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ENERGY COST OF CONTRACTION IN FAST AND SLOW MUSCLE FIBRES ISOLATED FROM AN ELASMOBRANCH AND AN ANTARCTIC TELEOST FISH

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SUMMARY

1. Single fast and small bundles of slow fibres were isolated from the muscles of an elasmobranch (dogfish, *Scyliorhinus canicula*) and an Antarctic teleost (*Notothenia neglecta*). A third fibre type present in the dogfish (superficial fibre) was also isolated. Fibres were chemically skinned with a non-ionic detergent.

2. Tension generation and ATPase activity were measured during isometric activations. ATPase activity was estimated by measuring the release of ADP into the experimental solutions using high performance liquid chromatography.

3. In the dogfish fibre types, both tension and ATPase activity increased in the order superficial < slow < fast, even after corrections were made for differences in myofibrillar density. The economy of isometric contraction (tension/ATPase activity) was 50–60% higher in the slow and superficial fibres than in the fast.

4. In the Antarctic species, both tension and ATPase activity of the fast fibres were higher than those of the slow fibres, and the slow fibres were 30% more economical than fast fibres. After correction for differences in myofibrillar density, tensions were very similar.

5. The results are discussed with reference to the energy supply, recruitment pattern and function of the various fibre types.

INTRODUCTION

The muscle tissues of all vertebrates are composed of several fibre types readily distinguishable on the basis of their electrophysiological, mechanical and biochemical properties (Hess, 1970; Guthe, 1981; Johnston, 1985). The broadest division is into fast and slow twitch fibres and slow tonic fibres. It is generally accepted that this diversity of fibre type has functional significance. The fast fibres are thought to be used for rapid, brief movements, the slow fibres for slow, maintained contractions and in the maintenance of posture. This proposal has led to the suggestion that the different fibre types may differ in their energy requirements during contraction. It would be energetically advantageous if the slow fibres, which

Key words: muscle, mechanics, energetics, skinned fibres, Antarctic fish.

may be active for long periods, had a lower cost of contraction than the briefly active fast fibres. This idea is supported by the work of a number of groups (e.g. Awan & Goldspink, 1972; Crow & Kushmerick, 1982) conducted on mammalian fast and slow twitch muscles, in which phosphocreatine breakdown and oxygen consumption were measured before and after contraction. These measurements include the effects of differences in the type and local availability of substrates intrinsic to each fibre type. Furthermore, they do not always control for the mass of the muscle or the dimensions and arrangement of fibres within the muscle, which determine its mechanical properties. The skinned fibre is essentially a system of isolated myofibrils, with an energy supply under the control of the experimenter. It is therefore possible to study the 'economy' intrinsic to the force-generating mechanism itself. In the present study, we have measured the myofibrillar ATPase activity during isometric contractions of skinned fibres isolated from the fast and slow muscles of two species of fish, the dogfish (*Scyliorhinus canicula*) and an Antarctic teleost, *Notothenia neglecta*. In addition, the large diameter, slow, superficial fibres of the dogfish, which Bone, Johnston, Pulsford & Ryan (1985) have recently reported to generate unusually low tensions, have also been studied.

MATERIALS AND METHODS

Fibre preparation and solutions

Dogfish (*Scyliorhinus canicula*) were obtained from the Millport Marine Laboratory, Gt Cumbrae, Firth of Clyde, and kept in filtered, recirculated sea water at 10°C for up to 8 weeks. *Notothenia neglecta* were obtained by courtesy of the British Antarctic Survey from the South Orkney Islands, British Antarctic Territories, and were kept in filtered, recirculated sea water at 1°C for up to 9 months prior to experimentation.

Fish were killed by a blow to the head followed by pithing. Small fibre bundles were isolated from myotomes above the pelvic fins of the dogfish. In the Antarctic fish, fast (white) muscle was dissected from epaxial myotomes 4–6, counting from the head, and red (slow) muscle from the adductor pectoralis profundus muscle. Single fast and superficial fibres and small bundles of slow fibres were isolated in a small drop of relaxing solution, under silicone oil at 0–5 °C. The relaxing solution had the following composition: 65 mmol l^{-1} PIPES [piperazine-*N*,*N*′-bis(2-ethanesulphonic acid)] pH 7·4 at 0°C, 6·5 mmol l^{-1} $MgCl_2$, 15 mmol l^{-1} EGTA (ethyleneglycol-bis-(β-aminoethylether)*N*,*N*′-tetraacetic acid) and 6 mmol l^{-1} ATP. Activating solution was made by the addition of $CaCl_2$ to set pCa to 4·69, which was maximally activating for all fibre types. The concentration of buffer was adjusted to maintain a pMg of 3·02, a pMgATP of 2·29 and an ionic strength of 0·175 mol l^{-1} in both solutions. The concentrations of the various ionic species were calculated with the aid of an iterative computer program similar to that described by Fabiato & Fabiato (1979). Skinning solution was made by the addition of 1% Brij 58 (polyoxyethylene 20 cetyl ether) to relaxing solution. 50 μg ml^{-1} oligomycin was

added to the solutions in some experiments to study the activity of the mitochondrial ATPase.

Apparatus

Fibres were attached to the apparatus by wrapping the ends around stainless steel wire hooks, and a drop of methacrylate polymer (Perspex)/acetone glue was added for additional security, and to cover the part of the fibre not involved in force generation. One hook was attached to a rigid mounting, the other to an AME 801 silicon beam strain gauge (AME, Horten, Norway), sensitivity $0{\cdot}5\,\mathrm{mN\,V^{-1}}$, compliance $<4\,\mu\mathrm{m\,mN^{-1}}$. Sarcomere length, measured by laser diffraction, was set to $2{\cdot}3\,\mu\mathrm{m}$ (for optimum overlap of actin and myosin filaments), and fibre diameter and length measured. The skinning and relaxing solutions were held in 2-ml capacity chambers set into a temperature-controlled Perspex block. A 200-μl capacity chamber contained the activating solution. Solution changes could be effected in 1–2 s. Solutions were stirred by gentle vibration of the chambers.

Experimental protocol

All experiments were conducted at 0°C. Fibres were initially skinned for 20 min before being transferred to relaxing solution for 10 min. Unpublished electron microscopic studies reveal extensive damage to all cell membranes. Fibres were then activated in 175 μl of activating solution, and force was measured throughout isometric contractions of 2–6 min duration. After the fibre had been returned to relaxing solution, the activating solution was removed and frozen (−30°C) for later analysis. Fibres were equilibrated in relaxing solution for 10 min between activations.

ATPase assay and data analysis

ATP utilization by the fibres was measured as an increase in the total ADP content of the post-contraction activating solution. ADP diffusion from the fibre into the solution was assumed to be rapid relative to the length of the contraction (Kushmerick & Podolsky, 1969; see Results). A Gilson High Performance Liquid Chromatography system was used to separate and measure the concentrations of the various nucleotides present in the solution. 100-μl samples were injected onto an ODS Ultrasphere (reversed phase) column (Altex, Berkeley, California). AMP and ADP were extracted on an isocratic gradient of 20% (v/v) methanol against an aqueous buffer of $20\,\mathrm{mmol\,l^{-1}}$ KH_2PO_4, $5\,\mathrm{mmol\,l^{-1}}$ tetrabutyl ammonium hydroxide, set to pH 2·65 with H_3PO_4. The methanol was increased to 50% to release the ATP. Nucleotides were monitored as they came off the column with a 254 nm detector. ADP production was calculated by integrating the area under the chromatograph. Standards (100 μl) containing 0–50 nmol ADP were used to calibrate the column, and the area was linearly related to the amount of ADP. The retention time of ADP varied slightly from column to column, and with column age, but a typical value for a day's experiments was 14 min ± 2 s (mean ± S.E., $N = 13$). Typically, around five

controls were analysed on a given day (with their associated experimental samples), with an S.E. of 0·05 nmol ADP about the mean.

If it is assumed that the two myosin S_1 heads on each molecule act independently, then the rate of ATP hydrolysis ($ATP\,S_1^{-1}\,s^{-1}$) can be calculated. For this calculation, myosin was assumed to account for 8% of wet weight in fibres composed of 80% myofibrils (Bendall, 1969). The wet weight was calculated from fibre volume, assuming a specific gravity of 1·1, and it is assumed initially that all fibres are 100% myofibrils. Although these assumptions may lead to small systematic errors, they obviate the need for direct measurement of myosin content, which is difficult and inaccurate on small preparations. Maximum isometric tension is expressed as $kN\,m^{-2}$. Since the proportion of space occupied by myofibrils varies with species and fibre type, both tension and ATP turnover rate data must be corrected for percentage myofibrillar volume, which has been determined from stereological analysis of electron micrographs. These corrected values are given along with the uncorrected data. In dogfish, myofibrils occupy 75·7%, 62·2% and 77·8% cell volume in superficial, slow and fast fibres, respectively (Bone *et al.* 1985), and in *N. neglecta* 86·3% and 54·4% in fast and slow fibres, respectively (Camm & Johnston, 1985).

The energy cost of contraction is obtained by dividing the tension by the ATPase activity, assuming tension does not decrease during contraction. Errors introduced by this assumption are <7% for dogfish fast fibres, and <3% for all others. The rate of rise of tension was not significantly different between fibre types, and would not, therefore, lead to systematic error.

RESULTS

Preliminary work was performed to determine the most appropriate experimental protocol. Cross contamination of chambers due to solutions carried on the fibres was found to be negligible provided the relaxing solution was regularly changed. ATP solutions always contained trace quantities of contaminating ADP. ATP hydrolysis in relaxed fibres was insignificant. Thus two types of control were used with the same result: (1) relaxing solutions in which the fibres had been incubated for a time equal to the subsequent activation, and (2) activating solutions which had not been exposed to the fibre. In Fig. 1 a control chromatogram is shown, together with post-contraction chromatograms from four consecutive 3-min activations of a slow fibre preparation from *N. neglecta*.

Fish slow muscle is rich in mitochondria. To determine whether or not the skinning treatment leaves the mitochondrial ATP synthase active, myofibrillar ATPase activity was determined in the presence and absence of $50\,\mu g\,ml^{-1}$ oligomycin, controls being performed on the same fibres. The results are summarized in Table 1. ATPase activity was greater, and showed considerably less scatter, in the presence of oligomycin, although a paired *t*-test did not reveal a significant difference between the two data sets ($P < 0{\cdot}08$). Oligomycin was included in the solutions in all experiments on slow fibres. Oligomycin had no effect on ATPase activity in fast fibres. This was to be expected, given the low mitochondrial

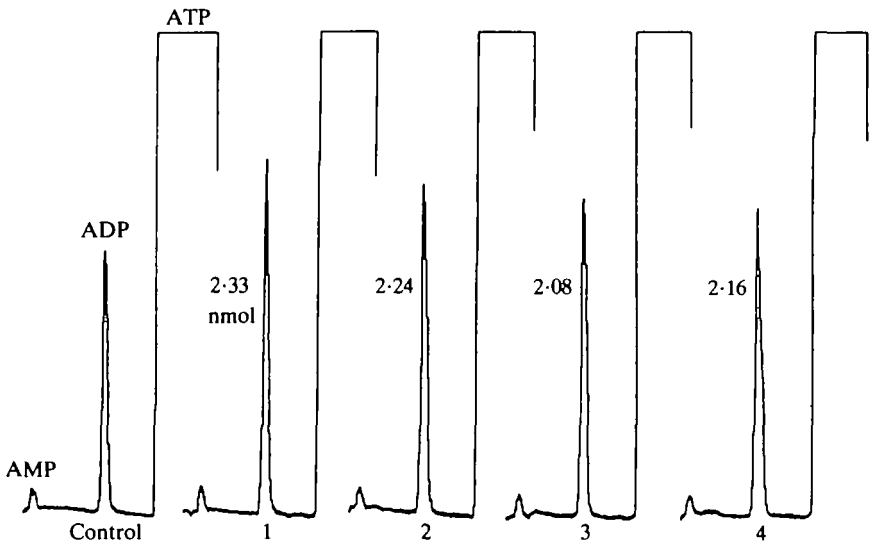

Fig. 1. Post-contraction chromatograms from four consecutive 3-min activations of a slow fibre bundle from *Notothenia neglecta*, together with a control. The figures refer to the increase in total ADP (in nmol) in each sample.

density (Walesby & Johnston, 1980). Maximum isometric tension was not affected by oligomycin.

ATPase activity in both fast and slow fibres was measured after activations of different durations up to 6 min in length. ATPase activity was found to be independent of the duration of the contraction between 2 and 6 min. In Fig. 2, results are shown from four fibres with different cross sectional areas and rates of ADP release, to illustrate this point. The long contractions needed in these experiments did not allow more than two 'active' points to be obtained from fast fibres before tension began to decline. However, any deviation from linearity after 2 min not detected by these experiments would be small.

A further potential complication was the effect of fibre diameter on the diffusion of ADP out of the fibre, and hence the measured ATPase activity. No correlation was found between fibre diameter and ATPase activity, suggesting that the measurements are not significantly influenced by diffusion: this is illustrated for

Table 1. *The effect of oligomycin on myofibrillar ATPase activity in skinned fibres from* Notothenia neglecta

	ATPase activity ($S_1^{-1}s^{-1}$)	
	+ Oligomycin	Control
Slow fibres	0·71 ± 0·04	0·57 ± 0·07
N	10	10
Fast fibres	1·86 ± 0·17	1·89 ± 0·25
N	4	4

N = number of fibres (means ± S.E.).

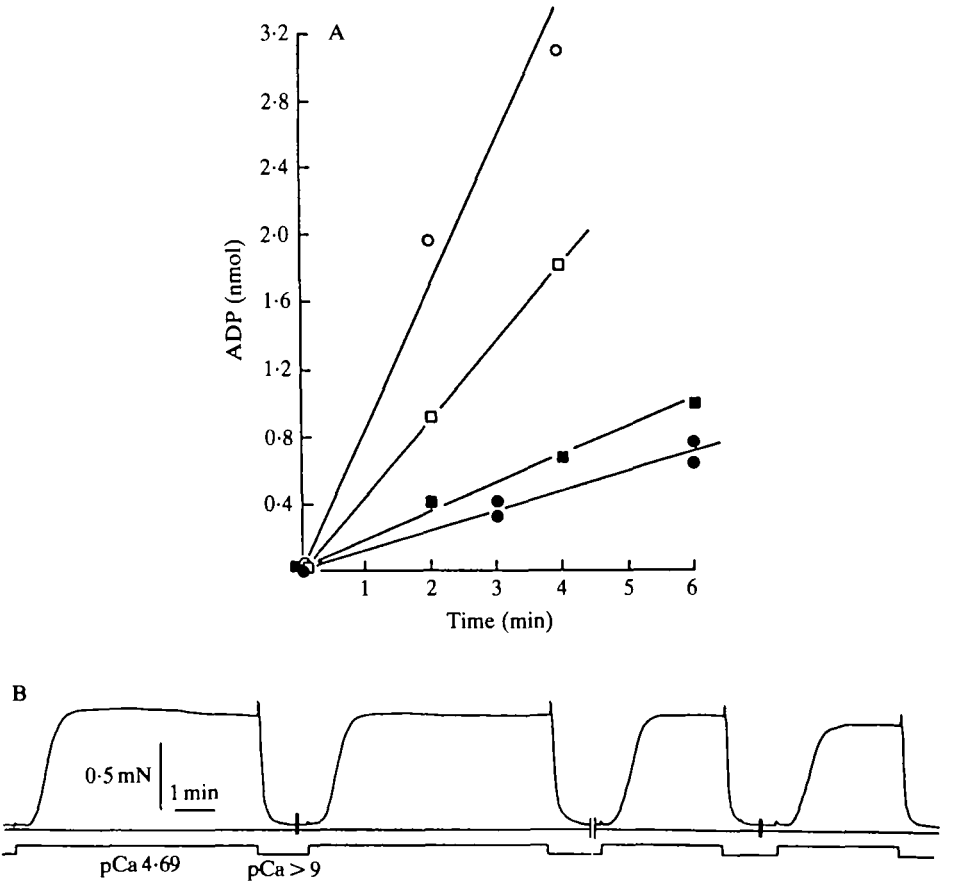

Fig. 2. (A) ADP released plotted against time for four preparations. Open circles, *Notothenia neglecta* fast fibre, cross sectional area (CSA) = 0·0095 mm^2; open squares, *N. neglecta* fast fibre, CSA = 0·0073 mm^2; closed circles, dogfish slow fibre bundle, CSA = 0·0149 mm^2; closed squares, *N. neglecta* slow fibre bundle, CSA = 0·0079 mm^2. (B) Isometric force against time records from the dogfish slow fibre bundle used in A.

N. neglecta fast fibres in Fig. 3, together with a plot of maximum tension against fibre diameter. Since tension is independent of diameter, it can be assumed that ATP levels in the centre of the fibre are saturating.

In comparing the ATPase activity and maximum isometric tensions of the various fibre types, measurements were taken over at least two contractions per fibre (2–4 min for fast fibres, 2–6 min for slow fibres). The decline in maximum isometric tension (P_0) in fast fibres ranged from 1–5 % P_0 min^{-1}, and in slow and superficial fibres was consistently <2 % P_0 min^{-1}. ATPase activity showed no consistent tendency to increase or decrease from activation to activation. The results from all fibre types are summarized in Table 2. In both species, fast fibres generate greater isometric tensions than slow fibres, but the amount of tension generated per ATP utilized by the slow fibres is 30–50 % greater than in the fast fibres. The superficial fibres of the dogfish also generate low tensions and are some 60 % more economical than the fast fibres.

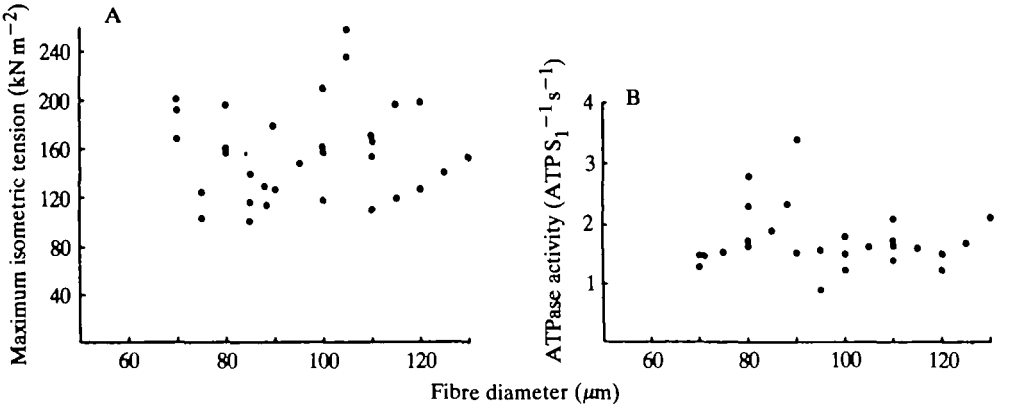

Fig. 3. Maximum isometric tension and ATPase activity plotted against fibre diameter. Data from *Notothenia neglecta* fast fibres.

DISCUSSION

Maximum isometric tensions are a little lower than those reported by Altringham & Johnston (1982) and Johnston & Harrison (1985). In the case of the dogfish, this is largely due to the higher temperature used in the previous experiments. Differences in the experimental solutions may explain some of the discrepancy in both species. ATP turnover rates are comparable to those obtained from carp fast muscle (1·85 ATP s^{-1}, 7°C, Altringham & Johnston, 1985*a*) and rabbit soleus (0·93 s^{-1}, 22°C, Krasner & Maughan, 1984), using a similar technique. Actomyosin ATPase activity has been extensively studied in the frog (at 0°C), using a wide variety of techniques, yielding similar results. For example, Levy, Umazume & Kushmerick (1976) reported a value of 0·96 s^{-1} for fully activated, isometric skinned fibres, and Homsher, Irving & Wallner (1981), who measured phosphocreatine breakdown after long tetani in whole muscles, reported a value of 1·1 s^{-1}.

Goldspink, Larson & Davies (1970) and Awan & Goldspink (1972) reported a six-fold difference in isometric economy in a range of fast and slow twitch muscles from the hamster. Even larger differences (>200-fold) were found between bird and reptile twitch and tonic fibres (Goldspink, 1975). However, these results reflect differences in economy of the intact muscle and include extrinsic factors such as variation in cross sectional area relative to muscle mass. More recent work by Crow & Kushmerick (1982), which includes corrections for differences in muscle geometry, found only small differences in economy between fast and slow twitch muscles of the mouse. During 12-s tetani the energy cost of the extensor digitorum longus muscle (fast) was only 50% greater than that of the soleus (slow). The present work supports these findings. In the two species studied, slow fibres are some 25–50% more economical in their energy demands than the fast fibres. Very recently, however, Elzinga & Lännergren (1985) have reported a 10-fold greater variation in stable maintenance heat rate relative to tension, in single fibres isolated from the toad *Xenopus laevis*.

The results presented here, and those of Crow & Kushmerick (1982) and Elzinga & Lännergren (1985), presumably reflect differences in the myosin cross bridges that

Table 2. *Maximum isometric tension and ATPase activity of the various fibre types in* Notothenia neglecta *and the dogfish* Scyliorhinus canicula

	Dogfish *S. canicula*			*N. neglecta*	
Fibre type	Fast	Slow	Superficial	Fast	Slow
Maximum isometric tension ($kN\,m^{-2}$)	$132 \pm 6^{***}$	49 ± 7 NS	40 ± 4	$156 \pm 7^{***}$	81 ± 9
(corrected)	(170)	(79)	(53)	(181)	(149)
N	6	11	5	31	19
ATPase activity ($S_1^{-1}\,s^{-1}$)	$0{\cdot}79 \pm 0{\cdot}06^{***}$	$0{\cdot}20 \pm 0{\cdot}04$ NS	$0{\cdot}15 \pm 0{\cdot}02$	$1{\cdot}74 \pm 0{\cdot}10^{***}$	$0{\cdot}71 \pm 0{\cdot}05$
(corrected)	(1·02)	(0·32)	(0·20)	(2·02)	(1·31)
N	6	11	5	27	16
'Economy' P_0/ATPase activity (expressed relative to fast fibres)	1	1·48	1·62	1	1·27

The values in brackets have been corrected for differences in myofibrillar density between fibre types (see text for details). Economies are expressed relative to that of the fast fibres in each species.

All values are mean ± S.E., *N* = number of fibres.

*** = $P < 0{\cdot}001$, NS = not significant (standard *t*-test).

have some functional significance. It is therefore of interest to discuss them in relation to the energy supply, biochemical and mechanical properties and function of the various fibre types. Slow and superficial fibres in dogfish are multiply innervated by a number of different motor axons (Bone, 1978). The energy supply to slow fibres is primarily aerobic – the mitochondria ocupying 34% of fibre volume (Bone *et al.* 1985) – and is sufficient to meet the normal demands of slow, sustained swimming, during which only the slow fibres are active. In contrast, superficial fibres are pale, have a low mitochondrial volume density (<5%), and are more densely packed with myofibrils. Superficial fibres are thought to have a tonic role in this benthic shark, holding the head and tail in a raised position above the sea bed (Bone *et al.* 1985). The finding of a high isometric economy and low rate of isometric ATP turnover for superficial fibres is consistent with their specialization for long-term tension production at low metabolic cost. The ultrastructure and contractile properties of the superficial fibres are similar to those described for the pale, multiply innervated (type 5) fibres of the iliofibularis muscle of the toad, *Xenopus laevis* (Lännergren, 1978; Smith & Ovalle, 1973).

In contrast to the slow and superficial fibres, white fibres in the dogfish are focally innervated by two motor axons (Bone, 1978). Maximum unloaded contraction velocities are two- and eight-fold greater than those of slow and superficial fibres, respectively (Altringham & Johnson, 1982; Bone *et al.* 1985). Fast fibres have low mitochondrial volume densities and relatively high power outputs (Bone *et al.* 1985). They are recruited exclusively for burst swimming, and fatigue after 1–2 min activity, coincident with the exhaustion of glycogen stores (Bone, 1966). Our studies have shown these fibres to have the highest ATP turnover rates, and to produce the highest tension, consistent with their need to deliver a high power output for short periods.

The various myosin light and heavy chains are expressed at different genetic loci, and can be synthesized separately, producing fibres with very different compositions. Lännergren & Hoh (1984) have recently examined the native myosin isoenzyme composition of single toad fibres from which they had previously obtained force–velocity curves, and found evidence for at least five myosin isotypes. If the maximal tensions produced by the three fibre types in dogfish are corrected for differences in myofibrillar packing, the stresses range by a factor of 3 (Table 2). One explanation of this could be that the force generated per cross bridge cycle is different in each fibre type, providing functional evidence for at least three myosin isotypes. The variation in force may result from fundamental differences in the properties of the myosin heads, or from differences in the proportion of time spent in the force-generating state.

In contrast to the elasmobranchs, the fast and slow fibres of advanced teleost fish, such as *N. neglecta,* are both multiply innervated (Johnston, 1981). In some teleost species the functional role and metabolic differentiation of different fibre types is less clear cut than for elasmobranchs (Johnston & Moon, 1980, 1981). Slow, sustained activity in *N. neglecta* is achieved largely through the use of large fan-shaped pectoral fins. Muscle fibres in the fins are predominantly slow, with a high mitochondrial

volume density and high activities of enzymes associated with aerobic metabolism (Walesby & Johnston, 1980; Johnston & Harrison, 1985). The trunk muscles of Notothenids are composed almost exclusively of fast fibres, and are usually recruited for only a few tailbeat cycles at a time (Montgomery & MacDonald, 1984). The fast fibres contain <3% mitochondria, and have low activities of both aerobic and anaerobic enzymes (Johnston & Harrison, 1985), but high activities of enzymes associated with the immediate supply of energy for contraction, e.g. creatine phosphokinase, adenylate kinase and 5'AMP aminohydrolase (Walesby & Johnston, 1980). These results suggest that the energy supply to these fibres is probably based on phosphocreatine breakdown, which may be sufficient for short bursts of activity. Phosphocreatine stores are probably replenished during rest periods by aerobic metabolism (Johnston & Harrison, 1985).

Contraction speeds of fast trunk and slow pectoral fibres from several Notothenid species are rather similar, at around 1 and 0·7 muscle lengths s^{-1}, respectively (Johnston & Harrison, 1985), as are their isometric tensions and ATP turnover rates when corrected for differences in myofibrillar density (Table 2). In contrast to dogfish, fast and slow fibres therefore appear to have myosin isotypes with rather similar contractile properties. However, they do differ in their sensitivity to inhibition by inorganic phosphate (Altringham & Johnston, 1985*b*). 10 mmol l^{-1} phosphate was found to decrease maximum isometric tension by 30% in slow fibres, but by only 11% in fast fibres.

It is known that Antarctic fish muscles have an unstable type of myosin (Johnston, Walesby, Davison & Goldspink, 1975; Johnston & Walesby, 1977). Such variation in structure is thought to be related to functional adaptations for operation at temperatures at or below 0°C (Johnston & Walesby, 1977; Johnston & Harrison, 1985). Normal temperatures experienced by *N. neglecta* are −2 to +1°C compared to 6–18°C for the dogfish. The higher tensions and ATP turnover rates at 0°C of homologous fibre types in the Antarctic fish relative to the dogfish may reflect these structural adaptations in the different myosins.

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