length when released after stretch. This indicates that there must be some material left in the A bands connecting the ends of the actin filaments. Hanson and Huxley called the hypothetical structure S filaments, but they have never been seen by electron microscopy. Their possible significance in relation to resting tension is unclear.

A further possibility is that the actin and myosin filaments are connected in some way. Hill (1968) proposed the idea that even in resting muscle some cross bridges are attached to the actin filaments. He furthermore assumed that these bridges are activated, and generate active "filamentary resting tension." Evidence for attached bridges derives from the behavior of a muscle (whole sartorius) stretched at constant speed. Tension rises rapidly during the first part of the stretch but remains constant after the "elastic limit" is reached, corresponding to about 0.2% change of muscle length. The "short-range elasticity" is equated with spring-like properties of the attached bridges which can be extended about 2 nm before their contacts slip. The concept of a "filamentary resting tension" is founded on the behavior of the muscle in hypertonic solutions in which a parallel increase in elastic modulus of the short-range elastic component and in steady-state tension was observed.

A short-range elastic component can be observed also in isolated fibers (Lännergren, 1971). The E value in isotonic solution is about the same as for the whole muscle. Hypertonic solutions up to about 1.7T increase E also in isolated fibers. In the absence of tetracaine, solutions above 1.7T cause the form of the stretch-response to change and no clear elastic limit

is seen (Fig. 8). The form of the stretch response in this range was similar to that seen when fibers are activated by caffeine or with elevated $[K]_o$ (Lännergren, 1971). This is consistent with the idea that strong hypertonic solutions activate the normal tension-generating mechanism. This activation is prevented by tetracaine and the time-course of the stretch-response is preserved in the high tonicity range. In the presence of tetracaine the effect on the steady-state tension and on the elastic modulus became dissociated, as can be seen when Figs. 4 and 9 are compared. This finding does not exclude cross-bridge action as a basis for the tension increase. However, the idea that each attached cross bridge generates a fixed amount of tension would not seem valid.

The large increase in the stretch-response relative to the tension increase is probably not due to increased internal viscosity since the resistance to stretch was only little velocity sensitive as demonstrated by Hill (at 1.85T, his Fig. 6) and confirmed by us (at 2.0T, Table I). It may still be that the internal milieu is altered in such a way in strongly hypertonic solution that attached bridges are able only to resist displacement but not to generate tension. This is an ad hoc assumption which has not been tested by the present experiments. It receives some support, however, by the results of Gordon and Godt (1969) who found that increased ionic strength markedly reduced isometric tension generation of skinned fibers under maximal calcium activation.

ADDENDUM

After submitting this manuscript for publication our attention was drawn by Prof. T. Sakai to a paper by K. Saito: The first major component of tension of the skeletal muscle in hypertonic solution (*Jikeikai Med. J.*, **18**:67 (1971). We regret that we had not previously noticed this article but are pleased to see that the results presented in our previous paper (Lännergren and Noth, 1973) agree well with Dr. Saito's findings.

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