THE pCa–TENSION AND FORCE–VELOCITY CHARACTERISTICS OF SKINNED FIBRES ISOLATED FROM FISH FAST AND SLOW MUSCLES

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SUMMARY

1. Single fast fibres and small bundles of two to six slow fibres were dissected from the myotomal muscles of the cod, Gadus morhua, and the dogfish, Scyliorhinus canicula. Fibres were chemically skinned with the non-ionic detergent Brij 58.

2. The isometric tension properties were investigated. Maximal isometric tensions (mean ± s.e. of mean) were 18.65 ± 1.18 (n = 11) and 8.34 ± 0.98 (n = 13) N cm⁻² for cod fast and slow fibres, and 18.34 ± 0.88 (n = 28) and 8.24 ± 0.39 (n = 12) N cm⁻² for dogfish fast and slow fibres respectively. The values are comparable to those observed in mammalian and amphibian skinned fibres. The lower tensions generated by the slow fibres cannot be fully explained on the basis of their lower myofibrillar fractional volume.

3. In common with previous studies, a steep sigmoid relationship between pCa and tension was observed. The threshold for tension generation was around pCa 7.2. Half-maximal pCas were 6.08 and 6.42 for cod fast and slow muscle, and 6.41 and 6.50 for dogfish fast and slow fibres respectively. Cod fibres were maximally activated at around pCa 5.18, and dogfish fibres at pCa 5.62.

4. Contraction-induced residual tensions were observed in cod fast fibres after return to relaxing solution. This phenomenon is a feature common to many skinned fibre studies, but the mechanism behind it has yet to be resolved.

5. The force–velocity characteristics of fast and slow fibres have been investigated (at 8 °C).

6. Points below 0.6 P₀ on the P–V curves could be fitted to a linear form of the Hill equation. Extrapolated Vₘₐₓs were calculated as follows: cod fast fibre Vₘₐₓ = 1.01 muscle length sec⁻¹ (Lsec⁻¹) (a = 0.21 P₀; b = 0.21 Lsec⁻¹). Slow fibre = 0.53 Lsec⁻¹ (a = 0.28 P₀; b = 0.21 Lsec⁻¹). Dogfish fast fibre Vₘₐₓ = 2.34 Lsec⁻¹ (a = 0.06 P₀; b = 0.14 Lsec⁻¹). Slow fibre = 0.67 Lsec⁻¹ (a = 0.19 P₀; b = 0.13 Lsec⁻¹).

7. Contraction velocity in cod slow fibres decreased continuously to produce markedly non-linear velocity transients, similar to those reported for amphibian slow fibres.

8. The effect of altering Ca²⁺ concentration on the shape of the isotonic velocity curve (at low loads) was studied in dogfish fast fibres (0.5–1 °C). Contraction velocity

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decreased continuously during shortening, at both maximal and half-maximal Ca²⁺ concentration. The rate of decay of velocity with shortening was greater at low Ca²⁺ concentration.

INTRODUCTION

Fish slow muscle fibres are multiply innervated, and activated by junction potentials (Stanfield, 1972). They differ from the corresponding fibres in amphibia with respect to their high aerobic capacity and well developed sarcoplasmic reticulum (Kryvi, 1977; Johnston & Moon, 1981; McArdle & Johnston, 1981), and their involvement in sustained locomotory activity (Bone, 1966; Johnston, Davison & Goldspink, 1977). Fast fibres show a diversity of innervation between different fish orders (Bone, 1964, 1970). In elasmobranchs (such as the dogfish), chondrosteans, dipnoans and some primitive teleosts, fast fibres receive a single end-plate innervated by two axons, and stimulation elicits propagated action potentials (Hagiwara & Takahashi, 1967; Hudson, 1969). The more advanced teleost orders have a complex distributed innervation somewhat analogous to that of the slow fibres (Barets, 1961; Bone, 1964; 1970). For example, fast fibres of cod receive nine to twenty-three discrete end-plates derived from a large number of different axons (Altringham & Johnston, 1981a). Distributed fast muscle innervation is associated with a higher aerobic capacity and greater involvement in sustained locomotion than the focally innervated type (Johnston, 1980; Johnston, 1981b).

While there have been a number of electrophysiological (Bone, 1966; Hudson, 1969; Stanfield, 1972; Hidaka & Toida, 1969), ultrastructural (Kryvi, 1977; Patterson & Goldspink, 1972; Johnston, 1981a) and biochemical (Johnston et al. 1977; Johnston & Moon, 1980) studies of fish fast and slow muscle fibres, very little is known about their mechanical properties. The complex orientation of fibres within fish myotomes (see Alexander, 1969) makes mechanical studies difficult; the few experiments performed on fish muscle to date have been restricted mainly to fin and jaw muscles (see Bone, 1978; Johnston, 1981b for reviews).

There have been few studies made of the mechanical properties of multiply innervated slow fibres in vertebrates. Aidley (1965) studied isotonic contractions of frog rectus abdominus muscle during K⁺ contractures. Floyd & Smith (1971) investigated the mechanical properties and heat production of frog iliofibularis muscle using a nerve–muscle preparation in which the fast fibres were selectively blocked. However, both these preparations suffer from the disadvantage that the slow fibres only constitute around 2–3% of total muscle cross-sectional area (Kruger, Duspiva & Furlinges, 1933). Previous to the present work, there have been only two studies on the mechanical properties of isolated slow fibres. Constantin, Podolsky & Tice (1967) looked at sarcomere shortening using high speed photography during the local application of droplets of Ca²⁺ solutions. Contraction velocity was ten times slower than in iliofibularis twitch fibres. Lännergren (1978, 1979) conducted a series of elegant experiments on inact single fibres isolated from a Anuran toad, Xenopus laevis. Force–velocity curves were obtained for iliofibularis twitch and slow fibres; maximum contraction velocities were 6·34 and 1·10 Lsec⁻¹ respectively (22·5 °C). The present studies on fish skinned fibre preparations represent not only the first such
experiments on myotomal muscle but also add to the rather sparse knowledge of slow fibre mechanics.

A preliminary account of some of this work has previously been given (Altringham & Johnston, 1981b).

METHODS

Fibre preparation

Cod, Gadus morhua, 45-60 cm in length were caught by commercial fishermen in the Firth of Forth. Dogfish, Scyliorhinus canicula, 50-65 cm in length were obtained from the Millport Marine Laboratory, Great Cumbrae, in the Firth of Clyde. Both species were kept in the laboratory in recirculated, filtered sea water at 10 ± 1 °C. Fish were killed by a blow to the head, and subsequently pithed. Small strip of red (slow) and white (fast) muscle were taken from the larger myotomes. In cod, these are immediately behind the head, and below the lateral line. Myotomes midway along the body were used from dogfish (Fig. 1). The initial dissection was done in ice-cold Ringer solution. Teleost Ringer solution contained (mM): NaCl, 142.2; KCl, 2.6; CaCl₂, 1.35; MgCl₂, 0.4; NaHCO₃, 18.5; NaH₂PO₄, 3.2; pH 7.0; (Hudson, 1969). Elasmobranch Ringer solution contained (mM): NaCl, 134; KCl, 6.8; CaCl₂, 6.1; MgCl₂, 1.1; urea, 208; set to pH 7.4 with NaHCO₃; (Meiss et al. 1974).

Bundles of ten to twenty fibres were gently dissected from a myotome by cutting them free from the myosepta, and teasing them from the main fibre mass. Care was taken to touch only the ends of the fibres. Bundles were placed immediately in a shallow (2 mm deep) glass trough containing silicone oil at 0-5 °C. A small drop of standard relaxing solution was injected into the oil around the fibres.

Fibre attachment and skinning

Single fast fibres, or small bundles of slow fibres, were teased from the main bundle. Because of the difficulties encountered in mechanically skinning cod fibres, chemical skinning was routinely adopted in all experiments as described below.

The fibres, 1-2.5 mm in length, were rapidly transferred to the apparatus between the tips of fine forceps, and mounted on fine glass hooks. The ends were wrapped around the hooks and held in place by a drop of plexiglass/acetone glue. Each fibre was then immersed in the first incubation solution. The thin covering of silicone fluid helps to prevent dehydration, and transference of the fibre was usually complete within 15-30 sec. The first incubation solution contained normal relaxing solutions (see below), with 1 % Brij 58 (polyoxyethylene 20 cetyl ether) a non-ionic detergent. A 30 min incubation makes the membrane completely permeable to external solutes (Orentilicher, Rueben, Grundfest & Brandt, 1974). Fibres were then transferred to relaxing solution without Brij 58 for > 3 min.

Sarcomere length was measured using laser diffraction. Immediately after attachment, the sarcomere length was set at 2.3 µm, and the fibre length and diameter measured. After the first activation cycle sarcomere length may decrease to 2.1-2.2 µm at the centre of the preparation, possibly due to damage and subsequent extension at the ends. For this reason, sarcomere length was always checked after the first activation, and reset to 2.3 µm if necessary. Further shortening may occur, but this was generally < 0.1 µm.

Fibres or fibre bundles used in all experiments ranged from 50-200 µm in diameter.

Solutions

All chemicals were analar grade reagents. The following were obtained from Sigma Chemical Company (London); EGTA (ethyleneglycol-bis(β-aminoethylether)N,N'-tetraacetic acid), creatine kinase, phosphocreatine, ATP. All other chemicals were obtained from BDH. BDH 'extra pure' imidazole was used in all experiments.

The solution compositions were determined in part from a series of preliminary experiments, described in the Results section, and in part from information in the literature (for details, see Altringham, 1981). The basic relaxing solution has the following composition: 10 mM-imidazole pH 7.0 at 8 °C. 110 mM-KCl. 3 mM-MgCl₂. 5 mM-EGTA. 10 mM-phosphocreatine. 2.5 mM-ATP.
Fig. 1. A, sampling positions, shown as dotted rectangles, for the myotomal strips used to isolate skinned fibres. B, fibres arrangement in fish myotomes.
Creatine kinase in solid form is added to a final concentration of > 20 u. ml\(^{-1}\) just before each experiment. Imidazole, EGTA, phosphocreatine and ATP stock solutions were all set to pH 7.0 at (8 °C). The exact quantities of KOH or HCl added to set the pH was noted in all cases. An iterative computer programme (Perrin & Sayce, 1967), modified by White & Thorson (1972) was used to calculate the concentrations of the various ionic species in the solutions. Activating solutions were made by the addition of CaCl\(_2\) from 0-5 mM to the basic relaxing solution. The concentrations of selected ionic species are given in Table 1.

### Table 1. The concentrations of selected ionic species in the relaxing and activating solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>[CaCl(_2)] (mM)</th>
<th>[Ca(^{2+})] free (µM)</th>
<th>pCa</th>
<th>[Mg(^{2+})] free (mM)</th>
<th>[MgATP(^{4-})] (mM)</th>
<th>Ionic strength (mM)</th>
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<tr>
<td>Relaxing</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>0.48</td>
<td>2.24</td>
<td>180.2</td>
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<tr>
<td>Activating</td>
<td>0.25</td>
<td>0.04</td>
<td>7.41</td>
<td>0.48</td>
<td>2.24</td>
<td>180.4</td>
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<td>0.50</td>
<td>0.08</td>
<td>7.08</td>
<td>0.48</td>
<td>2.24</td>
<td>180.6</td>
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<td>1.00</td>
<td>0.19</td>
<td>6.73</td>
<td>0.49</td>
<td>2.24</td>
<td>181.0</td>
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<td>0.49</td>
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<td>1.75</td>
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<td>6.39</td>
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<td>2.25</td>
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<td>1.12</td>
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<td>183.1</td>
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<td>0.52</td>
<td>2.25</td>
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<td>2.99</td>
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<td>184.1</td>
</tr>
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<td>4.50</td>
<td>6.58</td>
<td>5.18</td>
<td>0.53</td>
<td>2.25</td>
<td>185.0</td>
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<td>37.80</td>
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Dogfish muscle, in common with other elasmobranchs, contains high concentrations of osmoregulatory solutes, mainly in the form of urea and trimethylamine oxide (TMAO) (see e.g. Prosser, 1973; Robertson, 1975). The effects of these solutes on tension generation have been reported elsewhere (Altringham, Yancy & Johnston, 1982). The absence of both of these solutes does not significantly affect maximum isometric tension. Due to the uncertainties which would be introduced into the calculations of species concentrations, urea and TMAO have been omitted in the present studies.

**Apparatus**

The apparatus developed for these studies allows the independent measurement of tension and length. A block diagram and details of the mechanical section are shown in Fig. 2. Fibres are attached to the two glass hooks shown in the Figure. One hook is attached directly to the silicon beam of an AES803 strain gauge element (A.M.E., Horton, Norway). The element is held rigidly in a screened, aluminium and perspex adaptor, mounted on a one-way micromanipulator. This allows the distance between the hooks to be adjusted. The output from the element is fed to a bridge circuit and amplifier unit. The sensitivity varies slightly with the element in use, a typical value would be 0.35 V mN\(^{-1}\). Noise is < 3 mV, drift < 1 mV hr\(^{-1}\). The other hook is attached to a 3.7 cm long balsa wood lever. During isometric contractions, a brass pin, glued to the armature of a miniature relay, holds the free end of the lever against a stop. The total compliance of the system is < 4 µm mN\(^{-1}\).

The other end of the lever is attached to a meter movement taken from a sensitive moving coil galvanometer. The isotonic afterload is generated by passing a current through the coil, to produce forces of 0 to > 2.5 mN. This is displayed as voltage on a digital voltmeter. Voltage is linearly proportional to load. Activation of the relay draws back the brass pin, allowing a contracting fibre to shorten. The equivalent mass of the lever is < 0.2 mN.

During an isotonic release, an aluminium flag attached to the lever crosses the path of an L.E.D.-photodiode assembly, allowing the measurement of displacement against time.
Fig. 2. A, diagram of the apparatus used in the study of the force–velocity relationships of skinned muscle fibres. T, tension; L, length. B, details of the mechanical section of the apparatus. B, incubation chamber; F, foil flag; H, glass hooks; L, balsa wood lever; P, photodiode assembly; S, mechanical stops.
Sensitivity = 43 μm/100 mV. After a release, the lever can be brought back to its stop by applying a load > P0 to the fibre. The relay is then deactivated, returning the brass pin to its position in front of the lever. A second stop is placed so as to allow a maximum fibre shortening of 400 μm.

A block of perspex mounted on a two way micromanipulator slides in a channel beneath the hooks. The lower half contains circulating ethylene glycol/water from a Grant cooling system, to regulate temperature (+/-0.5 °C). The upper half has three chambers, each of 1.5 ml capacity. The block can be raised to immerse the fibre in any of these three chambers. A change from one bath to another can be effected in < 5 sec. Thin perspex windows set in the block allow the beam from a He-Ne laser to be passed through the fibre from below, for the measurement of sarcomere length. Fibre length and diameter are measured in situ. Tension and length are recorded on a Tektronix 5113 dual beam oscilloscope. A 35 mm camera attachment is used to record the transients, and tension is continuously monitored on a Brvans 28000 chart recorder.

**Experimental protocol used in isometric experiments**

Fibres were immersed in activating solutions until a steady tension developed before being returned to relaxing solution. The activating solutions were used in a random order, with maximally activating Ca²⁺ concentrations at intervals to monitor any decrease in P0. A solution change could be effected in 5 sec. In some experiments, a fibre was transferred directly from one activating solution to another of higher or lower free Ca²⁺ concentration. Results obtained in this way did not differ from those obtained by relaxing the fibre after each activation.

The pCa-tension curves were linearized according to the Hill equation:

$$\log_{10} \left( \frac{P}{P_0 - P} \right) = n \log_{10} [Ca^{2+}] + h,$$

where n and h are constants. A straight line is obtained by plotting $\log_{10} (P/P_0 - P)$ against pCa. When $P = 0.5 P_0$, then $\log_{10} (P/P_0 - P) = 0$. The Ca²⁺ concentration at this point is therefore that concentration required to give half maximal tension. Regression lines were fitted and 95% confidence limits calculated by computer. In all experiments, fibres were discarded when the diffraction pattern became unclear, or P0 fell below 75% of the maximal isometric tension of the first activation. The laser beam has a diameter of 1 mm, and thus samples upwards of 40% of the fibre length. If the tension generated by a fibre declined with successive activations at a given pCa, maximal or near maximal activations were performed at intervals during the experiments to monitor this decay. In this way the fall in P0 with each activation could be extrapolated, and used to obtain normalized values of $P/P_0$. As will be seen in the results section, the decline in P0 was usually small (< 10%).

In those cod fast fibres which showed residual tensions, experiments were terminated when this rose to 20% P0. Note, however, that the magnitude of this tension did not affect P0 (see also Thames, Teichholz & Podolsky, 1974).

All experiments were carried out at 8 °C.

**Experimental protocol of P-V determinations**

Fibres were activated after post-skinning incubation in relaxing solution. When tension reached a steady value, releases were given against a series of pre-set loads. In slow fibre preparations, up to ten releases could be given during a single maximal activation, without a significant decrease in tension. In a number of experiments, only one release was performed in each of 10-15 activation-relaxation cycles. The results obtained by the two methods did not differ. In experiments on fast fibres, only three to five releases were given in each cycle. Releases were usually given in a series of ascending or descending loads. Wherever possible, velocity measurements at low loads (< 0.2 P0) were repeated in the same or subsequent activation cycles. Results were not included in the final analysis if the contraction velocity showed a consistent decrease at these low loads. Experiments were terminated when P0 fell to < 70% P0 of the first activation. All experiments were carried out at 8 °C.

**Data analysis**

Velocity transients were recorded from the oscilloscope with a 35 mm camera. Negatives were projected directly onto graph paper for analysis. A typical transient is shown in Fig. 3 to demonstrate the method of analysis. Isometric tension (P0) is dropped to a new value (P).
Concomitant with this step force change, there is a rapid decrease in length, due to the series elastic component (s.e.c.) of the muscle. The early events of the active contraction cycle are obscured to a varying extent by mechanical oscillation. Velocity measurements were taken over the second 50 msec interval after the onset of the step change in load. Some extrapolation through the oscillations was necessary at low loads.

Fig. 3. An isotonic release to illustrate the method of transient analysis. S.e.c. = series elastic component. Tension is expressed as $P/P_0$. Mean velocity over the second 50 msec interval after the onset of release ($a/b$) is measured.

Velocity in muscle length sec$^{-1}$ (Lsec$^{-1}$) was plotted against the relative tension $P/P_0$. Force–velocity curves obtained in this way could be fitted to Hill’s equation (1938):

$$(P + a)v = b(P_0 - P),$$

where $P = load$, $P_0 = maximal isometric tension$, $v = velocity$, and $a$ and $b$ are constants. Curves were linearized by plotting $(P_0 - P)/P_0$ against $P/P_0$. $1/V_{max}$ is given by the intercept with the y axis, $a/P_0$ by the intercept with the x axis, and $1/b$ by the gradient. Lines were fitted to the force–velocity curves by computer, using the constants $a$ and $b$ derived from the above analyses.

It has been shown that at high loads the experimental points on a force–velocity curve deviate markedly from the rectangular hyperbola predicted by Hill’s equation (e.g., Hill, 1970; Edman, Mulieri & Scubon-Mulieri, 1976; Lällnergren, 1978), and points beyond 0.6–0.8 $P_0$ are usually omitted from the linear analysis. For this reason, velocity at high loads was not studied in the present work.
RESULTS

Effect of repeated activation-relaxation cycles on tension development

A number of experiments were performed to look at the effect of repeated activation-relaxation cycles on steady isometric tension (\(P\)) and resting tension. Maximal isometric tension (\(P_0\)) and the rate of rise of tension were not increased by raising the concentrations of MgATP\(^2-\), creatine kinase or phosphocreatine.

![Fig. 4. A series of successive maximal isometric activations on a fast cod fibre. Note the steady decline in \(P_0\) and the rate of rise of tension, and the progressive increase in residual tension on transfer to relaxing solution. Arrows indicate changes to activating/relaxing solutions.](image)

Repeated activation-relaxation cycles could be performed at high and low Ca\(^{2+}\) concentrations with no increase in resting tension in all but cod fast fibres (e.g. Figs. 5 and 6). In many cod fast fibre preparations, a residual tension in relaxing solution of 5–10\% of \(P_0\) began to build up in successive activations after the first two to three cycles (Fig. 4). In most experiments on slow fibres little or no decrease in \(P_0\) was seen over five to ten cycles. Fibres would occasionally undergo up to fifteen cycles without a significant deterioration in \(P_0\) or a loss in clarity of the diffraction pattern. \(P_0\) in fast fibre preparations often remained constant over five successive cycles, but commonly decreased by 5–10\%, most noticeably in cod (Fig. 4). This phenomenon was prevalent only at Ca\(^{2+}\) concentrations greater than half-maximal. The residual tensions observed in cod fast fibres were also seen only at high Ca\(^{2+}\) concentrations. An increase in ionic strength, from 180 to 210 mM decreased \(P_0\), but had no effect on the relative magnitude of the residual tension.

The pCa–tension relationship

The results obtained from a typical single cod slow fibre preparation are shown in Fig. 5 to illustrate the experimental protocol. Typical results from dogfish fast and slow fibres are given in Fig. 6. Pooled data on the pCa–tension relationship from six fast and six slow fibres from cod are given in Fig. 7. In Fig. 8 data from eight fast
Fig. 5. A series of isometric activations at different free Ca$^{2+}$ concentrations in a single cod slow fibre preparation. Arrows indicate changes between activating/relaxing solutions. The figures above the activation curves are the pCa values of the activating solutions.

Fig. 6. Activation curves from dogfish fast (A) and slow (B) fibre preparations (See Fig. 5 for notation).
Fig. 7. A. pCa-tension relationship for cod myotomal muscle. ○, fast fibres (n = 6); ●, slow fibres (n = 6). Numbers refer to the number of observations. Values are mean ± s.e. of mean. B. Hill plot of log \((P/P_0)/(1 - P/P_0)\) against pCa for cod fast (○; \(r = 0.99\)) and slow (●; \(r = 0.98\)) fibres.

Fig. 8. A. pCa-tension relationship for dogfish myotomal muscle. ○, fast fibres (n = 8); ●, slow fibres (n = 8). Numbers refer to the number of observations. Values are mean ± s.e. of mean. B. Hill plot of log \((P/P_0)/(1 - P/P_0)\) against pCa for dogfish fast (○; \(r = 0.94\)) and slow (●; \(r = 0.97\)) fibres.
and five slow dogfish fibres are presented. Each curve represents results from three fish. Tension is expressed relative to the maximum isometric tension obtained from each fibre. The threshold for tension generation in cod fibres is around pCa 7.2 (0.06 µM-free Ca²⁺ concentration) and maximum tension is reached at pCa 5.18

![Diagram A](image1)

![Diagram B](image2)

Fig. 9. A typical experiment (from cod slow fibres) to illustrate the experimental protocol. A, isometric records of second and third activations in a cycle of three. Numbers refer to the data points given below. B, force–velocity curve from single slow fibre preparation. P/P₀ = relative tension (load/maximum isometric tension). Velocity is expressed in fibre lengths sec⁻¹. Numbers against data points refer to the order in which they were derived.

(7 µM-free Ca²⁺ concentration). The dogfish curves are somewhat steeper, maximum tension being reached at pCa 5.52 (3 µM-free Ca²⁺ concentration). Half-maximal pCa₅ were significantly different for cod fast (6.08) and slow (6.42) fibres (P < 0.05) but similar for the dogfish fibres (fast fibres, 6.41; slow fibres, 6.50).

The constant n in the Hill equation gives an estimate of the number of Ca²⁺ binding sites on the troponic C molecule (note, however, that the other Ca²⁺ binding sites
cannot be excluded, for example those on the myosin LC2 (DTNB) light chain – see Discussion). Gradients of 1·9 and 1·6 for cod fast and slow fibres suggest a minimum of two sites, with a higher degree of co-operativity in the fast muscle. The steeper dorfish curves give values of 3·5 and 3·2 for fast and slow muscles respectively. A
minimum of four sites is therefore postulated. The shape of the pCa–tension curve is determined by the number of sites, and the degree of interaction between them. A single kinetic analysis such as this cannot separate these factors, but can only give information about their combined effects. The situation is further complicated if the sites do not have identical $K_m$'s for Ca$^{2+}$, and if the degree of interaction also varies between sites.

![Diagrams of isometric tensions](image)

Fig. 11. Isotonic releases from dogfish fast and slow fibres (maximally activated, pCa 5.52).

- A. transients from two fast fibres.
- B. transients from one slow fibre preparation.

**Steady isometric tensions**

Force in skinned fibres has been shown to be proportional to cross-sectional area (e.g. Hellam & Podolsky, 1969; Wise, Rondinone & Briggs, 1971). Cross-sectional area was calculated from the mean diameter, assuming circularity of the fibres. The small error which may arise from this assumption is minimized by taking the mean diameter, and by pooling results from a number of fibres. Maximum isometric tensions were $18.65 \pm 1.18$ (n = 11) and $8.34 \pm 0.98$ (n = 13) N cm$^{-2}$ for cod fast and
FISH SKINNED MUSCLE FIBRES

slow, and 18.34 ± 0.88 (n = 28) and 8.24 ± 0.39 (n = 12) N cm⁻² for dogfish fast and slow fibres respectively. All values are mean ± s.e. of mean.

P-V relation

Results from a typical experiment (from cod slow fibres) are shown in Fig. 9 to illustrate the experimental protocol. Both dogfish fibre types, and cod slow fibres,

were very robust at all Ca²⁺ concentrations. It was difficult, however, to obtain more than two or three releases from maximally activated cod fibres before they showed visible signs of non-uniformity and a marked deterioration in the clarity of the diffraction pattern.

Typical isotonic releases from cod fast and slow fibres at maximal and sub-maximal Ca²⁺ concentrations are shown in Fig. 10. Release from maximally activated dogfish
fast and slow fibres are given in Fig. 11. A noticeable feature of all velocity transients was their departure from linearity. Transients from maximally activated fast fibres and dogfish slow fibres resemble those from previous studies on skinned fast fibres from frog (e.g. Podolak & Teichholz, 1970; Thames et al., 1974), in that deviations from linearity at low loads were small (velocity decreased < 25% in 250 ms). In all fibre types, the transients became increasingly more curved at higher loads.

Fig. 13. Force--velocity curves from cod slow fibres. Horizontal bars indicate range of data, vertical bars ± s.e. of mean, numbers are number of observations. ○: data from maximally activated fibres (pCa 5.18); ●: data from half maximally activated fibres (pCa 6.30).

Velocity in cod slow fibres decreased rapidly initially, before slowing towards a uniform velocity, producing transients very similar to those reported by Aidley (1965) and Floyd & Smith (1971) from the slow fibres of frog rectus abdominus muscle.

Transients from half-maximally activated cod fast fibres showed a greater departure from linearity than maximally activated fibres (Fig. 10). The poor viability of cod fast fibres did not allow this effect to be studied in detail.
Fig. 14. Force–velocity curves from dogfish myotomal muscle. ○: fast fibres (three animals, seven fibres). ●: slow fibres (three animals, five fibres).

Table 2. Summary of $V_{\text{max}}$, $a/P_0$ and $b$, data for cod and dogfish muscles from the present study.

<table>
<thead>
<tr>
<th></th>
<th>$V_{\text{max}}$ (L sec$^{-1}$)</th>
<th>$a/P_0$</th>
<th>$b$ (L sec$^{-1}$)</th>
<th>Experimental details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fast muscle</td>
<td>Slow muscle</td>
<td>Fast muscle</td>
<td>Slow muscle</td>
</tr>
<tr>
<td>Cod, Gadus morhua</td>
<td>1·01</td>
<td>0·53</td>
<td>0·21</td>
<td>0·28</td>
</tr>
<tr>
<td>Dogfish, Sphyraena canicula</td>
<td>2·34</td>
<td>0·67</td>
<td>0·06</td>
<td>0·19</td>
</tr>
</tbody>
</table>
Due to the poor viability of cod fast fibres, and the curved nature and interfibre variability in slow fibres, no consistent differences were found in shortening velocity for submaximally activated fibres. The data were therefore combined.

Fig. 15. Top: isometric activation curves for fibre 2441. Arrows indicate transfer between activating and relaxing solutions. Bottom: isotonic releases a-d made following the above activations. 0, zero tension; $P_o$, maximum isometric tension; $P_o'$, maximum tension at low (Ca$^{2+}$); $P$, load. Shortening is from top to bottom.

$P-V$ curves obtained from cod fibres are shown in Figs. 12 and 13, and curves from dogfish fibres in Fig. 14. The details are summarized in Table 2.

The effect of Ca$^{2+}$ concentration on contraction velocity

In contrast, a number of releases at maximal and submaximal Ca$^{2+}$ concentration could be obtained from the same fibre in dogfish preparations. The effect of Ca$^{2+}$ on the isotonic release could therefore be reliably quantified. A series of experiments were
carried out at 0·5–1 °C to investigate this phenomenon. Each fibre was alternately maximally (pCa 5·52) and half-maximally (pCa 6·39) activated and released once against a low load, before being returned to relaxing solution. The load at half-maximal Ca\(^{2+}\) concentration was chosen to give the same relative load as at maximal Ca\(^{2+}\) concentration, in effect normalizing the velocity curves for comparison (see e.g. Gulati & Podolsky, 1981).

A typical experiment is illustrated in Fig. 15. Although contraction velocity continuously declines with increased shortening in all fibres, this effect is much more marked at half-maximal Ca\(^{2+}\) concentration. In Fig. 16 the curves illustrated in Fig. 15 have been replotted to show the mean contraction velocity at various times after release. It can be seen that the observed contraction velocity is dependent on the time after release at which the measurements are made, particularly for sub-maximal activations. This effect was clearly evident in all fibres studied, including a number of experiments performed at loads between 0·1–0·3 P\(_o\). The velocities 50 ms after release for eight fibres activated at pCa 5·52 and pCa 6·39 are shown in Table 3. A highly significant difference in contraction velocity at submaximal and maximal Ca\(^{2+}\) concentration was found when records were analysed using a paired t test; \( P < 0·001 \).
The step change in tension in our experiments was too slow to resolve the events during the first 50 ms of the release (the true displacement being masked by overshoot and oscillations of the lever following the rapid series elastic length change). However, extrapolation of the results shown in Fig. 16 back to the moment of release suggests that 'initial contraction' velocity may be independent of Ca$^{2+}$ concentration. Qualitatively similar results were obtained for all fibres studied.

**Table 3. Initial contraction velocities at maximal and submaximal [Ca$^{2+}$] free for the eight dogfish fast fibres studied**

<table>
<thead>
<tr>
<th>Fibre</th>
<th>$P_o(6.39)/P_o(5.32)$</th>
<th>$P_o/l_o$ (load)</th>
<th>$V(5.52)/V(6.39)$ (La/sec$^{-1}$)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2441</td>
<td>0.62</td>
<td>0.07</td>
<td>0.69</td>
<td>0.55</td>
</tr>
<tr>
<td>2741</td>
<td>0.58</td>
<td>0.05</td>
<td>0.72</td>
<td>0.45</td>
</tr>
<tr>
<td>0752</td>
<td>0.63</td>
<td>0.04</td>
<td>0.80</td>
<td>0.67</td>
</tr>
<tr>
<td>0753</td>
<td>0.57</td>
<td>0.05</td>
<td>0.76</td>
<td>0.57</td>
</tr>
<tr>
<td>2051</td>
<td>0.60</td>
<td>0.04</td>
<td>0.88</td>
<td>0.68</td>
</tr>
<tr>
<td>2052</td>
<td>0.63</td>
<td>0.02</td>
<td>0.86</td>
<td>0.69</td>
</tr>
<tr>
<td>2053</td>
<td>0.48</td>
<td>0.07</td>
<td>0.81</td>
<td>0.75</td>
</tr>
<tr>
<td>2054</td>
<td>0.46</td>
<td>0.04</td>
<td>0.83</td>
<td>0.64</td>
</tr>
</tbody>
</table>

$X \pm$ s.e. of mean

0.73 ± 0.03  0.55 ± 0.04  0.18 ± 0.018

Range

0.46–0.68  0.02–0.07

* $P < 0.001$ (from paired t test).

**DISCUSSION**

Steady isometric tension

It is evident from these studies that maximum isometric tension is comparable to values found for other vertebrate skinned muscle fibres. For example, 14.7 and 16.7 N cm$^{-2}$ for frog semitendinosus (Gordon, Godt, Donaldson & Harris, 1973; Hellam & Podolsky, 1969, respectively) and 13.2 for rabbit psoas (Wise et al. 1971). The values for frog muscle are somewhat lower than those obtained from live preparations of 20–30 N cm$^{-2}$ (Ramsey & Street, 1940; Gordon, Huxley & Julian, 1966). In contrast, the tensions produced by intact teleost fish fibres (4.02 N cm$^{-2}$ for fast fibres, and 1.67 N cm$^{-2}$ for slow fibres; Flitney & Johnston, 1979) are very much lower than those generated by the skinned fibres of the present study. This may be caused by damage during dissection, due to the distributed nature of teleost innervation.

The lower tensions produced by skinned fibres, relative to live fibres, in other species may be due to differences between the intracellular environment of the intact cell and the bathing solutions used for skinned fibres. A mechanically skinned fibre swells in relaxing solution, the filament lattice distance increasing by around 15% (Matsubara & Elliot, 1972), and this has been proposed as a possible source of the discrepancies. April & Brandt (1973), however, found no effect on tension generation of changes in the interfilament spacing caused by the swelling of intact cells in
hypotonic solutions. The swelling itself may lead to an over-estimate of cross-sectional area if it is not taken into account in the calculations.

The swelling in mechanically skinned fibres is caused, in part at least, by swelling of the sarcoplasmic reticulum (Taylor & Godt, 1976). Fibre diameters in this study were measured in relaxing solution before and after treatment with Brij 58. No evidence for swelling was obtained. Chemical skinning with detergents disrupts both the sarcolemma and the s.r. (Orentlicher et al. 1974), and this may account for the absence of swelling. As yet, it is not known if the tensions generated by chemically and mechanically skinned fibres are significantly different.

Maximum isometric tension in slow fibres was approximately half that of the fast fibres. This would be predicted qualitatively from ultra-structural studies on fish muscle. The fractional volume occupied by myofibrils varies from 60–86% in fast muscle, to 40–60% in slow (for review, see Johnston, 1980). However, the difference is not fully explained by the relative fractional myofibrillar volume. Although no quantitative data are available for cod or dogfish, studies with other fish indicate that there are only around 50% more myofibrils in fast fibres than in slow fibres. On this basis, it would appear that the tension generated per myofibril is greater in fast than in slow fibres.

Residual tension

The residual tensions observed in cod fast fibres after several activation cycles are not an unusual feature. They have been observed and studied by a number of workers. Unfortunately, no clear picture has arisen, due to differences in the temperature, ionic strength and experimental protocol used by the various groups. Two consistent conclusions can however be drawn (see Gordon et al. 1973; Thames et al. 1974). Residual tensions are greater at low ionic strength (< 140 mM), and high temperatures (~ 20 °C, Hellam & Podolsky, 1969; Donaldson & Kerrick, 1975). Thames et al. (1974) found residual tensions to be absent at an ionic strength of 180 mM, and changes in ionic strength above this value did not affect contraction velocity. Similar findings were reported by Julian & Moss (1981). For this reason, the present experiments were performed at an ionic strength of 180 mM.

It is interesting to note that residual tensions were observed only in cod fast fibres, the least viable of the preparations studied. A number of unpublished experiments were performed at 0–2.5 °C, and at this temperature residual tensions were observed less frequently, and the decline in P0 with successive activations was less marked. Jewell & Kentish (1981) have recently studied this decline in P0 and associated rise in resting tension in cardiac trabeculae. Both can be largely abolished by the inclusion of dithiothreitol (DTT) in the incubation medium. DTT is commonly used as an SH-bond protector, to prevent protein denaturation. Thus both the residual tensions, and the decline in P0 may simply be due to protein denaturation. Since the publication of this work we have included DTT in our experimental media, however, preliminary experiments have shown little improvement in fibre viability.

pCa-tension relationship

Biochemical studies on the Ca²⁺ regulatory proteins of fast and slow skeletal and cardiac muscles have revealed a number of structural differences. Three forms of
rabbit troponin I have been identified on the basis of molecular weight and amino acid sequence (Syska, Perry & Trayer, 1974). Slow skeletal and cardiac troponin Cs have a similar primary structure in rabbit, but that of fast skeletal troponin C is very different (Perry, 1979). Perry (1979) also identified three forms of troponin T on the basis of molecular weight. Differences in the light chains of fast, slow and cardiac muscles are also well documented (e.g. Lowey & Risby, 1971; Sarkar, Sreter & Gergely, 1971; Weeds, Hall & Spurway, 1975).

Are these biochemical differences reflected to the functional differences observed between fast and slow muscles? In cod the slow fibre curve is shifted to lower free $\text{Ca}^{2+}$ relative to the fast fibres, indicating a lower apparent $K_m$ for $\text{Ca}^{2+}$, and the fast muscle exhibits a greater degree of co-operativity. These findings are very similar to those of Kerrick, Secrist, Coby & Lucas (1976), who studied the $\text{pCa}$-tension relationship of fast and slow fibres isolated from rabbit muscle. They found that the $\text{pCa}$-tension curve was shifted around 0.3 $\text{pCa}$ units to the right in fast compared to slow fibres. In contrast, the $\text{pCa}$-tension curves for dogfish fast and slow fibres were more similar. In cases where fast fibres have a higher apparent $K_m$ for $\text{Ca}^{2+}$, for a given rate of removal of $\text{Ca}^{2+}$ by the sarcoplasmic reticulum, fast muscle will relax more quickly. A more rapid rate of relaxation is also aided by the higher fractional volumes (Eisenberg, Kuda & Peter, 1974; Eisenberg & Kuda, 1975; Kryvi, 1977; Johnston, 1980b) and greater rates of $\text{Ca}^{2+}$ uptake (Briggs, Poland & Solaro 1977; McArdle & Johnston, 1981) of fast muscle sarcoplasmic reticulum. The fast muscles of fish also contain high concentrations of parvalbumins, cytoplasmic $\text{Ca}^{2+}$ binding proteins, which are thought to aid rapid relaxation (Gerday & Gillis, 1976; Pechère, Derancourt & Harech, 1977).

The data suggest that a minimum of two $\text{Ca}^{2+}$ binding sites are involved in the activation of cod fibres, and four in dogfish. $\text{Ca}^{2+}$ binding sites have been identified on both the thick and thin filaments in skeletal muscle. Four sites are found on troponin C (e.g. Collins, Potter, Horn, Wilshire & Jackson, 1973; Potter & Gergely, 1975), and two on the LC$_2$ light chains of myosin (e.g. Kendrick-Jones, Szentkiralyi & Szent-Györgyi, 1976). Many invertebrates possess only thick filament regulation (Szent-Györgyi, 1975). Most muscles including those of the higher vertebrates have both regulatory complexes. Although the physiological importance of the LC$_2$ sites is in question, evidence does exist for a functional myosin linked regulation in vertebrates (Huxley, 1972; Chaplain & Gergs, 1974; Lehman, 1978). A kinetic analysis of isometric tension transients from frog skinned fibres led Moisescu (1976) to propose a minimum of six $\text{Ca}^{2+}$ ions per functional unit in frog. It would appear that the activation of both cod and dogfish myotomal muscle involves a minimum of four $\text{Ca}^{2+}$ binding sites, located on the troponin C molecules. In addition, the two $\text{Ca}^{2+}$ binding sites on the myosin LC$_2$ light chains may be involved.

**Maximum contraction velocity**

Values of $V_{\text{max}}$ and $a/P_0$ obtained in the present study, and those taken from the literature are summarized in Tables 2 and 3 respectively. $V_{\text{max}}$ for cod and dogfish fast fibres is two to three times greater than in slow fibres. Flitney & Johnston (1979) report an approximately two-fold difference in *Talapia*. This is in marked contrast to the values found for amphibian muscles, where a six to ten-fold difference has been
reported (Costantin et al. 1967; Lännergren, 1978). The results obtained from fish are in fact more similar to those from mammalian fast and slow twitch fibres. As can be seen in Table 4, mammalian fast fibre $V_{\text{max}}$ are two to three times greater than slow. Note that the table is not comprehensive, merely representative of the data in the literature.

It is thought that amphibian slow fibres are responsible only for maintaining posture, and therefore have no requirement for a high contraction velocity. However, the slow fibres of fish play an active role in locomotion. At slow and intermediate swimming speeds in elasmobranchs and other primitive fish, it is the slow fibre population alone which is active (Bone, 1966; Bone et al. 1978). In function, therefore, they have a role more analogous to the slow twitch fibres of mammalian skeletal muscle. This may be reflected in the small differences in $V_{\text{max}}$ seen between fast and slow muscle.

The force-velocity relationship

The degree of curvature of the force-velocity relationship is determined by the value of $a/P_0$. As $a/P_0$ decreases, the relationship becomes more curved. The values obtained for $a/P_0$ in the present study (Table 2) lie within the range found in other vertebrate muscles, listed in Table 4.

The degree of curvature of the force-velocity curve is thought to have functional significance. Hill (1938) suggested that the exact shape was connected with the heat produced, and that the rate of heat production ($h$) could be described by:

$$h = av + f(t),$$

where $f(t)$ is a rate of heat production which falls during a tetanus to reach a steady value equal to $a.b$. From this, it follows that as $a/P_0$ decreases the ratio of work rate ($P.v$) to (work + heat) increases.

Woledge (1968) tested those predictions by studying the thermal and mechanical properties of tortoise rectus femoris muscle ($a/P_0 = 0.07$ at $0 \, ^\circ\text{C}$), and comparing his results with those of Hill (1938) on frog sartorius ($a/P_0 = 0.26$ at $0 \, ^\circ\text{C}$). Woledge concluded that tortoise muscle has a work rate to (work + heat) ratio 70% greater than that of frog muscle. He also found the efficiency of the whole cycle of contraction and recovery to be 70% greater. Evidence was presented to suggest that tortoise muscle can obtain a greater amount of work per mole of phosphocreatine than frog muscle. Although the exact quantitative relations are not known, Woledge suggested that a more curved $P-V$ relationship is associated with a more efficient conversion of free energy into work.

In any muscle, there must be a compromise between isotonic efficiency and power output, since an increased curvature of the $P-V$ relationship will lead to a decrease in force at a given velocity. From a study of Tables 2 and 4, it can be seen that with the exception of dogfish fast muscle, all the fish fibre types investigated have similar values of $a/P_0$, within the range given for amphibian and mammalian fast muscles, suggesting an adaptation towards higher power output. It is interesting to note that in higher teleosts such as the cod there is good evidence to suggest that fast fibres are recruited at intermediate sustainable swimming speeds, in addition to providing power for burst swimming (Johnston et al. 1977; Bone et al. 1978; Johnston & Moon,
<table>
<thead>
<tr>
<th></th>
<th>$V_{\text{max}}$ (L sec$^{-1}$)</th>
<th>$\alpha/\rho_a$</th>
<th>Experimental details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tilapia mossambica</em></td>
<td>2.57</td>
<td>1.50</td>
<td>18 °C; intact fibre bundles, opercular muscles</td>
</tr>
<tr>
<td>Amphibia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Frog, Rana temporaria</em></td>
<td>1.4-2.39</td>
<td>0.17-0.29</td>
<td>4-7 °C skinned fibres, semitendinosus muscle</td>
</tr>
<tr>
<td><em>Rana temporaria</em></td>
<td>1.29</td>
<td>0.26</td>
<td>6 °C, whole sartorius muscle</td>
</tr>
<tr>
<td><em>Anuran toad, Xenopus laevis</em></td>
<td>6.34</td>
<td>0.18-0.33</td>
<td>0 °C, whole sartorius muscle</td>
</tr>
<tr>
<td><strong>Reptiles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tortoise, Testudo spp.</em></td>
<td></td>
<td>0.07-0.16</td>
<td>0 °C, whole retractor penis muscle</td>
</tr>
<tr>
<td><em>Testudo spp.</em></td>
<td></td>
<td>0.23</td>
<td>0 °C, large fibres bundle, rectus femoris muscle</td>
</tr>
<tr>
<td><strong>Mammals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>2.3</td>
<td>0.7</td>
<td>15 °C e.d.l. (fast twitch) and soleus (slow twitch) skinned fibres</td>
</tr>
<tr>
<td>Rat$^{10}$</td>
<td>43 μm sarcomere$^{-1}$</td>
<td>18 μm sarcomere$^{-1}$</td>
<td>35 °C whole e.d.l. and soleus</td>
</tr>
<tr>
<td>Mouse$^{11}$</td>
<td>50 μm sarcomere$^{-1}$</td>
<td>23 μm sarcomere$^{-1}$</td>
<td>35 °C whole e.d.l. and soleus</td>
</tr>
</tbody>
</table>

1980). This similarity of function may be reflected in the small differences seen in $a/P_0$ between cod fast and slow fibres. However, in discussing efficiency, it is necessary to consider the mechanical constraints under which the muscles operate. Alexander (1969) has studied fibre geometry in a large number of fish, and made estimates of the degree of fibre shortening during the body flexures associated with swimming. He calculated that in cod, the fast fibres operate almost isometrically during swimming, shortening by only 2–3%. In dogfish, shortening is thought to be 7–9%, very similar to the 10% predicted for slow fibres in both fish. As an example, Goldspink (1975) has shown that the chicken anterior latissimus dorsus (a.l.d.), a true slow muscle, is fifteen to eighteen times more efficient (in terms of cost of a 1 min contraction in $\mu$mol ATP) than the fast twitch posterior latissimus dorsus during isometric contractions. However, the a.l.d. is very inefficient under isotonic conditions.

Further studies involving simultaneous measurements of heat production and chemical breakdown under isometric and isotonic conditions are required to elucidate the significance of different $a/P_0$ values in different muscles.

The shape of the isotonic velocity curve

In all fibres studied, isotonic velocity decreased with shortening. In slow fibres, this phenomenon was particularly marked, producing very curved displacement traces. These results are consistent with many previous studies in skinned and intact fibres (see references below). A small departure from linearity in isotonic velocity curves is a common feature of maximally activated skinned fibre preparations. The more marked decrease in velocity observed in slow fibres has also been noted in amphibian slow fibres (Aidley, 1965; Floyd & Smith, 1971; Lannergren, 1978), and in cardiac muscle (Forman et al. 1972). It would seem that this length-dependent decrease in contraction velocity was a fundamental property of the contractile apparatus.

The increase in this length-dependent decrease in velocity seen in submaximally activated fibres, and here studied in dogfish, have recently been investigated by other workers. Similar results to those in the present study have been obtained by Brenner (1980) using glycerinated rabbit psoas fibres, and by Gulati & Podolsky (1981) on mechanically skinned frog fibres. In both studies, it was observed that the progressive decrease in shortening velocity was markedly increased at submaximal Ca$^{2+}$ concentrations. These findings may help to explain some of the inconsistencies in studies which have assumed uniform or near uniform shortening. For example, contraction velocity in skinned fibres has been variously reported as being independent (Podolsky & Teichholz, 1970; Thames et al. 1974; Gulati & Podolsky, 1978) or dependent (Julian, 1971; Wise et al. 1971; Julian & Moss, 1981) on calcium concentration.

Brenner (1980) has suggested that this decrease in contraction velocity at submaximal [Ca$^{2+}$] is a result of an increase in cross-bridge load. Another possibility is that such length-dependent effects in skeletal muscle are related to change in affinity of troponin-C for Ca$^{2+}$ as has been suggested for cardiac fibres. For example, in cardiac cells loaded with the Ca$^{2+}$-sensitive photo-protein aequorin, a rapid, step decrease in length is accompanied by a transient increase in intracellular [Ca$^{2+}$] suggesting Ca$^{2+}$ release from the thin filaments (Allen & Kurihara, 1981). However, such a mechanism would seem unlikely to explain the still substantial non-linearity of isotonic transients at saturating Ca$^{2+}$ levels.
Various other length-dependent phenomena have been reported in skeletal muscle which appear to be related to the degree of activation of the fibres. For example, Edman (1975, 1976; 1980) has described a length-dependent deactivation in intact frog fibres. The degree of tension deactivation was found to be greater when a step decrease in length was given during a twitch than during a fused tetanus, again suggesting a correlation with the level of activation.

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REFERENCES


FISH SKINNED MUSCLE FIBRES


