Hydrostatic compression in glycerinated rabbit muscle fibers

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ABSTRACT Glycerinated muscle fibers isolated from rabbit psoas muscle, and a number of other nonmuscle elastic fibers including glass, rubber, and collagen, were exposed to hydrostatic pressures of up to 10 MPa (100 Atm) to determine the pressure sensitivity of their isometric tension. The isometric tension of muscle fibers in the relaxed state (passive tension) was insensitive to increased pressure, whereas the muscle fiber tension in rigor state increased linearly with pressure. The tension of all other fiber types (except rubber) also increased with pressure; the rubber tension was pressure insensitive. The pressure sensitivity of rigor tension was 2.3 kN/m^2/MPa and, in comparison with force/extension relation determined at atmospheric pressure, the hydrostatic compression in rigor muscle fibers was estimated to be 0.03% Lo/MPa. As reported previously, the active muscle fiber tension is depressed by increased pressure. The possible underlying basis of the different pressure-dependent tension behavior in relaxed, rigor, and active muscle is discussed.

INTRODUCTION

The basic event which underlies muscle contraction is thought to be a cyclic interaction of cross-bridges (myosin heads) between thick (myosin) and thin (actin) filaments (Huxley, 1980; Huxley, 1985). However, the nature of the exact molecular process which results in development of interfilamentary force by attached cross-bridges remains unclear. Considerable work has been done on the interaction between isolated actin and myosin subfragment-1 in solution using hydrostatic pressure perturbation (Coates et al., 1985). Previously, we presented some observations on the effects of increased hydrostatic pressure on the isometric tension of skinned muscle fibers and examined the results in relation to biochemical findings. Basically, it was observed that the steady isometric tension in a maximally Ca-activated muscle fiber was depressed when exposed to high pressure (~1% per MPa; Geeves and Ranatunga, 1987) and that the extent of depression was dependent on the presence of the products of ATP hydrolysis (Fortune et al., 1989*a*).

The main aim of the present study was to examine the mechanical behavior of skinned muscle fibers in rigor and relaxed conditions under high pressure. The experimentally determined effects of increased pressure on the isometric tension of elastic fibers of known mechanical characteristics (e.g., glass, rubber) as well as of other biological materials (e.g., keratin, silk, collagen) are also presented for comparison with muscle fiber responses. A large body of knowledge is available on the mechanical behavior of various solid materials under high pressure (see Brandes, 1970) and, for convenient presentation and discussion of our results, the basic principles dealing with the mechanical behavior of an elastic body under high pressure are summarized in Methods.

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METHODS

Pressure chamber

The pressure chamber used in most of the present experiments was demonstrated to the Physiological Society (Geeves and Ranatunga, 1990). The chamber had a volume of 3 ml (1 × 1 × 3 cm) milled in a stainless block of 5 × 7 × 14 cm (see Fig. 1 *a*). Along its horizontal plane were seven ports; two of them were inlet/outlet ports for solution exchange, a third was connected to a high pressure line from a High Performance Liquid Chromatography pump (302 pump with 802C control unit; Gilson Medical Electronics, Villiers-le-Bel, France) and a fourth was used to house the pressure transducer (601A; Kistler Instrument Corp., Amherst, NY). The remaining three ports were for especially designed "transducer plugs" (see below); one of the transducer plugs carried a tension transducer (AE 801; Aksjeselskapet Mikro-Elektronikk, Horten, Norway), a second one carried a micrometer for length adjustment, and the third was not used in the present experiments. The top and the bottom faces of the chamber were fitted with Perspex (ICI Plastics, Welwyn Garden City, England) windows for illumination and visualization of the fiber, and the chamber could be opened for fiber mounting by removing the top window (see Fig. 1 *b*). The design of the apparatus was such that the entire length of the fiber, its attachments, and the force transducer were within the fluid-filled pressure chamber of 3 ml.

A transducer plug consisted of a brass cylindrical body and a stainless steel cap, and it was so designed that a steel tube or rod (3 mm diam) could be fitted through it and pressure sealed (see Fig. 1 *c*). In the tension transducer plug, an AE 801 element (Akers) was glued (with Araldite) at its base to the steel tube and its electrical connections were

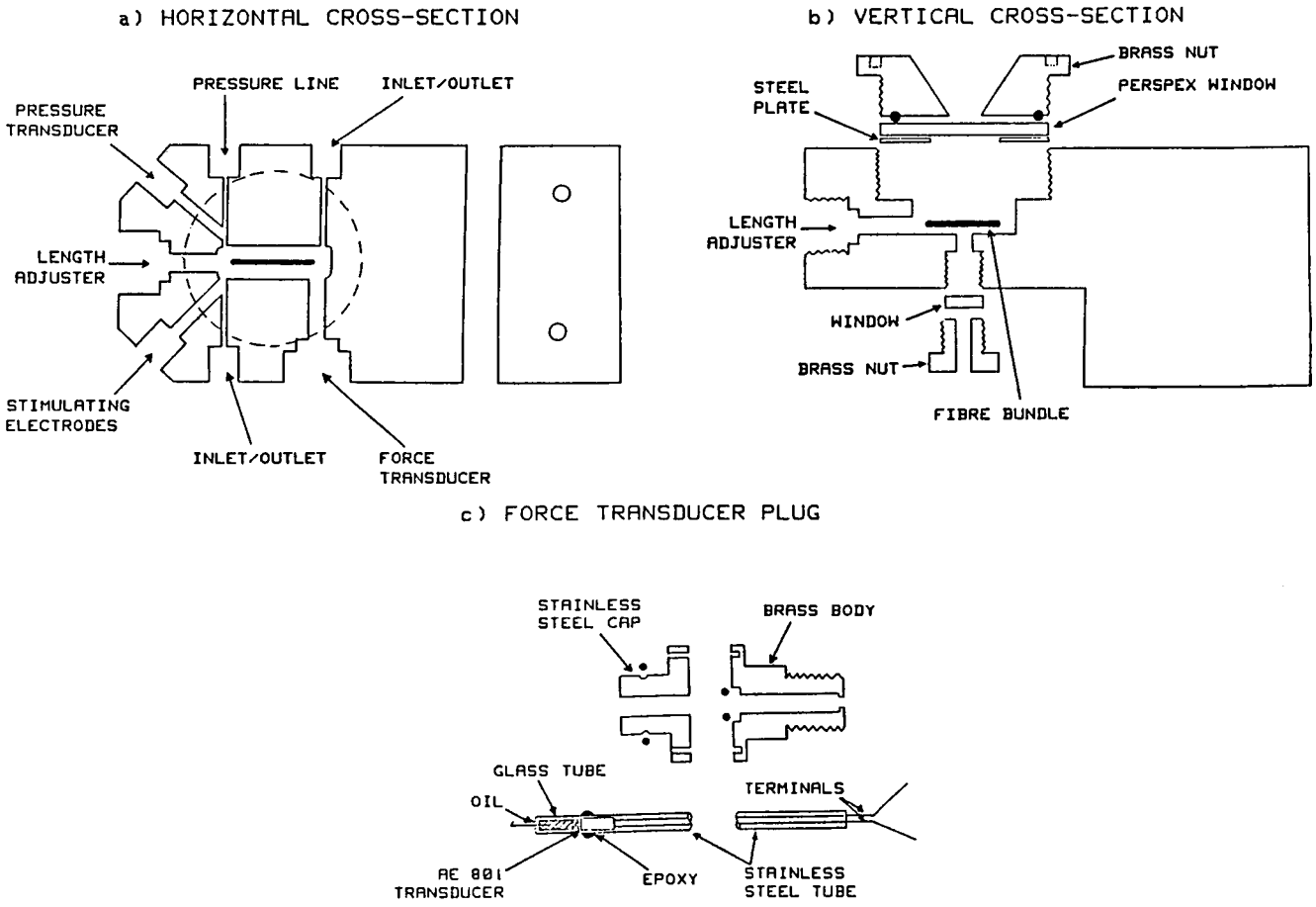

FIGURE 1 Schematic diagrams of cross-sections of the pressure chamber. *a* is a top view showing the seven ports in the horizontal plane. *b* is a section of the chamber in the vertical plane showing the two perspex windows (see text for details). *c* shows the design of the transducer plug. An AE 801 transducer element was glued to a stainless steel tube which could be pressure sealed. The beam of the element was enclosed in a close-fitting glass tube filled with paraffin. The entire beam and body of the transducer was within the pressure chamber. The filled circles indicate positions of some of the rubber O-rings used for pressure sealing.

made through the tube. The beam itself and part of the base of the transducer were enclosed within a close-fitting glass tube (see Geeves and Ranatunga, 1987) which was filled with paraffin oil for electrical insulation from the experimental solution. The natural resonant frequency of the transducer was 5–6 kHz, and the compliance <3 μm/mN. The output of the unloaded tension transducer showed little or no change on increase of pressure (<10 μN for 5–10 MPa).

Experimental materials

The materials used in the experiments were copper wires, glass fibers, rubber strands, human hair (keratin), silk threads (fibroin), collagen fibers, and glycerinated muscle fibers or fiber bundles. Copper wires were from a transformer coil, glass fibers were isolated from Glass Wool (BDH Chemicals, Eastleigh, Hants, England) and rubber filaments were drawn out from a rubber solution (Original Cow Gum; Cow Proofings Ltd., Slough, Berks, England) and dried at 70°C to remove petroleum. Individual silk filaments were isolated from surgical silk threads (Davis and Geck Cyanamid of Great Britain, Gosport, Hants., England), spun collagen fibers were a gift from Dr. W. Harrington (from Ethicon Inc., Somerville, NJ), and glycerinated muscle fibers were prepared as described previously from rabbit psoas muscle (Geeves and Ranatunga, 1987). Each fiber type was approximately cylindrical, and for convenience, all of them will be referred to as fibers. The width of different fibers ranged from 20 μm (glass) to 160 μm (rubber); muscle fiber bundles (3–6 fibers) were used to obtain comparable tensions. The lengths of preparations ranged between 4–15 mm in different experiments. In muscle fiber experiments under rigor and Ca-activated conditions, the fibers were set at rest length (sarcomere length ~2–2.6 μm) and the rigor tension was increased when necessary by small stretches (~1–2% initial length). In experiments on relaxed fibers, the fiber lengths were increased by stretching up to 1.6–1.8 times the initial length, to produce different passive tensions. Longer muscle fiber bundles were used for obtaining force/extension curves in rigor and shorter bundles for force/extension curves in relaxed condition.

Experimental procedure

A fiber was glued (with nitrocellulose adhesive) between two steel hooks, one of which was glued (with Araldite) to the tension transducer beam

and the other to the steel rod in the length adjustment plug; the latter was used to set an appropriate level of initial tension in the fiber. In the experiments reported here, the initial steady tension ranged from 0.3–5 mN. The chamber was filled with an appropriate aqueous solution (see below), the top Perspex window fixed and the chamber was sealed. Exchange of solution during an experiment could be done via inlet/outlet ports which were fitted with high pressure taps. The pressure was increased by means of the HPLC pump connected directly to the fiber chamber, and the pressure in the chamber was monitored by a separate pressure transducer. The compositions of the solutions used for muscle fibers are given in Table 1; experiments on nonmuscle fibers used the rigor solution.

With some fibers (e.g., glass and rubber) there were occassional indications of irreversible changes in tension, but in all the experiments reported here the tension recovery after an exposure to a high pressure was within 12% of initial tension.

Pressure effects on an isotropic fiber: basic principles

On the basis of the generalized Hooke's law, the axial strain (ϵ_a) for a cylindrical fiber of an isotropic material is given by

$$\epsilon_a = \frac{\sigma_a}{E} - \frac{\nu}{E}(\sigma_r + \sigma_t),$$

where (σ_a), (σ_r), and (σ_t) are, respectively, the axial, the radial, and the tangential (uniaxial) components of tensile stress (a, r, and t are equivalent to Cartesian coordinates), E is the elastic modulus and υ is Poisson's ratio (defined as, ν = − lateral strain/axial strain, i.e., ϵ_r/ϵ_a or ϵ_t/ϵ_a). Hydrostatic pressure imposes a volumetric stress, so that an increased hydrostatic pressure acts as a uniform compressive stress ($-p$). Thus, when a fiber, which is under tensile stress, is exposed to elevated hydrostatic pressure, it's net axial strain (ϵ_a^P) will be,

$$\epsilon_a^P = \frac{(\sigma_a - P)}{E} - \frac{\nu}{E}(\sigma_r + \sigma_t - 2P)$$

$$\text{i.e.} \quad E\epsilon_a^P = \underbrace{\sigma_a - \nu(\sigma_r + \sigma_t)}_{(1)\quad(2)} - \underbrace{P(1 - 2\nu)}_{(3)}.$$

The equation consists of a strain term (1), a stress or force term (2), and a pressure-dependent term (3). For most materials, Poisson's ratio (υ) is <0.5; therefore, a fiber of such a material with a constant endload (isotonic, i.e., component [2] constant) will undergo axial compression, or show a decrease in axial strain (ϵ_a), on exposure to high pressure. If axial compression is prevented, as when the fiber is held isometric, (i.e., component [1] constant) increased pressure will lead to an increase in axial force (term [2]). Rearranging the terms, the total axial force (= tension, F) is given by,

$$\frac{F}{A} = E\epsilon_a^P + P(1 - 2\nu),$$

where A is the cross-sectional area of the fiber. Thus, the axial tension would be independent of pressure if $\nu = 0.5$ but will increase with pressure if $\nu < 0.5$, the relation between axial tension and pressure being linear with a slope proportional to $(1-2\nu)$. The pressure sensitivity is independent of the value of E.

TABLE 1 Composition of experimental solutions

	Relaxing solution	Pre-activating solution	Activating solution
Imidazole	50	50	50
Mg-acetate	13	12	12
Na-ATP	11	11	11
Ca-EGTA	—	—	15
EGTA	15	0.5	—
K-propionate	61	106	62

Rigor solution contained 50 mM imidazole and 175 mM K-propionate only. Concentrations given in mM; ionic strength 200 mM; pH = 7 at 20°C.

RESULTS

"Nonmuscle" elastic fibers

Fig. 2 shows the effect of step increases in hydrostatic pressure on the tension developed by three fiber types, glass, collagen, and rubber; each fiber was stretched initially to maintain a steady tension. In glass and collagen, the tension increased in phase with pressure and the tension returned to the initial value on pressure release. In contrast, the tension in rubber changed little on pressurization. These records illustrate two useful points. Firstly, different tension changes are shown by different fiber types under similar experimental conditions; this indicates that the tension changes observed reflect properties of the fibers themselves and not in the elasticity in series with the fibers (e.g., glue and hooks). Secondly, the low pressure sensitivity obtained for rubber, in contrast to glass, was as expected on the basis of Poisson's ratios reported in the literature; glass has a ratio of 0.23 whereas that of rubber is close to 0.5. Fig. 3 shows plots of tension vs. pressure for glass and collagen, where data in each panel were obtained from the same preparation. These plots illustrate that as expected from the final equation given in Methods, the pressure sensitivity is approximately independent of the initial tension. Similar results were obtained for copper, keratin, and silk fibers (not shown).

Pooled data for all fiber types are given in Table 2. The differences in pressure sensitivity of tension among the different fibers are clearly evident. Poisson's ratios are available for glass, copper, and rubber (0.23, 0.35, and 0.5, respectively; Cowie, 1973) and the finding that pressure sensitivity decreases in sequence glass > copper > rubber is as expected. The Poisson's ratios calculated from our average data (Table 2) are 0.25, 0.47, and 0.5. The ratios and the average values of pressure sensitivity obtained for glass and rubber are of the expected magnitude; a discrepancy exists, however, with respect to copper. Because the width of the copper wire was 2–4-fold larger than glass, the compliance in the tension recording may have been a limiting factor. It is of interest to note

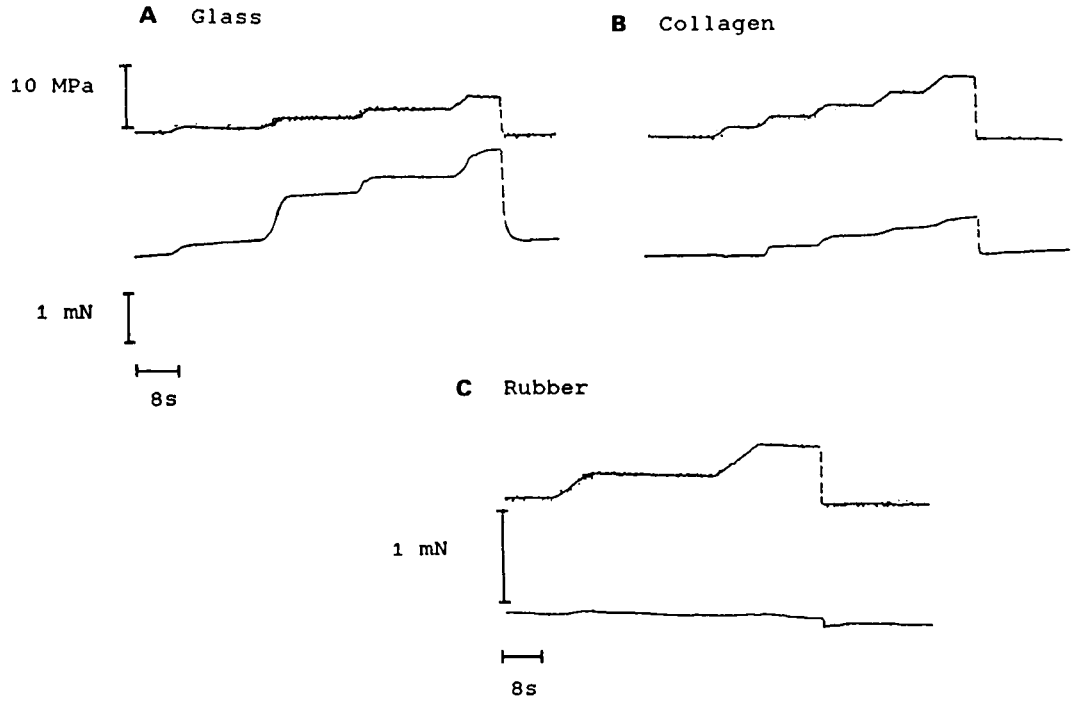

FIGURE 2 Oscilloscope traces of tension records from glass (*a*), Collagen (*b*), and rubber (*c*) fibers. Each panel shows the pressure trace (*top*) and the tension trace; zero tension is indicated by the horizontal bar near the base. In all nonmuscle fibers, except rubber, the tension increased proportionately with pressure. Note that some of the traces were retouched.

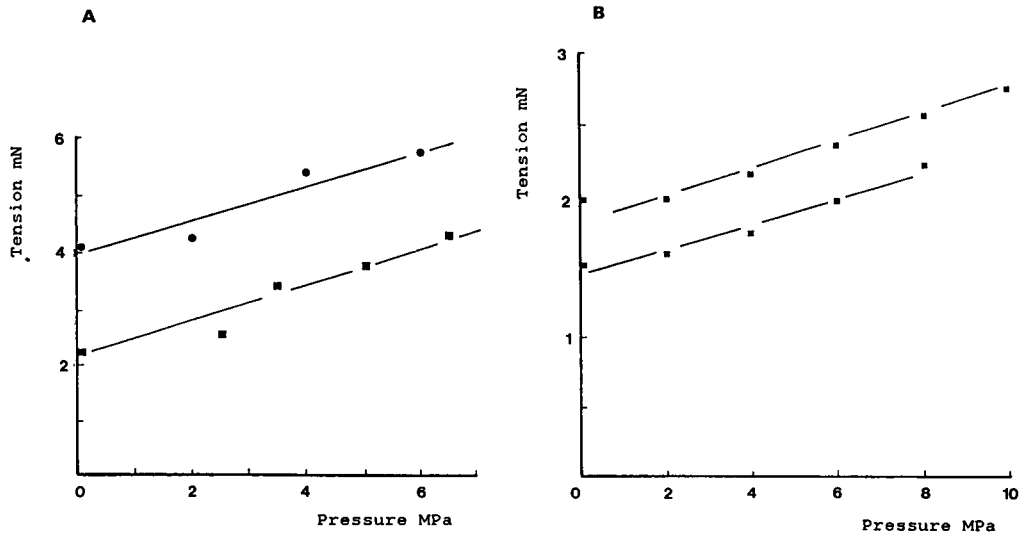

FIGURE 3 Plot of tension vs. pressure for a glass fiber (*a*) and a collagen fiber (*b*); lines were fitted by eye. The pressure sensitivity (*slope*) remains approximately similar at different initial tensions. The diameter and the length, respectively, were 17 μm and 8 mm for glass and 110 μm and 4 mm for collagen.

TABLE 2 **Pooled tension data from "nonmuscle" fibers**

	Glass	Copper	Keratin	Silk	Collagen	Rubber
No. of fibers	(3)	(1)	(2)	(2)	(3)	(4)
Initial tension (range, mN)	0.8–4	4–5	1.7–3.9	2.6–4	1.9–6.5	0.3–1
Initial tension (kN/m^2) mean	1978	380	1096	607	379	30.2
±S.E.	427	5	181	267	90	6.4
(n)	(11)	(3)	(5)	(4)	(10)	(8)
Tension change by pressure ($kN/m^2/MPa$)	503	58	63	42	8.9	−0.04
±S.E.	145	9	10	17	3.4	0.14
(n)	(11)	(3)	(5)	(4)	(10)	(8)

Each value in the bottom two rows is a mean ± S.E.M. and n, number of multiple pressurizations. The last row gives the pressure sensitivity of tension (or volumetric stress) of the fibers.

also that the glass–copper–rubber sequence for the elastic moduli (E) is different (60, 120, 0.02 GN/m^2, respectively) from that of Poisson's ratio (Cowie, 1973).

Muscle fibers

Fig. 4 shows representative tension records from a muscle fiber bundle exposed to different hydrostatic pressures. The passive tension in a relaxed fiber bundle was relatively insensitive to pressure changes (see Fig. 4 *a*). When the bundle was put in rigor, the measured tension increased with pressure (Fig. 4 *b*), whereas when the muscle fibers were maximally Ca-activated the steady active tension decreased with increase of pressure. The tension behavior of maximally Ca-activated muscle fibers has been examined in detail in the previous studies (Geeves and Ranatunga, 1987; Fortune et al., 1989*a*). The behavior of the fibers in relaxed and rigor conditions were examined in more detail in the present study.

Fig. 5 *a* contrasts the difference seen between the passive and the rigor tensions in a preparation, where the initial tensions were set at similar levels by stretching the fiber bundle. The experiments (n = 5) clearly showed that the difference in the tension behavior between a relaxed (passive) and rigor fiber was evident irrespective of the differences in the initial tension.

Rigor tension data in Fig. 5 *a* were collected after three series of sequential pressurization and release. Within certain tension and pressure limits (see below), data could be collected repeatedly from a fiber in rigor to character-

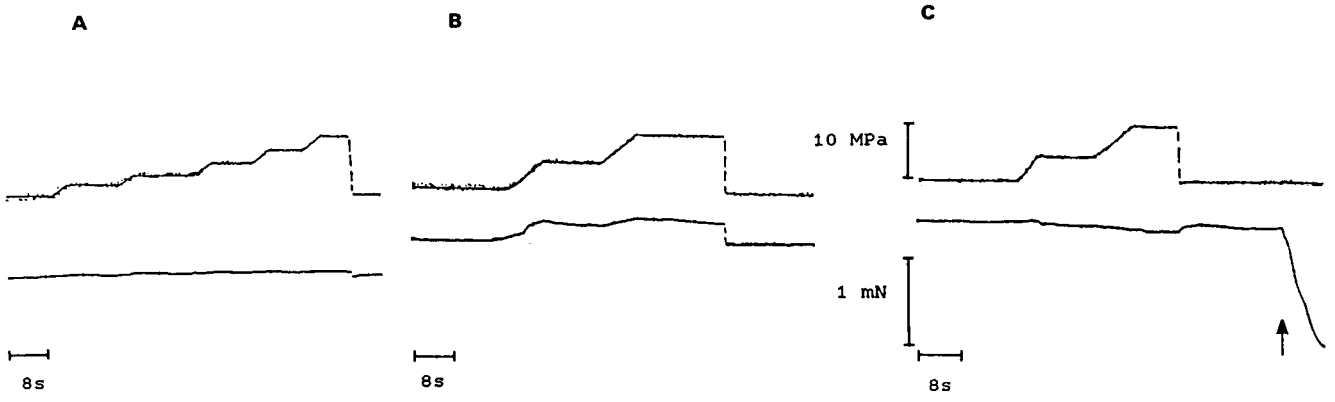

FIGURE 4 Tension records from a muscle fiber bundle exposed to different hydrostatic pressures. In (*a*) the bundle was relaxed but stretched to obtain a steady passive tension; (*b*) the bundle was put in rigor; (*c*) the bundle was maximally Ca-activated during pressure perturbation but was subsequently relaxed (indicated by the arrow). The fiber bundle contained 4–5 fibers with an overall cross-section of 0.12 mm and length of 4 mm. In each frame, the zero tension is indicated by the horizontal (time scale) bar. Note the qualitatively different tension behaviors.

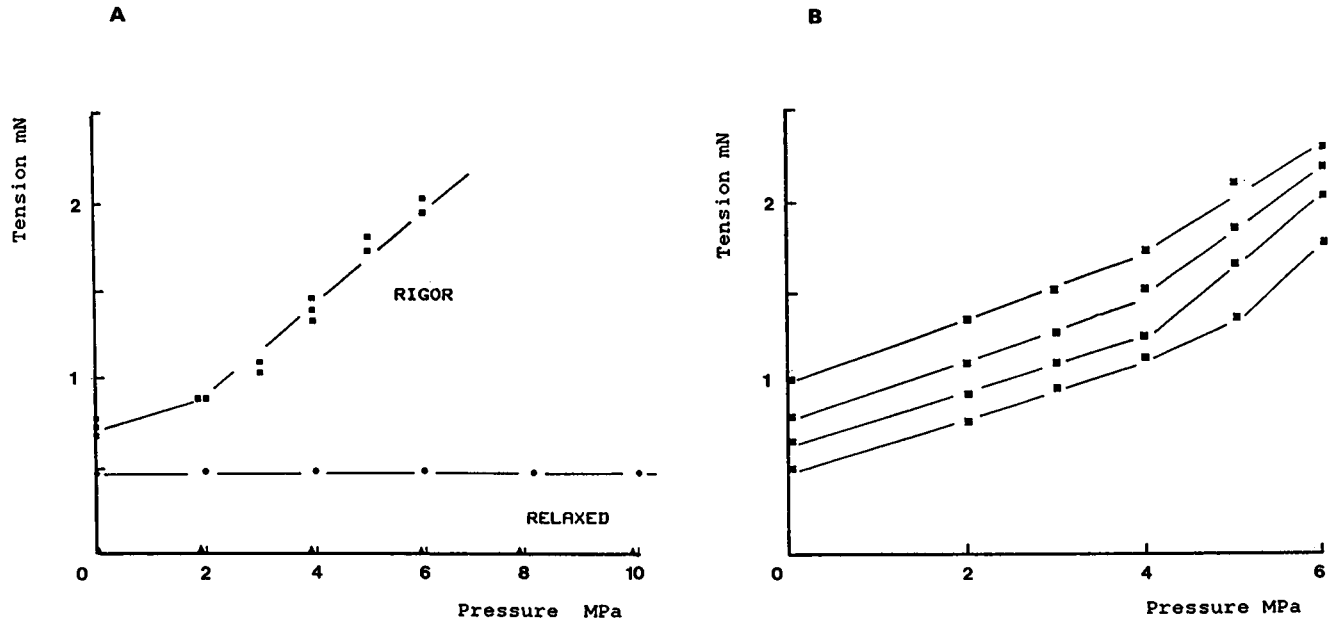

FIGURE 5 (*a*) A plot of tension versus pressure from one muscle fiber bundle, in relaxed (*circles and triangles*) and rigor (*squares*) conditions, where the initial tensions were adjusted to be approximately equal to illustrate the difference. Data for passive tension are averages from three separate pressure perturbations (range was within the diameter of the symbol), whereas those for rigor are individual measurements. (*b*) Data from the same preparation to show the pressure sensitivity of rigor tension at different initial rigor tensions. Within certain limits of pressure and tension (see text) the pressure/tension plots in a given preparation remained parallel (bundle diam 280 μm; length 5 mm).

ize the pressure sensitivity of its tension. The results show that the relation between increase of rigor tension and pressure was approximately linear, although a slight upward curvature was often indicated in the data. Additionally, the pressure sensitivity of rigor tension was found to be independent of the initial tension level; thus, as seen in Fig. 5 *b*, rigor tension vs. pressure curves at different initial tensions remained approximately parallel to one another. This overall behavior seen in Fig. 5 *b* is essentially similar to that shown by "nonmuscle" elastic fibers in Fig. 3. Table 3 summarizes the data from muscle fibers; the initial tension level and the pressure sensitivity are presented as in Table 2.

It was observed in several previous experiments that there was considerable variability between muscle fibers in the absolute pressure sensitivity of rigor tension and, in some experiments, the rigor tension was reduced to values considerably less than the control in release from high

TABLE 3 Pooled data for passive and rigor tension from glycerinated muscle fibers

	Relaxed	Rigor
No. of fibers	(6)	(9)
Initial tension (range, *mN*)	0.01–0.8	0.08–1.5
Initial tension		
(kN/m^2) mean	19.0	25.4
±S.E.	8.3	6.5
(*n*)	(10)	(30)
Tension change by pressure		
($kN/m^2/MPa$)	0.065	2.31
±S.E.	0.04	0.3
(*n*)	(10)	(30)

Data presentation is similar to Table 2. For active tension, the pressure sensitivity is negative; taking previously published data (Geeves and Ranatunga, 1987; Fortune et al., 1989*a*), the change of steady active tension by pressure would be -1 to -2 kN/m^2/MPa.

pressure (10 MPa). In two separate experiments we attempted to characterize this irreversible loss in rigor tension. The results showed that, in a given preparation, the loss of rigor tension upon release (to atmospheric pressure) was greater when it was pre-exposed to a higher pressure; for example, the mean (± S.E. of mean) recovered tension was only 38 ± 9% (n = 11) of the control when released from 10 MPa in contrast to a recovery of 105 ± 9% (n = 7) obtained after release from 5–6 MPa. Additionally, the loss of tension after release from the same hydrostatic pressure was greater when the rigor tension was initially set to a higher value by a slight stretch. The interaction between these factors which appear to determine the extent of tension of recovery is shown by the data in Fig. 6 *a*, where the percentage recovered tension is plotted against the initial tension for two different levels of pressurization. The possibility of "slippage" of rigor cross-bridges to low tension positions has been proposed (Ferenczi et al., 1984; Ferenczi et al., 1982) to explain lower tension in rigor than in active fibers; such an effect may be enhanced at release of high pressure.

In addition to estimating the pressure sensitivity of tension as described above, the force-extension relation (at atmospheric pressure) was examined for some fiber bundles. The data were used to determine the stretch required to produce an equivalent tension increase at atmospheric pressure and, thereby, estimate the extent of pressure-induced compression in a muscle fiber. The force-extension relation was shallow and curved for relaxed muscle fibers requiring large extensions (of 30–50% Lo; Horowits and Podolsky, 1987) whereas it was steep and linear in rigor fibers requiring extensions of only ~1% Lo (Lo = fiber length at rest). Data from one experiment are shown in Fig. 6 *b* where the circles and the line drawn through them represent the force extension relation of a fiber bundle in rigor, and for comparison that in the relaxed condition (*triangles*) is also plotted (line near the abscissa). The square symbols indicate the pressure sensitivity of rigor tension (per 10 MPa) obtained at two different length settings, so that the line connecting them represents the force extension relation at 10 MPa. The horizontal separation between the two force-extension relations (length of the interrupted line) would correspond to the extent of compression induced by 10 MPa increase of pressure. When represented as % Lo/MPa, the estimates for hydrostatic compression in rigor fibers ranged from 0.02 to 0.035; the mean ± S.E. of mean was

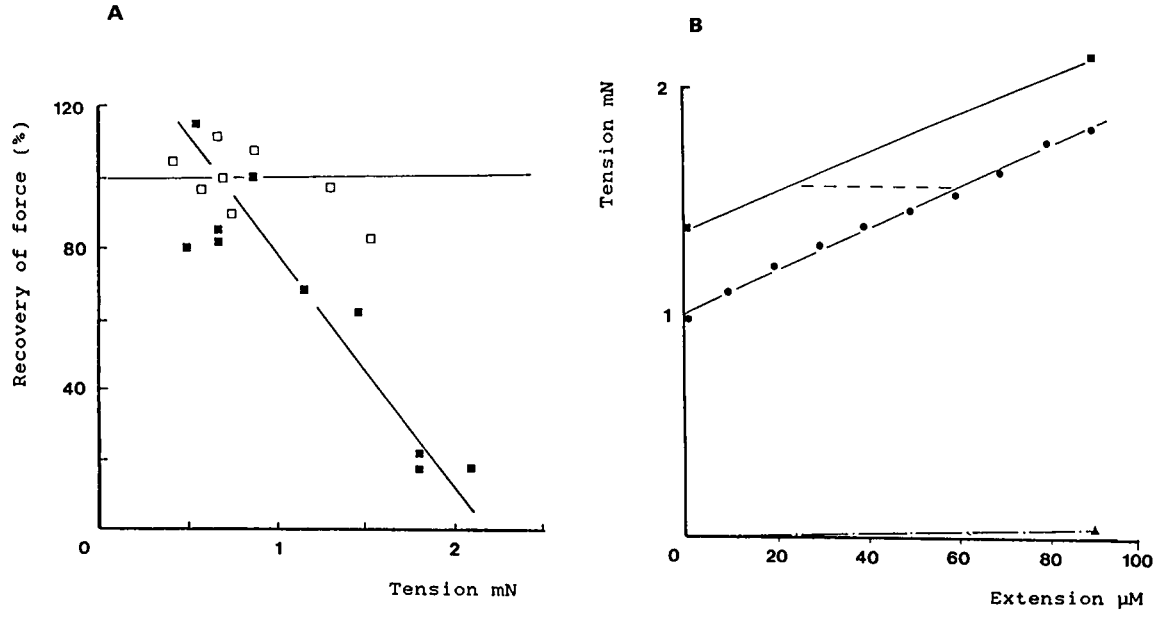

FIGURE 6 (*a*) Rigor tension recorded after release from two different high pressures (5 MPa-open squares, 10 MPa-filled squares) is plotted against the initial tension; the recovered tension is plotted as a percentage of initial tension. The data are from one preparation and they show that the loss of rigor tension after release is greater the higher the tension and pressure. (*b*) Filled circles and the line through them show the force-extension relation of a ribbon-shaped bundle of fibers in rigor at atmospheric pressure, and the triangle and the dotted interrupted line near abscissa show the change in passive tension (force-extension relation in relaxed condition) over the same extension range. The pressure sensitivity of rigor tension was determined at two different extensions and is shown by the two filled squares each indicating the average tension level at 10 MPa; the line joining the squares, thus, represents the force-extension relation at high pressure (10 MPa). The horizontal separation between the two relations (*dotted line*) is ~30 μm and it indicates the hydrostatic compression in the preparation (bundle width 600 μm, thickness 100 μm, and rest length [Lo] 15 mm).

0.028 ± 0.0026 (n = 5). Interestingly, this is similar to the isothermal compressibility of water at 20°C (0.046%/MPa; East, 1984).

Tension records in Fig. 7 show the main features of tension recovery on release from hydrostatic pressure. The tension records are from a single muscle fiber where a pressure release from 10 MPa was achieved complete in <10 ms; the experiment was carried out using the pressure chamber originally designed for solution studies (see Geeves and Ranatunga, 1987). Despite the oscillations seen at pressure release, the records show that the rigor tension decreased in phase with pressure change. A more complex recovery of tension was seen in active muscle fiber responses; the active tension initially decreased in phase with pressure and then recovered to the atmospheric level (Fortune et al., 1989*b*).

DISCUSSION

The data from nonprotein elastic fibers (glass, copper, and rubber) are approximately as expected from the known properties of these materials. Basically, a small stretch produces a nonequivalent compressive lateral strain, i.e., a volume increase, in glass and copper, but not in rubber. The data from protein fibers (keratin, silk, and collagen) show the same qualitative behavior as glass and copper. At a molecular level these fibers are nonisotropic and also, their cross-sectional areas contain a significant solvent phase. Moreover, different structures may be responsible for stresses in different directions. Therefore, calculation of Poisson's ratios would be meaningless and direct comparison with isotropic solid fibers clearly inappropriate. Our results show that keratin and silk are more pressure sensitive than collagen. Whether the differences can be attributed to protein secondary structure or their higher order structure remains unclear.

A muscle fiber is a complex polymeric structure consisting of a well-organized lattice of filaments of different mechanical properties lying in a large solvent (water) space. Therefore, the steady tension behaviors observed here under uniform compression may represent the net outcome of changes in a number of contributing elements, and identification of a particular behavior with any one or more structural elements remains difficult. Nevertheless, the results from muscle fibers show that the pressure-dependent behavior of steady tension is different in relaxed, rigor, and active muscle. On increasing hydrostatic pressure up to 10 MPa, the passive tension in a relaxed fiber showed little change, the tension in a fiber in rigor increased, and the tension in a maximally Ca-activated fiber decreased. The characteristic features of the three states are the absence of attached cross-bridges in the relaxed state, the presence of attached but noncycling cross-bridges in the rigor state, and the presence of cycling cross-bridges in the active state. Therefore, their different pressure-sensitive behaviors must be a consequence of the differences in cross-bridge involvement.

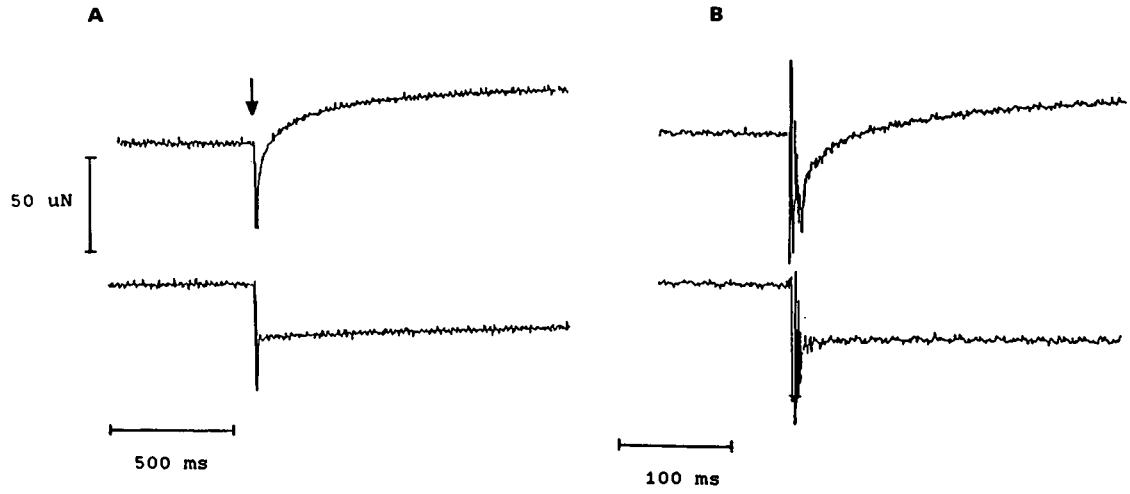

FIGURE 7 Features of tension recovery after release from high pressure. Records are from a single muscle fiber and they are shown at two different time scales in *a* and *b*. The fiber was maximally Ca-activated (*upper trace*) or put in rigor (*lower trace*) and exposed to a steady high pressure of 10 MPa; the pressure release (*arrow*) was complete in <10 ms. At atmospheric pressure, the steady active tension was 450 μN and the rigor tension was 180 μN. Note that the rigor tension was higher but the active tension was lower at high pressure and that the change of rigor tension occurs in phase with pressure release but the recovery of active tension is more complex; the tension decreased initially, as in rigor, and then recovered.

Filament lattice spacing

Before examining in detail the pressure effects on passive, rigor, and active tension, some consideration should be given to the possibility that high pressure may induce changes in filament lattice spacing. A number of studies have been made on both intact and skinned muscle fibers to investigate effects of altered filament lattice spacing on cross-bridge properties and behavior (Gulati and Babu, 1982, 1985, 1986; Metzger and Moss, 1987, and references therein). The active tension was found to be relatively unaffected except at extreme shrinkage of lattice spacing when the tension was depressed. Also, it appears that, in muscle fibers in rigor, an increase in filament lattice spacing augments isometric tension (Podolsky et al., 1982). An increase of lattice spacing of ~2 nm per 5 MPa would be required to account for tension changes observed in rigor muscle fibers. A change of lattice spacing, as a direct consequence of high pressure, would require a difference between the intermyofibrillar bulk water phase and the intramyofibrillar water phase in the extent of volumetric compression induced by high pressure. However, in so far that the filament lattice space is readily accessible to water, occurence of a steady hydrostatic pressure gradient across a skinned muscle fiber seems unlikely. Indeed, examination of x-ray equatorial diffraction pattern of muscle fibers in relaxed and rigor conditions did not show evidence of a lattice spacing change under high pressure (Knight et al., 1990).

Passive tension

In a glycerinated rabbit muscle fiber, passive tension may be developed in a number of components. The existence within vertebrate sarcomeres of structural filaments other than actin and myosin is well established (see Wang and Ramirez-Mitchell, 1983, and references therein) and some major proteins (e.g., titin, nebulin) have been recognized (see Wang et al., 1984; Trinick et al., 1984; Maruyama et al., 1989, and references therein). Titin (connectin) may form the gap filaments and intermediate filaments within the sarcomere (see Trinick et al., 1984). Evidence is accumulating that such titin filaments may bear almost all of the passive tension in skinned fibers (Horowits and Podolsky, 1987). Our results show that the structure or structures bearing passive tension in a skinned muscle fiber exhibit a net mechanical property characterized by the relative insensitivity of its isometric tension to increased hydrostatic pressure; a behavior shown also by rubber. Interestingly, the tension in a length of isolated connectin (titin) has been shown to have a positive temperature coefficient as in the case of rubber (Maruyama et al., 1977).

Rigor tension

Our results show that, to a first approximation, the steady tension behavior of a muscle fiber in rigor is similar to a uniform elastic body having a Poisson's ratio <0.5. The hydrostatic compression was estimated to be ~0.03% Lo/MPa. Tension changes experimentally observed may represent the net outcome of changes in a number of structural elements. However, the reduction in rigor tension upon pressure release was monotonic and in phase with pressure release on a millisecond time scale.

The main difference between a relaxed muscle fiber and a muscle fiber in rigor is that a rigor fiber has interfilamentary cross-bridges. Therefore, the characteristically different pressure dependence of rigor tension must be directly or indirectly linked to the presence of attached cross-bridges. Because Z-line, and perhaps the thick filament, may be involved in maintaining passive tension, the changed tension behavior in rigor fibers implies hydrostatic compression affects cross-bridges and/or thick and thin filaments. In a contracting muscle fiber, the compliance is largely resident in the cross-bridges (Ford et al., 1977, 1981). Therefore, the possibility that changes in rigor tension are induced by hydrostatic compression of a specific elastic component in a cross-bridge (e.g., S-2 segment) cannot be excluded.

It is clear from the data in Tables 2 and 3 that the volumetric stress or pressure sensitivity of muscle fibers is small in comparison with glass and other fibers (except rubber). However, a muscle fiber has a very large solvent space (see Matsubara et al., 1984) and thus the stress value for rigor myofibrils may be at least as high as for collagen. In general terms, the increase of tension at high pressure indicates the presence of lateral tension or stiffness in rigor fibers. It has been shown by calculation and by experiment that cross-bridges produce a lateral force, the magnitude and the direction of which may change with lattice spacing (see Discussion by Matsubara et al., 1984). It would be of interest to determine pressure sensitivity of rigor tension at specifically altered lateral spacing and filament overlap to examine these findings more quantitatively.

Active tension

The steady tension in a maximally Ca-activated fiber is reversibly reduced by pressure, but the examination of recovery of tension after release indicates interaction of a number of processes. Of specific interest is the initial drop in tension as in a rigor fiber, before the subsequent tension recovery. Analysis of the tension recovery provides useful information about the pressure sensitive events in cross-bridge operation (Fortune et al., 1989*b*). It is also of

interest to note the qualitative similarity between the change of muscle fiber tension after a pressure release and that after a temperature jump (see Goldman et al., 1987). Either situation represents a release of volumetric stress (i.e., hydrostatic decompression in one and thermal expansion in the other) and an initial drop in tension is seen in both cases.

We thank The Wellcome Trust for financial support and Dr. D. J. Pitt of The Department of Mechanical Engineering, University of Bristol, for explaining the theoretical basis of the pressure effects on isotropic solid materials. Dr. Geeves is a Royal Society University Research Fellow.

Received for publication 15 May 1990 and in final form 13 August 1990.

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