# Tension in Isolated Frog Muscle Fibers Induced by Hypertonic Solutions

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ABSTRACT  The effect of hypertonic solutions on the tension of isolated
twitch muscle fibers of the frog has been investigated. Increased tonicity up to
about 1.7 times normal (1.7 T) caused a very small, graded, maintained
tension increase. Above about 1.7 T a large, transient contracture response
was superimposed on the small tension. The contracture response was graded
with tonicity and reached a maximum at 2.5 T of $108 \pm 25$ mN·mm$^{-2}$, a
third of the maximum tetanic tension in isotonic solution. Contracture tension
developed with a delay which decreased with increased tonicity. The con-
tracture threshold was lower and the delay shorter in small fibers than in
large. Contractures were obtained equally well in depolarized as in polarized
fibers. They were completely suppressed by 0.1–0.5 mM tetracaine. The pos-
sible mechanism responsible for the tension-inducing effect of hypertonic
solutions is discussed in terms of the close similarity between the properties of
these contractures and those caused by caffeine, and it is suggested that the
effect is due to a release of calcium from internal stores.

## INTRODUCTION

It is well known that the mechanical activity of skeletal muscle is suppressed in
hypertonic solutions (Overton, 1902; Ernst, 1926) although the excitability of
the cell membrane is little affected (Hodgkin and Horowicz 1957, Varga-
Mányi and Tigyi, 1962). The main suppressive effect is probably exerted
directly on the contractile elements themselves (April, Brandt, Reuben, and
Grundfest, 1968; April and Reuben, 1968; Isaacson, 1969, Gordon and Godt,
1970), but it cannot be excluded that the excitation-contraction mechanism is
also affected (Caputo, 1966, Gordon and Godt, 1970). It has recently been
found that hypertonic solutions also induce tension development. This was
first noted by Hill (1968) who found, using whole frog sartorius muscles, that
a change from isotonic to hypertonic bathing solution was associated with a
small, long-lasting, and reversible increase in tension. This was interpreted as
an increased efficiency of a process which is responsible for a part of the resting

tension. Gordon and Godt (1970) on the other hand, who used bundles of 2–40 fibers, found that the tension response to a two- to threefold increase in tonicity was large and mainly transient. Lännergren (1971) also observed transient tension responses when solutions of 1.5–2 times normal tonicity were applied to single frog fibers. Thus it seems clear that hypertonic solutions can elicit mechanical activity. However, no systematic study has been made of these contractures. In the present report details will be given about reversible and reproducible contractures induced by hypertonic solutions; it will furthermore be shown that the tension response can be divided into two separate components.

A preliminary account of this work has been given (Lännergren and Noth, 1972).

## METHODS

PREPARATION  The experiments were performed from August 1971 to March 1972 on Irish frogs (*Rana temporaria*) which were kept at 4°–7°C up to the time of use. The dorsal head of the semitendinosus muscle was dissected free and placed in a Ringer-filled Perspex trough. A single twitch fiber was isolated with the aid of jeweller's forceps and iris scissors. Fine stainless-steel hooks (50 and 80 $\mu$m, respectively) were attached to each trimmed tendon with two separate knots tied with 50 $\mu$m monofilament nylon thread. The distance between the nearest knot and the end of the muscle fiber was 150–200 $\mu$m. The fiber was then mounted in a horizontal channel (cross-section area 6.4 mm$^2$) in the dissection trough between a stiff hook, located near the inlet to the channel, and the moveable element of a force transducer. Two platinum wires at the bottom of the channel served as electrodes for electric stimulation. A continuous flow of Ringer solution was maintained through the channel between tests. A stop-cock system enabled quick changes to be made between different solutions. Shortly before a change to a test solution the rate of flow was increased to about 4.5 ml·min$^{-1}$ corresponding to a mean velocity in the channel of 12 mm·s$^{-1}$. The test solution was allowed to run in with the same speed. Tests with dye solutions indicated that it took about 1 s for a solution with this velocity to reach the distal end of the fiber after the stop-cock arm was turned. Tests were also made in which twitch tension was recorded when a fiber was stimulated electrically with 3 pulses·s$^{-1}$. A change was made from normal Ringer solution to a test solution in which all NaCl was replaced by choline chloride. The time between turning the stop-cock arm and disappearance of twitches was less than 2 s. The instant of turning the stop-cock arm was marked on the recording paper and is indicated by an arrow in the figures. Flow rates greater than 12 mm·s$^{-1}$ were used when potassium contractures were recorded.

TENSION RECORDING  The conditions for tension recording were very nearly isometric. A variable-capacitance force transducer was used with a mechanical resonance frequency of about 300 Hz and a compliance of 0.03 m·N$^{-1}$. The signal from the transducer was recorded on a chart recorder (for details see Lännergren, 1971).

MICROSCOPY   During the dissection the tendons of the preparation were held in clamps made from a split nylon rod and a stainless-steel tube. The clamps could be turned, which allowed the preparation to be viewed from desired directions. After a fiber had been isolated it was held just taut with its largest diameter perpendicular to the optical axis of the dissecting microscope and a measurement of the diameter was made with the aid of an ocular micrometer. The fiber was then turned so that the smallest diameter could be measured. The cross-section area ($A$) was approximated as $A = \frac{1}{4} \cdot \pi \cdot a \cdot b$ where $a$ is the largest diameter, $b$ the smallest; the mean diameter ($d$) was calculated as $d = (a \cdot b)^{1/2}$.

In the majority of the experiments the fiber was held at a length of 0.5 mm above slack length. Control measurements with a high-power microscope ($\times 1000$ magnification) indicated that this corresponded to a sarcomere length of about 2.2 $\mu$m. A few experiments were performed at a sarcomere length of 3.0 $\mu$m. This value was checked with the aid of the high-power microscope. In some experiments determinations of fiber volume were performed simultaneously with the registration of tension. The method was the following. A portion of a fiber, which was visually as nearly circular as possible, was photographed through the high-power microscope at $\times 138$ magnification at 5-s intervals before and during the application of hypertonic solution. The diameter of the fiber was afterwards measured directly from the film with the aid of a dissecting microscope fitted with an ocular scale. Since the length of the fiber was held constant, changes in fiber volume could be approximated as changes in diameter square. Volumes in hypertonic solutions are given relative to the volume in Ringer solution.

*Solutions*

The normal Ringer 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   Hypertonic solutions were in most cases prepared by the addition of solid sucrose to Ringer solution. The amount of sucrose required for a given osmolality was calculated from the equation used by Dydyńska and Wilkie (1963) which takes into account the small deviation from linearity for the relation between the amount of sucrose present and osmolality. The tonicity of the Ringer solution and of the various hypertonic solutions used was controlled with a freezing point osmometer and was found to be within $\pm 2\%$ of the calculated value. The normal Ringer solution had a tonicity of 234 mosmol/kg $H_2O$. The tonicity of the test solutions will be given relative to that of Ringer solution. Thus a 2 T solution contained 78.4 g sucrose/kg $H_2O$ and had a tonicity of 469 mosmol/kg $H_2O$; a 3 T solution contained 153.7 g sucrose/kg $H_2O$ and had a tonicity of 704 mosmol/kg $H_2O$. In some experiments hypertonic solutions with altered calcium concentration were used (0.2 and 10.0 mM, respectively). The alteration in tonicity caused by this change was compensated for by the addition or removal, respectively, of an osmotically equivalent amount of NaCl. Solutions with 10 mM Ca contained tris(hydroxymethyl)aminomethane (Tris) buffer (5 mM) instead of phosphate buffer.

POTASSIUM METHYL SULPHATE SOLUTIONS   In these all NaCl and KCl in the normal Ringer solution was substituted by $KCH_3SO_4$ (Hopkin & Williams, Ltd.,

Essex, England). When required the tonicity was increased by the addition of an appropriate amount of solid sucrose.

SOLUTIONS CONTAINING CAFFEINE OR TETRACAINE    Caffeine was added as solid substance (E. Merck AG, Darmstadt, Germany) and tetracaine as 2% stock solution (ACO Läkemedel AB, Solna, Sweden). No correction for the change in tonicity was made in these cases.

All solutions were made with double-distilled water from a quartz distiller. All chemicals, except $KCH_3SO_4$, caffeine, and tetracaine, were of analytical grade. The pH of all test solutions was 7.0–7.2. The experiments were performed at room temperature (20°–24°C).

### Experimental Procedure

The fibers were kept in the hypertonic solutions for times not exceeding 3 min since it has been shown (Gordon and Godt, 1970) that the recovery after long exposures is slow and sometimes incomplete. In many experiments, in which peak tension only was determined, a change back to Ringer solution was made soon after the time of maximum tension. The interval between application of test solutions was 15 min in a preliminary series of experiments; this however, was evidently too short since responses to repeated exposures became successively smaller and tetanus tension declined. In all later experiments 30 min rest was allowed between tests. With these precautions fibers remained in good condition for more than 10 exposures to hypertonic solutions in the range 1.5–2.5 T as judged by the lack of fall in tetanus tension (less than 15%) and the reproducibility of the response to a given test solution.

### RESULTS

### General Characteristics of the Tension Response to Hypertonic Solutions

The records in Fig. 1 are samples of the tension increase associated with a change from normal Ringer solution to a hypertonic medium (Ringer + sucrose). A solution with a tonicity 1.75 times normal caused a very small tension increase which started a few seconds after the stop-cock arm was turned and reached a plateau in 10–20 s (Fig. 1 A). Further increased tonicity caused a larger, transient response which started after a distinct delay of 10–15 s (Fig. 1 B and C). After the delay, tension rose rapidly, attained a maximum in about 25 s and then declined again. A solution with still higher tonicity (2.25 T) caused a large transient tension (Fig. 1 D).

Experiments on other fibers showed that the height of the plateau of the small, maintained tension increased with tonicity up to about 1.8 T. The maximum amplitude of the large transient tension was about 100 times greater than that of the small, maintained tension. Thus, with the amplification normally used to record the large response, the small tension increase could hardly be distinguished. In the following, reference will mainly be made to the large response and this will be called "contracture." Some properties of the

small, maintained tension change will be described in a later section of this paper; more details will be given in a later report.

The records in Fig. 2 were all taken at the same amplification and show the time-course of tension development at various tonicities. The contractures were in general transient but much longer lasting than potassium contractures in which spontaneous relaxation is complete within about 5 s at $[K]_o$ greater than 75 mM (Hodgkin and Horowicz, 1960). The latency decreased and the
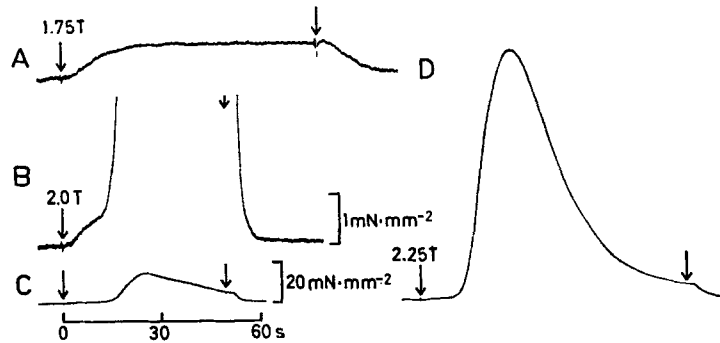


FIGURE 1. Tension increase in hypertonic solution. Change from Ringer solution to tes solution at first arrow, change back at second arrow. Tonicity increased by the addition of sucrose and given relative to that of Ringer solution. (A), small, maintained tension rise in 1.75 T. (B) and (C), simultaneous records of tension development in 2.0 T at high gain (B, same gain as in A) and low gain (C). Note that the first part of the response was similar to that in 1.75 T; after a delay contracture tension started and rose rapidly. Only the first and last part of the contracture is seen in (B). (D), tension development in 2.25 T recorded at low gain (same as in C). Fiber 35, mean diameter 112 $\mu$m. Tetanic tension (100 Hz) in Ringer was 306 mN·mm$^{-2}$.
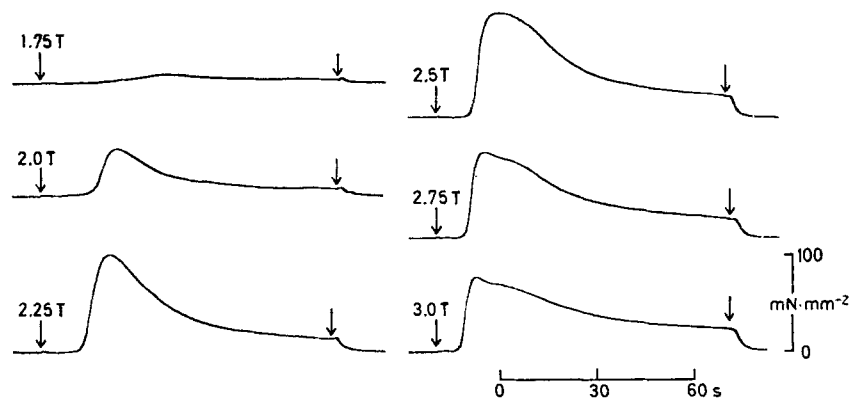


FIGURE 2. Time-course of contracture at different tonicities. Records from fiber 47, mean diameter 70 $\mu$m, taken in order of increasing tonicity. All records at same gain. Tetanic tension (100 Hz) in Ringer solution was 307 mN·mm$^{-2}$ at start of experiment and 300 mN·mm$^{-2}$ after last contracture.

rate of rise increased with increased tonicity. The rate of fall of tension was usually slower at high tonicities than at low. When the hypertonic solution was replaced by normal Ringer solution tension decreased towards the resting level after a short delay.

### The Relation between Tonicity and Peak Contracture Tension

The relation between peak tension per cross-section area and tonicity is shown in Fig. 3 which includes measurements from 34 fibers with mean diameters ranging between 43 and 149 $\mu$m. The curve fitted to the mean value for each tonicity is sigmoid in shape and starts between 1.5 and 1.75 T; it has a maxi-
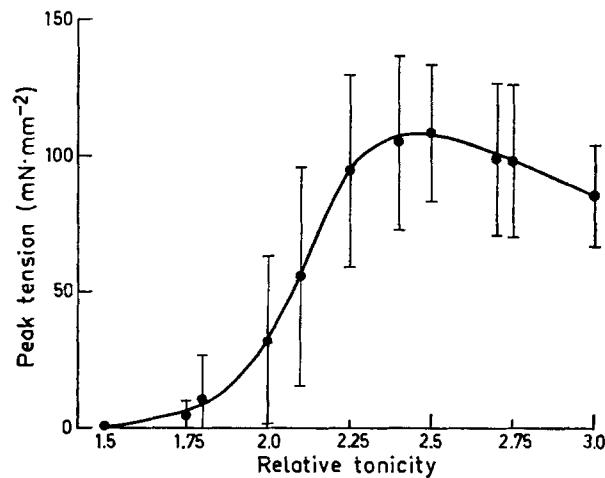


FIGURE 3. Relation between peak contracture tension and tonicity of test solution. Symbols represent means of observations from 34 fibers (10–14 fibers for each tonicity value). Vertical bars indicate ±SD. The mean value for tetanic tension in Ringer solution was 328 mN·mm$^{-2}$.

mum at 2.5 T and a slight fall at higher tonicities. The peak contracture tension at 2.5 T was 108 ± 25 mN·mm$^{-2}$ (mean ± SD, n = 14) which was 33% of the mean value for the maximum tetanic tension in isotonic solution (328 ± 25 mN·mm$^{-2}$).

In three fibers the tonicity-peak tension relation was determined at two different sarcomere spacings, 2.2 and 3.0 $\mu$m. The threshold and tonicity for maximum tension were unaffected by sarcomere length. However, the maximum tension at 3.0 $\mu$m sarcomere length was only 50–60% of that obtained at 2.2 $\mu$m.

The maximum tension elicited by hypertonic solution was about 110 mN·mm$^{-2}$. This is only about a third of the value reached with electrical stimulation, with high [K]$_o$ or with caffeine in which cases tensions of 300–350 mN·mm$^{-2}$ are attained (Hodgkin and Horowicz, 1960; Lüttgau and

Oetliker, 1968). This finding was analyzed further in experiments in which the maximum tension caused by tetanic stimulation, application of 117.5 mM $KCH_3SO_4$ and of 5 mM caffeine was measured in solutions with various tonicities. The results of such measurements from six different fibers are given by the filled symbols in Fig. 4. In the tonicity range where the hypertonic solution itself caused tension development the symbols represent the tension level obtained with hypertonicity plus caffeine or potassium, added a few seconds after
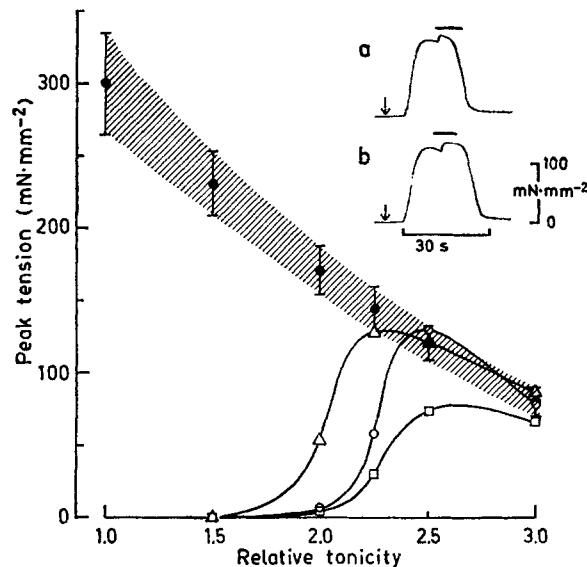


FIGURE 4.  Maximum tension at various tonicities. Modes of stimulation: at 1.0 and 1.5 T: electrical stimulation (100 Hz) or elevated $[K]_o$ (117.5 mM); at 2.0 T and higher tonicities: hypertonicity plus high $[K]_o$ or hypertonicity plus caffeine (5 mM). Mean values from six fibers indicated by filled symbols, ±SD shown by vertical bars and hatched area. Open symbols indicate peak value of tension elicited by hypertonic solutions only. $\triangle$, fiber 50, mean diameter ($d$): 66 $\mu$m; $\bigcirc$, fiber 51, $d$: 84 $\mu$m; $\square$, fiber 52, $d$: 114 $\mu$m. Inset: the effect of raising $[K]_o$ ($a$), or applying caffeine ($b$) at the moment when peak contracture tension (in 2.5 T) was reached. Period of application of test solution indicated by horizontal bar.

the peak of this tension was reached. The hatched band represents ± SD of a mean curve fitted to these points. It was found, in agreement with the results of Gordon and Godt (1970), that tension decreased with increased tonicity, regardless of the method of stimulation. The open symbols represent measurements of the tonicity-peak tension relation for three of the fibers with different diameters. It is seen that the curves fitted to the points for the two smaller fibers coincide with the maximum tension curve at about 2 T and that the curve for the larger fiber joins the upper curve at about 3 T. This means that above a certain tonicity both caffeine and potassium were ineffective in caus-

ing extra tension. This is also demonstrated in the records in the inset of the figure. It thus seems likely that the relatively small amplitude of the hypertonicity contracture is not due to incomplete activation of the fiber, but depends on a general limitation of the contractile response at high tonicities.

*The Influence of Fiber Size on the Tonicity for Half-Activation and on Contracture Latency*

The tonicity-peak tension curves of Fig. 4 suggested that the contracture threshold might vary with fiber size. In order to test this possibility data from 25 fibers with different diameters were analyzed. The tonicity required for half-maximum activation was determined from a curve fitted to tonicity-peak tension values for each fiber. Values so obtained were then plotted against mean fiber diameter and are given in Fig. 5. It is evident, in spite of a rather larger scatter, that fibers with a large diameter require a higher tonicity in order to be activated than thin fibers.

Fiber size also influence the time interval between the application of test solution and contracture tension development. This is shown in Fig. 6 which is a plot of the time between the change to hypertonic solution (2.75 T) and the time at which tension was half maximum. The plot clearly shows that the half-activation time increased with fiber diameter.
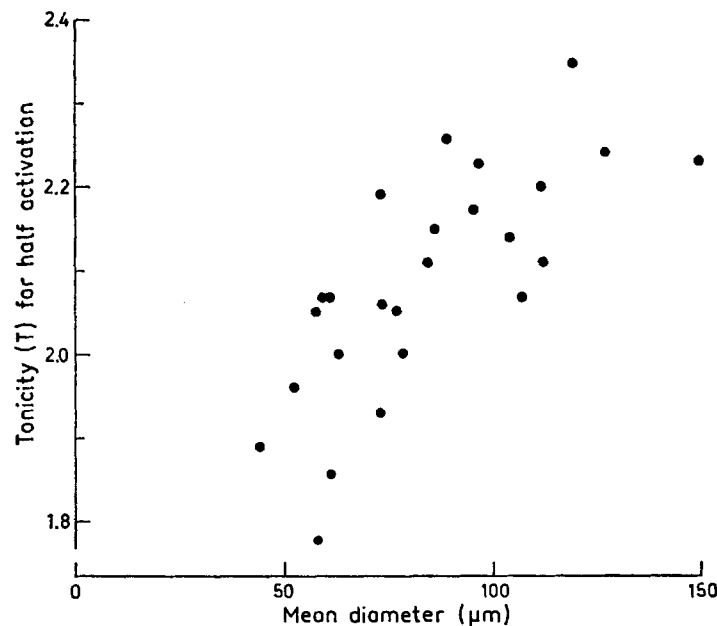


FIGURE 5.   Relation between tonicity of test solution needed to give half-maximum contracture tension and fiber diameter. Data from 25 fibers with range of mean diameter 43–149 μm.
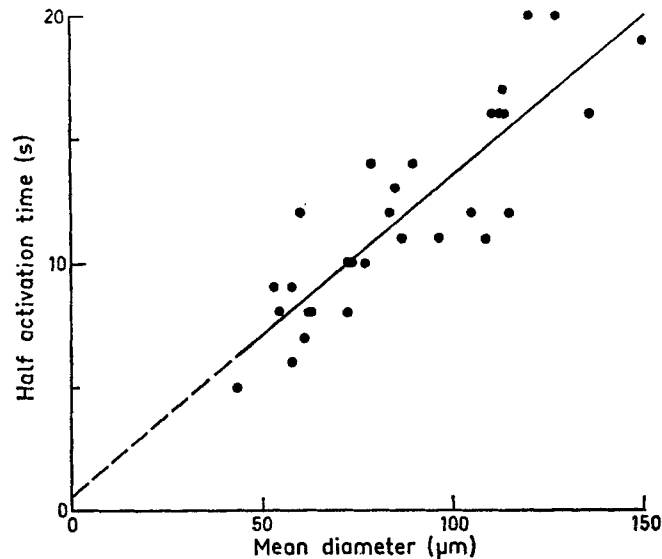
FIGURE 6.   Relation between mean fiber diameter and time to half-maximum tension in 2.75 T solution. Straight line fitted to the values by method of least squares; $r = 0.87$.

## Effects of Varying the Composition of the Hypertonic Solution

In order to determine whether the stimulating effect was due to sucrose per se or to increased tonicity only, some experiments were performed with 1.5–2.75 T solutions in which either (a) all NaCl in the normal hypertonic solution was replaced by an osmotically equivalent amount of sucrose or (b) the desired tonicity was obtained by the addition of NaCl to Ringer solution. It was found that the contractures induced by these modified solutions had a similar time-course to those elicited by the standard hypertonic solutions; the tonicity-peak tension relation was also the same. It is concluded that the contractures in hypertonic solution are not due to a specific effect of sucrose and that they are unaffected by the ionic strength of the solution.

## The Effect of Membrane Potential on Contractures

It seemed to be of interest to determine to what extent the tension response to hypertonic solution was affected by changes in membrane potential. To obtain information on this point fibers were depolarized by increasing $[K]_o$. The response to hypertonic solution was then tested, while the fiber was still in high $[K]_o$.

Fig. 7 illustrates two experiments of this kind. The first record in Fig. 7 A is of a contracture in normal 2.25 T solution. After 30 min rest, isotonic $KCH_3SO_4$ solution was applied which elicited a normal, short-lasting potassium contracture (middle record). The fiber remained in the high [K] solution
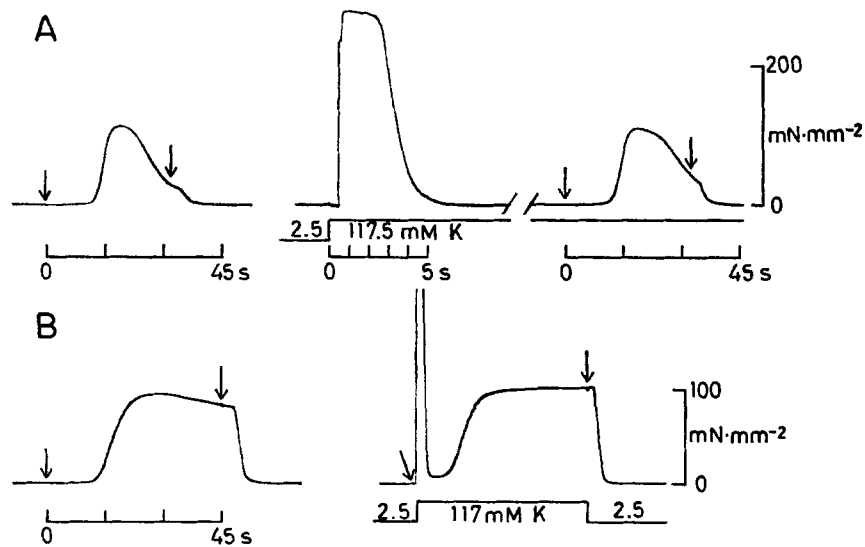
FIGURE 7. Contractures caused by hypertonic solution in depolarized fibers. (A), fiber 17, mean diameter 60 $\mu$m. Left, control contracture in 2.25 T; middle, change to 117.5 mM KCH$_3$SO$_4$ solution giving potassium contracture; right, response to increase in tonicity to 2.25 T 2 min later, when fiber was still in high [K]$_o$. Note different recording speed for response to hypertonic solution and high [K] solution. (B), fiber 53, mean diameter 96 $\mu$m. Left, response to 2.5 T solution, right, effect of increasing tonicity and [K]$_o$ simultaneously. The time scale to the left applies to both records in (B). Note different time-course of potassium contracture and contracture caused by hypertonic solution. Other experiments in which transducer gain was lower and the record not cut showed that the amplitude of the short latency response was little different from that of a K-contracture in isotonic solution.

for 2 min after which a hypertonic, high [K] solution was applied (right-hand record). This solution elicited a contracture with a time-course and amplitude strikingly similar to that of the control contracture. In the experiment of Fig. 7 B tonicity and potassium concentration were changed simultaneously (right-hand record). This caused a short latency, large, transient response, very similar to a normal potassium contracture and a long latency response which closely resembled the control contracture in normal hypertonic solution (left-hand record). The latency of the second response was in this case shorter than that of the control contracture. In other experiments of the same kind this tendency was less clear. The mean value for peak tension in hypertonic solution (2.4 or 2.5 T) applied 2 min after depolarization was 100 ± 26 mN·mm$^{-2}$ against 104 ± 34 mN·mm$^{-2}$ for the same 10 fibers in the polarized state.

From these results it seems clear that activation of fibers in hypertonic solution does not occur via membrane depolarization. Further evidence for this conclusion was the experimental finding that changes in the external calcium concentration from 2.0 to 10 mM (3 fibers) or from 2.0 to 0.2 mM (3 fibers)

neither changed the tonicity-peak tension relation nor the time-course of contractures which depend on $[Ca]_o$ as has been shown earlier (Lüttgau, 1963; Frankenhaeuser and Lännergren, 1967).

### The Effect of Tetracaine on Contracture Tension

The results presented above show that there is a close similarity between the stimulating effect of hypertonic solutions and caffeine. Caffeine contractures are long-lasting, they can be elicited in completely depolarized fibres (Axelsson and Thesleff, 1958; Lüttgau and Oetliker, 1968) and they are little influenced by $[Ca]_o$ (Frank, 1960, 1962). This similarity made it worthwhile to test whether or not local anaesthetics, which competetively inhibit caffeine contractures (Feinstein, 1963), also block contractures caused by hypertonic solutions. Tetracaine was chosen as the test substance since it was found to be the most potent of the agents tested by Feinstein (1963).

Figure 8 A, lower record, shows that tetracaine did have a blocking effect. About 2 s after a small dose (0.2 mM) was applied to a fiber, which exhibited a lasting contracture in 2.25 T, tension started to fall with a half-relaxation time of about 7 s. This relaxation time was longer than that after a change back to Ringer solution (about 2 s). Further information about the rate of action was obtained in experiments in which tetracaine and hypertonic solution were applied simultaneously. This resulted in a short-lasting contracture of reduced amplitude. When tetracaine was applied 15 s before the hypertonic solution complete block was observed.

The minimum concentration of tetracaine required to prevent contracture tension development varied with tonicity. It was 0.1 mM at 2.0 T and increased by about 0.1 mM for each 0.25 T increment so that 0.5 mM was required at 3.0 T.

Figure 8 B is from another fiber which gave a transient contracture in 2.0 T (top and bottom record). The middle record shows the effect of applying tetracaine (1 mM) before the tonicity was changed and then washing it away while the fiber was still in the hypertonic solution. Tension rose after a long delay and reached about 65% of the peak value without tetracaine. This was clearly much higher than that of the control contractures at the corresponding time (2 min exposure to hypertonic solution). This result, which was verified on three other fibers, shows that a tonicity change constitutes a lasting stimulus, the tension-evoking effect of which can be deferred.

### The Effects of Tetracaine on the Small, Maintained Tension Increase

In contrast to contracture tension, the small, maintained tension increase (see above) was unaffected by tetracaine. This is shown in Fig. 9. The response of a large fiber, i.e. fiber for which the contracture threshold would be expected to be high, was recorded at high amplification in 1.75 T solution (Fig. 9 A).
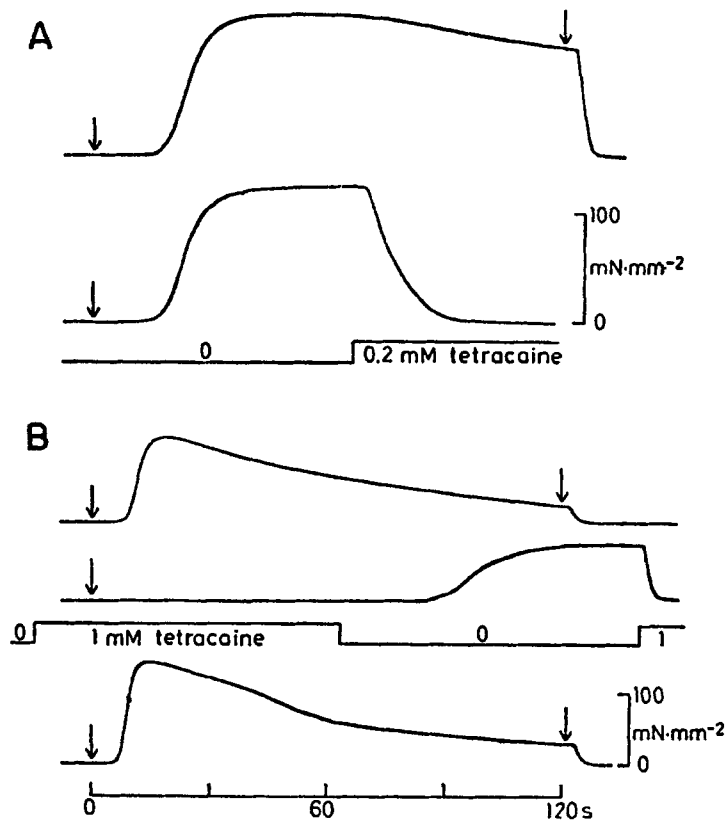
FIGURE 8.   (A), upper part, control record obtained when 2.25 T solution was applied (first arrow) and subsequently replaced by Ringer solution (second arrow). Lower record, effect of applying tetracaine during a lasting contracture. Note complete relaxation in 2.25 T solution. Fiber 38, mean diameter 107 $\mu$m. (B), upper and lower records are controls taken in 2.0 T solution. Middle record shows complete suppression of response when tetracaine was present. Tension started to rise after a delay when tetracaine was removed and reached 65% of control tension. Fiber 41, mean diameter 62 $\mu$m.

After the normal rest period in Ringer solution (30 min), tetracaine (0.2 mM) was applied for 15 s and a new record in 1.75 T (with tetracaine) taken (Fig. 9 B). It can be seen that neither the amplitude nor the time-course of the response was changed. A higher concentration of tetracaine (0.6 mM; Fig. 9 C) caused by itself a very slight, slowly developing tension rise, but again the response to the hypertonic solution was little affected.

### Volume Change and Tension Development

The time-course of the small tension change was roughly exponential with a time constant of 11.2 $\pm$ 2.1 s (mean $\pm$ SD, n = 20). This is about the same time constant as that for a volume change seen when hypertonic solution is

applied (Hodgkin and Horowicz, 1959, p. 145). This suggested that the two phenomena might be associated with each other. This possibility was tested in experiments in which tension and diameter measurements were performed simultaneously (Fig. 10). The change in volume was calculated from diameter measurements. This way of determining volume may lead to large errors
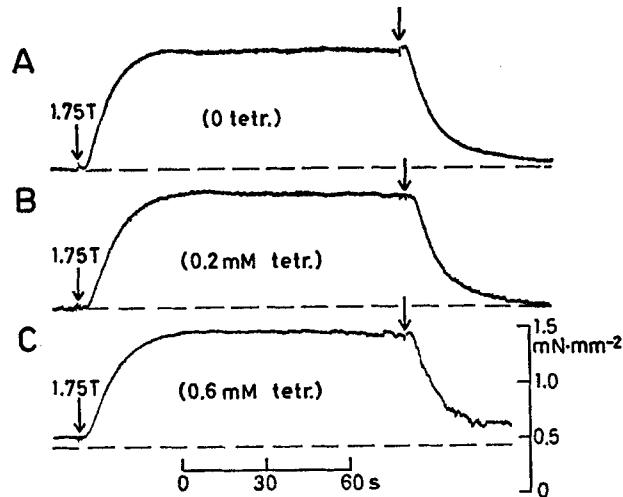
FIGURE 9. Effect of tetracaine on small, maintained tension increase at a tonicity (1.75 T) which was subthreshold for contracture development. Upper record, no tetracaine added; middle, 0.2 mM given 15 s before tonicity change; lower, 0.6 mM given 60 s before tonicity change. Horizontal, interrupted lines indicate resting tension level before application of tetracaine. Fiber 49, mean diameter 128 $\mu$m, resting tension, determined by recording the drop in tension, which occurred when the fiber was detached from the transducer, was 0.44 mN·mm$^{-2}$.
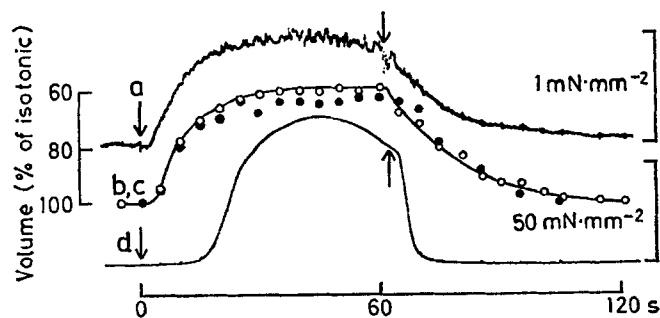
FIGURE 10. Tension increase ($a$) and volume change ($b$, open circles) associated with a change from isotonic to 2.5 T solution (first arrow) and back (second arrow) with 0.3 mM tetracaine present. Note the similarity in time-course of the two events. ($c$) and ($d$), volume change ($c$, filled circles) and tension development ($d$) in same type of experiment but without tetracaine. Note that the time-course of the volume change was little affected; contracture tension was delayed in onset, reached a high level and fell spontaneously. Fiber 44, mean diameter 150 $\mu$m.

because the cross-section of a fiber is usually irregular and the shape of the fiber may change during shrinkage (Blinks, 1965). We were aware of this risk and purposely selected for this experiment a large fiber with little difference between the largest and smallest diameter (160 and 141 $\mu$m, respectively). Two findings indicate that the diameter measurements during shrinkage did give reliable values for the relative volume: (a) the volume of a fiber equilibrated in 2.5 T solution would be 60% of that in isotonic solution if 67% of the total fiber water were osmotically active (Blinks, 1965). This is very nearly the value at the plateau in Fig. 10 *b*; (*b*) a second set of measurements taken 15 min later gave identical values (not included in the Figure). Thus, if it is assumed that the curve obtained from diameter measurements is a reliable index of volume change, then it appears that the time-course of the small, maintained tension increase was closely similar to that of the volume change. The situation was quite different when tetracaine was omitted and a contracture allowed to occur (Fig. 10 *d*). Contracture tension was delayed in onset and fell spontaneously after about 45 s. The change in volume, recorded simultaneously also in this case (Fig. 10 *c*), was little different from that seen when tetracaine was present.

DISCUSSION

*Comparison with Previous Investigations*

The main finding of the present investigation was that a single muscle fiber generated tension when a change was made from isotonic to hypertonic solution. Hypertonic solutions have been widely used to suppress the mechanical response to stimulation by electric current or by other means, but there are very few reports of tension development associated with the tonicity change itself. The first observation in this direction was probably that of Hill (1968) who described an "increase in resting tension" of whole sartorii occurring when the tonicity of the bathing fluid was raised. The increase was small and at 3.3 times normal tonicity reached about 8% of the maximum tetanic tension in isotonic solution; the increase was maintained at all tonicities. Isaacson (1969), also using whole sartorius muscles, similarly found a long-lasting contraction of 1–3% of tetanic tension at 2.5 times normal tonicity. He found an increased transmembrane calcium flux in hypertonic media and suggested the term "contracture" for the tension increase. Gordon and Godt (1970), working on small bundles of fibers, described phasic contractures in 2 T and 3 T solutions with mean values for the peak amplitude of 3.3 and 21.6% of tetanic tension, respectively. We found maximum tension to be developed at 2.5 T and the mean value at this tonicity was 33% of tetanic tension. As discussed earlier (Gordon and Godt, 1970; Lännergren, 1971) transient contraction of individual fibers together with a relatively slow diffusion in a whole muscle, causing

asynchronous activation of fibers, might explain why the contraction of an intact muscle is relatively weak and long-lasting.

### The Influence of Fiber Size on Contracture Parameters

Fiber size was found to affect contracture tension in several ways. The latency was shorter, the threshold was lower, and the rate of tension rise was higher in small fibers than in large. Peak tension per cross-sectional area was higher in small diameter fibers. The variation in latency and rate of tension rise with fiber size might be explained if it is assumed (a) that a critical relative volume has to be reached before tension starts to develop (see Fig. 10) and (b) that the permeability of the surface membrane is the main limiting factor for water efflux (and not diffusion within the fiber), which seems to be the case in muscle (Sorenson, 1971). The relation between surface area and fiber volume is larger the smaller the fiber, hence the time constant for water efflux will be shorter in a small fiber than in a large, and the critical volume will be reached earlier.

### Possible Mechanisms for Tension Generation in Hypertonic Solutions

It is generally accepted that normal contraction of muscle is due to a relative sliding of the two sets of filaments in each sarcomere. The first question that has to be considered in the present context is whether tension production in hypertonic solution depends on this normal mechanism or if it is caused in some other way.

It was found in the present experiments that the maximum contracture amplitude was only about a third of the tetanic tension in Ringer solution. However, hypertonic solutions, besides having an activating effect, also depress the mechanical response to all kinds of stimulation, setting an upper limit for tension development at about 50% of the isotonic value at 2 T (Fig. 4; Howarth, 1958; Gordon and Godt, 1970). If this limitation is taken into account, it appears that contractures in 2.25–3.0 T solutions have an amplitude which is 80–100% of the maximum possible in this tonicity range. This would seem difficult to obtain without involving the normal contraction mechanism. Our finding that the contracture amplitude was considerably reduced when the sarcomere spacing was changed from 2.2 to 3.0 $\mu$m, thus reducing the amount of overlap between the filaments, is in accordance with the idea that tension development in hypertonic solution is due to filamentary interaction.

It is generally believed that the intracellular free $Ca^{2+}$-concentration controls filamentary interaction under physiological conditions (Ebashi and Endo, 1968). In the resting state most of the calcium is stored in some part of the sarcoplasmic reticulum and the myoplasmic concentration is low. Activity is normally triggered by an action potential in the surface membrane which via the transverse tubular system causes a release of calcium from the stores. Tension development in hypertonic solution could either be due to interaction

with this normal mechanism or it could be the result of a more direct effect on the contractile elements. When a change is made to hypertonic solution, water is drawn out from the fiber which leads to an increased internal concentration of solutes and also to a decreased interfilamentary spacing which might start interaction and tension generation.

In order to try to decide between these two possibilities it might be of interest to compare the effect of hypertonic solution with that of caffeine, for which agent the mechanism of action is well known. Contractures caused by hypertonic solution and caffeine contractures have the following features in common: (a) Both types are relatively slow in onset and are long-lasting with respect to potassium contractures; recovery after a change back to normal solution is slow in both instances. (b) Both can be elicited in depolarized as well as in polarized fibres. (c) Both are little affected by changes in $[Ca]_o$. (d) Both can be completely and reversibly blocked by tetracaine in 0.1–1 mM concentration. Caffeine acts by causing a release of calcium from internal stores, most likely the sarcoplasmic reticulum (Bianchi, 1961; Frank, 1962; Isaacson and Sandow, 1967; Weber and Herz, 1968). Local anaesthetics, such as tetracaine and procaine, inhibit caffeine contractures by preventing calcium release (Feinstein, 1963; Gruener, 1967; Feinstain and Paimre, 1969); they do not affect the response to directly applied calcium (Gruener, 1967; Ford and Podolsky, 1972, p. 7). From the similarities mentioned above it may be inferred that hypertonic solutions exert their action via an effect on the calcium release mechanism.

The results of Homsher and Briggs (1968) from studies of transmembrane $^{45}Ca$ fluxes give further support for this idea. They found that $^{45}Ca$ efflux from frog sartorius muscles was more than doubled in 2.2 T solution whereas the influx was not changed. This requires the emptying of some internal stores, probably the sarcoplasmic reticulum. It is also interesting to note in this connection that Yamada (1970) found that the rate of heat production in 3 T solution rose 10–20 times above the level in isotonic solution which was interpreted as being due to a release of calcium and an increase in myosin ATPase activity. The threshold for this effect was slightly below 2 T which agrees well with the threshold for contracture tension development.

There is not sufficient information available at present to suggest a detailed mechanism for the calcium release. Two possibilities may be considered. It has been demonstrated that the longitudinal elements of the sarcoplasmic reticulum undergo marked swelling in hypertonic solutions (Birks and Davey, 1969). This might impair the binding capacity of the reticulum and allow calcium to escape into the sarcoplasm. Another alternative would be that the increased internal ion concentration, which results from the reduction in fiber volume, alters the Ca-accumulating properties of the sarcoplasmic reticulum.

The small tension increase seen in hypertonic solution when tetracaine was

present closely resembles the "filamentary resting tension" described by Hill (1968) in that there was no threshold for its development and that it was maintained as long as the test solution was left on. The present results give no clue to the mechanism behind the tension increase. It is clear, however, that in order to be able to study this kind of tension development at higher tonicities, tetracaine or some other blocking agent has to be used in order to prevent active tension production which is many times larger.

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