Journal of Physiology (1989), **410**, *pp.* 157–170
With 8 text-figures
Printed in Great Britain

VARIATION IN THE NORMALIZED TETANIC FORCE OF SINGLE FROG MUSCLE FIBRES

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(*Received 14 January 1988*)

SUMMARY

1. The forces produced in maximal fixed-end tetani of single fibres isolated from the anterior tibialis muscle of the frog *Rana temporaria* have been measured at sarcomere lengths of 2·2 μm and temperatures near 0 and 10 °C.

2. When normalized by either cross-sectional area or dry weight per unit length at a sarcomere length of 2·2 μm, the forces vary over a twofold range.

3. The normalized force is not significantly correlated with the velocity of unloaded shortening or the twitch characteristics of the fibres. Lack of variability of these two quantities (together with histochemical evidence) suggest that only one fibre type is present in the experimental sample.

4. The steady rate of energy liberation (stable heart rate) of the fibres during isometric tetani is positively correlated with the normalized force, indicating that extra ATP splitting is required to produce higher forces.

5. Fibres with a higher ratio of dry weight per unit length to cross-sectional area ('dry density') show a higher force when normalized by area, but not when normalized by dry weight per unit length.

6. Fibres with a more circular cross-sectional profile produce more force when normalized by either cross-sectional area or dry weight per unit length. The significance of this correlation is unclear.

7. The contribution of various sources to the total overall variation in normalized force is assessed. It is suggested that a diffusible substance or substances may be involved in modulating fibre force.

INTRODUCTION

The maximum force produced in an isometric contraction of a skeletal muscle fibre is expected to be proportional to the cross-sectional area of the fibre because most of the cross-section is occupied by myofibrils (Eisenberg, 1983) which are thought to be

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mechanically in parallel with one another (but see Street, 1983), so that the forces they produce sum. However, when single frog muscle fibres are compared, the normalized force, i.e. the ratio of the force to the cross-sectional area, is quite variable (e.g. Table 1 in Edman, 1979). This paper investigates some possible reasons for this variation.

The first possibility we consider is that the water content varies from one fibre to another. If this were so, cross-sectional area would not be a good estimator of the quantity of myofibrils within a fibre. Because most of the dry weight of the fibre is protein, and most of the protein is myofibrillar (Dubuisson, 1954), a better estimator would be the dry weight per unit fibre length. The second possible reason for variation between fibres is that they are of different 'types'. It is well known that in amphibian muscles there exist a number of histochemically distinct fibre types (Lännergren & Smith, 1966; Lännergren & Hoh, 1984; Pagani, Faris, Striz & Julian, 1985). In *Xenopus*, these types produce different amounts of force (Elzinga & Lännergren, 1985); this might also be the case in the frog. We have therefore characterized the type of the fibres, whose force we have measured, using histochemistry, force–velocity properties and twitch characteristics (another traditional way of dividing fibres into types). Thirdly, we have considered the possibility that the force is variable between fibres because the externally recorded force does not fully reflect that developed internally. This, for example, is what happens when muscles shorten beyond the plateau of the tension–length curve. At these lengths heat production is independent of length (Elzinga, Peckham & Woledge, 1984), presumably because it reflects the turnover of ATP by the myofibrils in any internal force development as well as that used in developing externally recorded force. We have therefore investigated whether the variability could be due to different proportions of externally recorded force by measuring the heat production of the fibres during tetanic contractions. In this paper, we assess the contribution of these and other sources of variation to the observed diversity of fibre properties.

Preliminary results of these experiments have been presented by Elzinga, Howarth, Wilson & Woledge (1985) and by Rall, Wilson & Woledge (1985).

METHODS

We used single fibres, dissected from the anterior tibialis muscle of the frog, *Rana temporaria*. No deliberate selection of fibres (for example, by diameter) was made. The Ringer solution contained (in mmol/l): NaCl, 115·5; KCl, 2·2; $MgCl_2$, 1; $CaCl_2$, 1·8; Ca-EGTA, 5; Na_2HPO_4, 2; NaH_2PO_4, 1.

Fibre diameter

The method used was essentially that described by Blinks (1965; modified after Sato, 1954). A muscle fibre was mounted horizontally in Ringer solution in a glass-walled chamber. At one end of the fibre, the tendon was crimped with a folded T-shaped aluminium foil clip and attached to a fixed metal hook. At the other end, a small hole was made in the tendon and a piece of stainless-steel wire 75 μm in diameter was passed through the hole and then pulled taut and secured at either end. Sarcomere length (measured by laser diffraction) was set to 2·2 μm. The fibre was viewed axially with a microscope fitted with a long-working-distance objective modified for water immersion (Leitz UM32/0·30 with an appropriate meniscus lens supplied by Ealing Optical). A short length (about 20 μm) of fibre at the focal plane of the microscope was illuminated with the image of a slit produced by focusing the light from a helium–neon laser on an adjustable aperture.

Photographs of the fibre cross-section so revealed were usually taken at two or more places along the fibre, by translating the fibre axially with respect to the objective.

The area and perimeter of the cross-sections were measured from tracings taken from the photographs. Calibration of the system was made by photographing a diffraction grating in Ringer solution. The cross-sectional areas measured at different points along a particular fibre were similar: the coefficient of variation of area was 4·6% (seventeen fibres: range 1·0–12·4%). This figure is very close to that reported by Blinks (1965).

Fibre weight and length

We weighed the fibres with an electrobalance (Cahn Instruments, Cerritos, CA, USA; Model 26). The tendons were trimmed off and the fibre transferred to a small, weighed piece of aluminium foil. Adhering Ringer solution was removed by placing the tip of a triangular wedge of filter paper on the foil beside the fibre. After placing the foil and fibre on the weighing pan and closing the door, fibres usually dried to constant weight in air within 10 min. Weights were read after 20 min. The presence of a container of silica gel (a dessicant) in the weighing chamber did not significantly affect the dry weight. In this study, fibre weights between 9·1 and 58·5 μg were recorded. The balance could be read to 0·1 μg, which corresponds to 0·2–1·1% of these weights. For normalization purposes, we divided the fibre weight by the length of the fibre (excluding tendons) at a sarcomere length of 2·2 μm. This was measured with eyepiece graticules in the dissecting microscope (Zeiss). The length of the fibres varied between 5·0 and 7·8 mm, and was estimated to be accurate to 0·1 mm, corresponding to 1·3–2·0% uncertainty. On the basis of these figures, we estimate the overall uncertainty in the weight per unit length to be no more than 3%.

Mechanical properties

Measurements of tension were made during fixed-end twitches and tetani of the fibres. The fibres in which cross-sectional area had been measured directly were transferred to another bath and mounted between a movable hook and a semiconductor strain gauge (Sensonor, Model AE801) to measure tension, and the sarcomere length was again adjusted to 2·2 μm. The fibres in which heat production was measured (see below) were stimulated by passing current through the T-shaped clips of platinum foil which were used to attach the tendons to the mechanical apparatus. In these fibres, tension was measured with a variable-capacitance transducer (Cambridge Technology, Inc., Model 400). Suprathreshold pulses of 1 ms width were applied with a frequency sufficient to produce a maximal, fused tetanus. At 10 °C, 1 s tetani were used. At 0 °C, for the purposes of the heat measurements, the tetani lasted 10 s. In these experiments, it was sometimes necessary to reduce the frequency of stimulation just below that required for complete fusion in order to sustain the tetanus.

Measurements of the velocity of unloaded shortening, V_0, were made during short (0·5 s) tetani near 0 °C by the method of Edman (1979). Releases were applied through a servomotor system (Cambridge Technology, Inc., Model 300S).

Heat production

The details of the methods have been presented previously (Curtin, Howarth & Woledge, 1983; Curtin, Howarth, Rall, Wilson & Woledge, 1984, 1986). The fibres were mounted isometrically in Ringer solution between the force transducer (see above) and a length controller (set at fixed position during heat production measurements). The chamber in which the fibre was held was made of anodized aluminium and was built on the flat (upper) surface of an aluminium heat sink. The lower surface of the heat sink was immersed in a close-fitting Dewar flask containing a stirred ice–water mixture. A lid to the chamber, also made of anodized aluminium, contained channels through which water from the Dewar flask was circulated. A Hill–Downing type thermopile was used to measure the temperature change of the fibre. It was similar in design to that described by Curtin *et al.* (1983), but the active region was 10 mm long and contained eighty junctions. The thermopile was provided with leads at 1 mm intervals and in most experiments records were made from the four or five sections underlying the central part of the fibre. For calibration purposes, Peltier current was usually passed through the whole thermopile.

Histochemistry

Histochemical staining of muscle sections for myosin ATPase was carried out essentially by the method of Guth & Samaha (1970), but with the following modifications which were suggested to us by Dr A. Rowlerson. Firstly, the sections were not fixed. Secondly, the alkaline buffer was sodium barbitone (29·4 g/l) and sodium acetate (11·7 g/l). Thirdly, all incubations were carried out at room temperature (about 22 °C). Finally, alkaline pre-incubations were for 20 min, and acid pre-incubations were for 5 min.

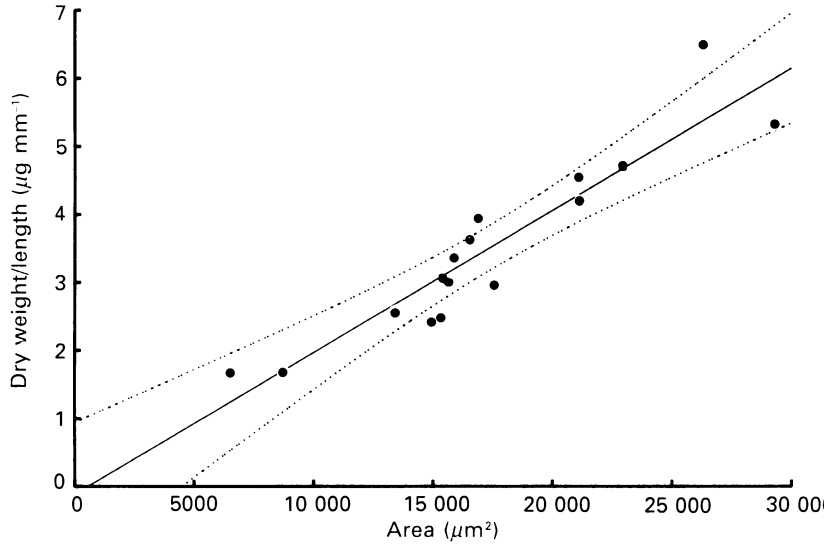

Fig. 1. The relationship between dry weight per unit length and cross-sectional area in sixteen fibres chosen to cover a reasonable range of areas. The continuous line shows the regression of dry weight/length on area; the dotted lines indicate the 95% confidence band for the population regression line.

RESULTS

Fibre diameter and weight/length ratio

Traditionally, cross-sectional area rather than dry weight has been used to normalize force. To compare these methods we measured (at 10 °C), by the method of Blinks (1965), the cross-sectional area of sixteen fibres together with their dry weight per unit length. (A total of seventeen fibres was used for the study, but we did not succeed in measuring force, weight and area in every fibre. This sample of fibres provided the results plotted in Figs 1–4.)

The dry weights per unit length plotted as a function of the cross-sectional areas are shown in Fig. 1. There is a strong correlation between these two measures ($r = 0{\cdot}934$) and the relationship is fairly well described by a straight line. From the slope of the line, one can estimate the average 'dry density', that is, the weight of solutes and insoluble material (specifically excluding water) per unit fibre volume, as 0·209 g ml^{-1} (209 kg m^{-3}). By using Hill's (1965) formula for the density of frog muscle as a function of x, the ratio of dry weight to wet weight ($1{\cdot}006 + 0{\cdot}288x$), we calculate for our fibres the wet:dry weight ratio to be 5·08 and the mean fibre water

content to be 80·3 %. Calculating the fibre diameter from the area assuming a circular cross-section, a weight per unit length of 1 μg mm^{-1} would correspond, on average, to a diameter of 73 μm, while 4 μg mm^{-1} would correspond to 146 μm and so on. A force of 1 N m (g dry wt)$^{-1}$ would thus correspond to 209 kPa (209 mN mm^{-2}). The scatter of points around the regression line is consistent with a variability of the 'dry density', and thus the water content, of these fibres. The range of 'dry densities' measured was 0·163–0·256 g ml^{-1}, corresponding to water contents in the range 84·5–76·2 %.

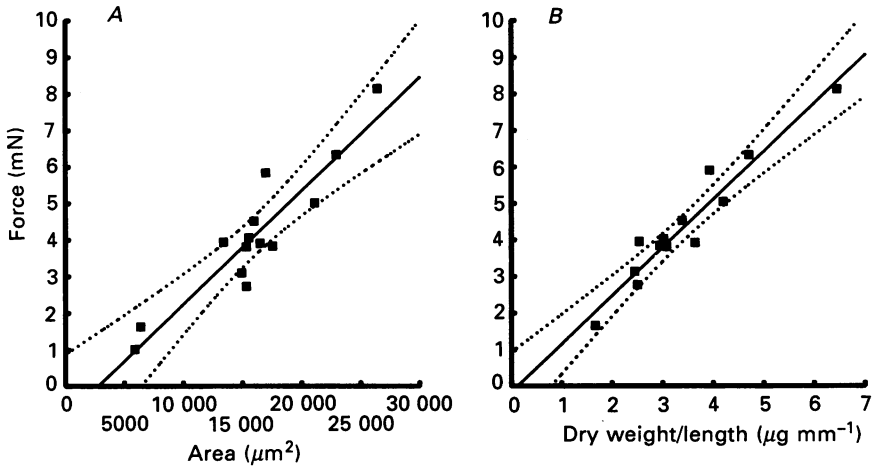

Fig. 2. The dependence of the maximum force produced in short isometric tetani near 10 °C on cross-sectional area (*A*) and dry weight/length (*B*) in the same fibres. The continuous line shows the regression of force on the measure of cross-section; the dotted lines indicate the 95 % confidence bands for the regression lines.

Mechanical properties

The tetanic force produced by these fibres is shown in Fig. 2 as a function of both cross-sectional area and of dry weight/length. The latter is a slightly (but not significantly, $P > 0{\cdot}2$) better predictor of force, but the scatter of points around the fitted lines shows that significant unexplained variation remains in both cases.

The coefficient of variation of force/area was 19·4 %, and for force/(dry weight/length) it was 12·4 %. Normalizing force production for the dry weight per unit length of the fibre is thus a reliable method, and convenient for studies of heat production in single fibres, where the weight is also required. Since it is at least as satisfactory to normalize force by dry weight per unit length as by cross-sectional area when the latter is determined very accurately, this normalization is to be preferred over that based on the more usual method of measuring only the minimum and maximum diameters. Blinks (1965) notes that simple measurements of fibre width also systematically overestimate area.

Comparing the force normalized by cross-sectional area with that normalized by dry weight per unit length reveals that the quantities are significantly correlated ($r = 0{\cdot}625$, $P < 0{\cdot}05$). If all the variation in normalized force were due to random errors in the determination of the normalizing factors, no such correlation would be

apparent. The fact that there is correlation suggests that normalized force varies because the properties of the fibres themselves are somehow different. Of the uncorrelated variation, only part can be explained by uncertainties in the estimates of the normalizing factors, namely area (about 5%) and weight per unit length (about 3%). Factors such as differences in water content and in composition of the 'dry weight' would accentuate this scatter.

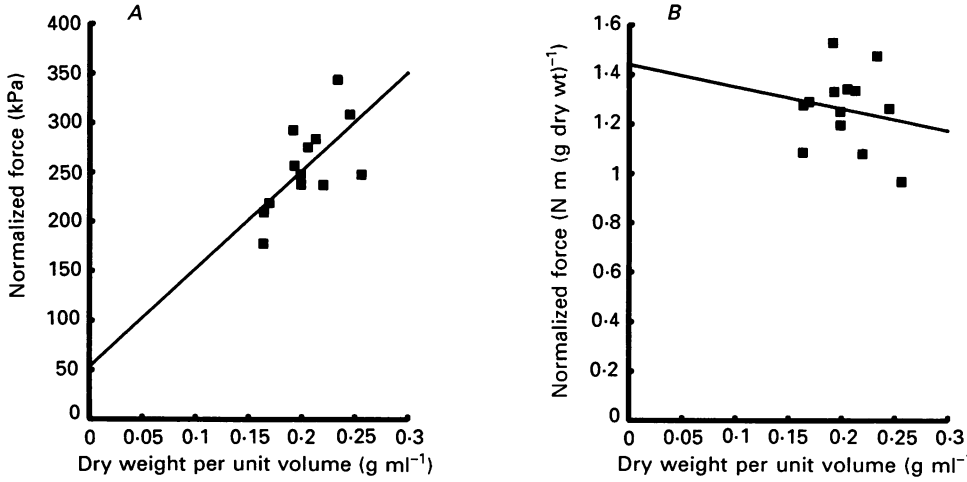

Fig. 3. The dependence of tetanic force normalized by cross-sectional area (*A*) and dry weight per unit length (*B*) on the ratio of dry weight per unit length to cross-sectional area ('dry density'). A higher dry weight per unit volume implies a lower water content of the fibre.

Force normalized by cross-sectional area was found to be significantly correlated with the ratio of dry weight per unit length to cross-sectional area, or 'dry density' ($r = 0{\cdot}658$, $n = 13$, $P < 0{\cdot}05$). This can be seen in Fig. 3*A*. From this we conclude that fibres with a lower water content produced a higher force per unit cross-sectional area. However, as Fig. 3*B* indicates, there was no correlation between force normalized by the dry weight per unit length and the 'dry density' ($r = -0{\cdot}168$, $n = 13$). This is to be expected, since variations in water content will be removed by drying.

Shape

Blinks' method also provides information on the shape of the fibre cross-section. None of the fibre cross-sections was exactly circular; to quantify the degree to which they deviated, we calculated for each fibre an index of circularity. This was defined as the ratio (d_a/d_p), where d_a and d_p are the diameters of circles having the same cross-sectional area and perimeter respectively as the actual cross-section. It was calculated as an average from photographs of the cross-section at several (two to five) places along the fibre (Fig. 4, inset). For a circular cross-section, the ratio (d_a/d_p) equals unity; for any other closed curve the ratio is less and tends to zero as the perimeter becomes more tortuous. The circularity (d_a/d_p) varied between 0·849 and 0·935 in the fibres studied (the same values would be calculated for elliptical

cross-sections with axial ratios in the range 1·508–1·154). The correlation between circularity and tetanic force normalized either for cross-sectional area ($r = 0{\cdot}865$, $n = 14$, $P < 0{\cdot}001$) or for dry weight per unit length ($r = 0{\cdot}734$, $n = 13$, $P < 0{\cdot}01$) is highly significant, as illustrated in Fig. 4). The more circular fibres thus tended to produce a higher normalized tetanic force. Since 'dry density' and circularity were found to be uncorrelated ($r = 0{\cdot}126$, $n = 16$), this effect of shape on force production appears to be independent of any influence of water content on force normalized by cross-sectional area.

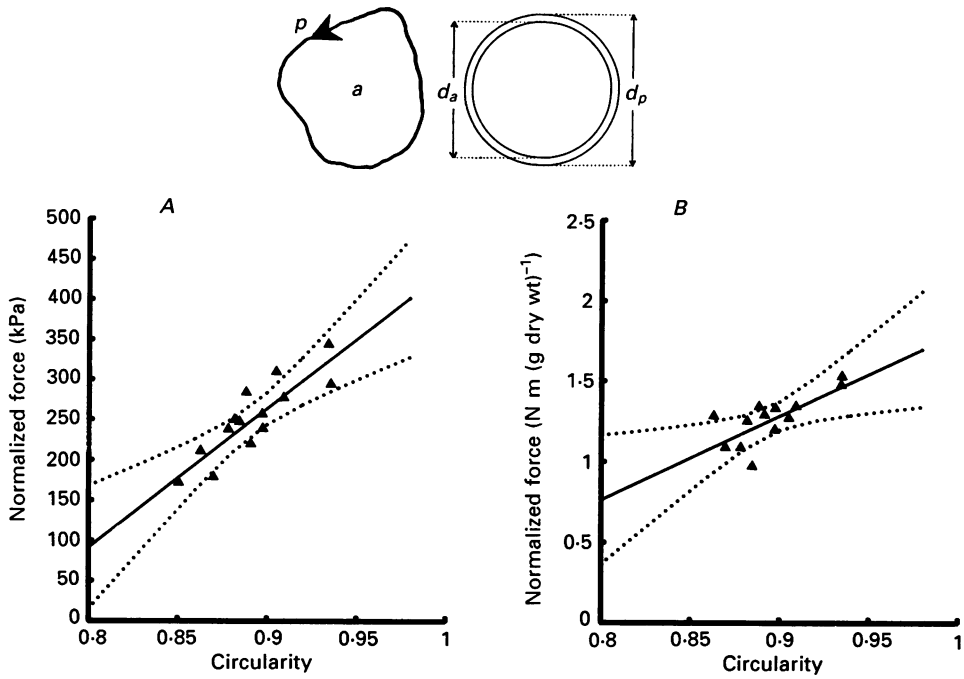

Fig. 4. The dependence of tetanic force normalized by cross-sectional area (*A*) and dry weight per unit length (*B*), on the circularity of the fibre cross-section. Inset, definition of circularity. From photographs (see Methods) of the fibre cross-section, one can measure a perimeter, p, and area, a. If the profile is not circular, a circle constructed with perimeter p (and diameter d_p) will be larger than one with area a (and diameter d_a). The ratio d_a/d_p is defined as an index of circularity of the cross-section.

Fibre types

Fibres of different histochemical type might well vary in size and force per unit cross-section in frog anterior tibialis as they do in *Xenopus* iliofibularis (Lännergren & Smith, 1966; Elzinga & Lännergren, 1985). We therefore tested whether, in a sample of twenty-two fibres at 0 °C, the normalized force was correlated with dry weight per unit length. (This sample provided the data for Figs 5,7 and 8.) No significant correlation was found ($r = 0{\cdot}087$). A similar lack of correlation ($r = 0{\cdot}100$) was found between the normalized tetanic force and twitch contraction time, another measure also often used to distinguish muscle fibres of different types (Lännergren,

Lindblom & Johansson, 1982). The mean time to peak tension in a twitch was 227 ± 6 ms (range 169–276 ms).

Different fibre types shorten at different speeds. We therefore measured V_0, the velocity of unloaded shortening, for each of the fibres (near 0 °C). In contrast to the approximately twofold variation in normalized tetanic force, V_0 was rather constant, with a mean value of $2{\cdot}13 \pm 0{\cdot}04$ μm s^{-1} half-sarcomere^{-1} (mean $\pm$ S.E.M., $n = 18$). In Fig. 5, V_0 is plotted as a function of maximum tetanic force to illustrate the striking difference in variability for these two intercepts of the force–velocity curve.

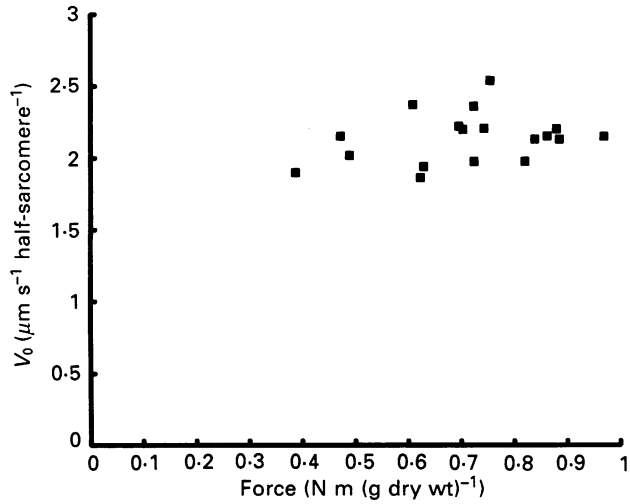

Fig. 5. Lack of correlation between the velocity of unloaded shortening and isometric tetanic force near 0 °C. The similarity of V_0 values is consistent with a single histochemical fibre type.

At an early stage of this work, we (Wilson & Woledge, 1984) obtained some fibres which shortened at significantly lower speeds: $V_0 = 1{\cdot}72 \pm 0{\cdot}05$ μm s^{-1} half-sarcomere^{-1} (mean $\pm$ S.E.M., $n = 13$). These fibres were all small, having dry weight/unit length values less than 4 μg mm^{-1}. Heat production was measured in a few of these fibres; the normalized heat rate appeared not to be significantly different from that of the larger, faster shortening fibres. These results are not included among those reported below, because later batches of frogs have not yielded fibres of this type; therefore no further investigation of them has yet been possible.

To investigate fibre type histochemically, anterior tibialis muscles were rapidly frozen, then sectioned and stained for ATPase activity (see Methods) and measurements of the area and staining characteristics of each fibre made. Figure 6 shows the results from one muscle, stained for residual ATPase activity after incubation at pH 10·1. Forty-one per cent of the fibres were classified as 'lightly stained' and 59% as 'darkly stained'. On acid pre-incubation using a serial section from the same muscle, the staining contrast was reversed. The (alkaline ATPase) lightly stained fibres were larger than those which stained darkly: they had a mean cross-sectional area of 13033 ± 494 μm^2 ($n = 128$) and contributed 77% of the total fibre

area. The darkly stained fibres had a mean cross-sectional area of 2704 ± 152 μm^2 ($n = 182$) and contributed 23% of the fibre area. The area contributed by tonic fibres (which showed an ATPase resistant to both acid and alkaline pre-incubation) was less than 1%.

The fibres whose force was measured at 0 °C had weights per unit length of at least 3·46 $\mu g\ mm^{-1}$. We estimate, from our results on the fibres in which size was measured both ways, that this would be equivalent to cross-sectional areas of at least

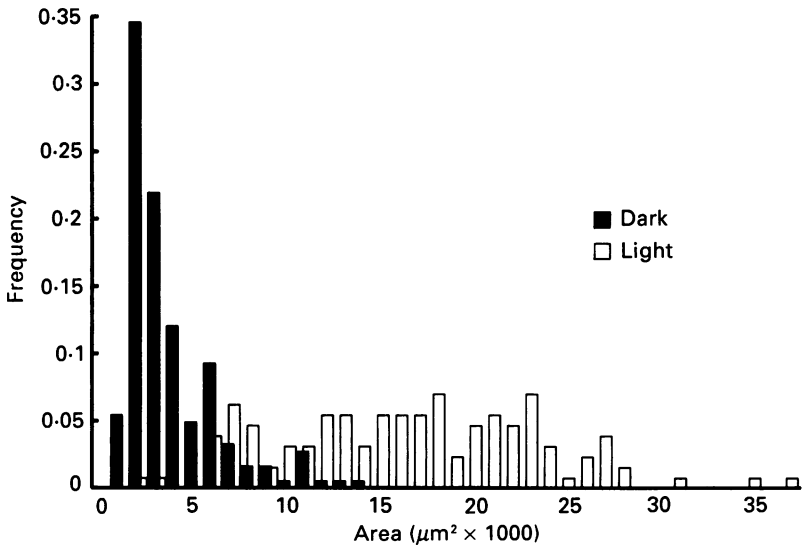

Fig. 6. Size distribution of anterior tibialis fibres staining strongly (filled columns) and weakly (open columns) for ATPase activity after incubation in alkaline conditions (pH 10·1, see Methods).

17000 μm^2. All would thus fall well within the group staining lightly for alkali-resistant ATPase and darkly for acid-resistant ATPase, a behaviour observed in the large fast-twitch fibres of frog sartorius muscle and other amphibian muscles (Spurway, 1982, 1984, 1985). To this extent, and on the basis of the lack of correlation with the other mechanical characteristics, it seems likely that any effects due to factors which determine fibre type (e.g. myosin isozyme composition) do not contribute significantly to the variation in normalized tetanic force observed here.

Heat production

As the variability is not due to differences of fibre type, the question then arises why fibres of the same type can produce different normalized forces. One possibility would be that there are forces within the fibre opposing tetanic force development. In this case one would expect that myofibrillar ATP splitting per unit weight would be approximately constant between fibres and independent of the normalized force. Variability between fibres in the normalized force and in the stable maintenance heat rate, which reflects ATP hydrolysis (Curtin & Woledge, 1979), can be illustrated by our results from a group of twenty-two fibres, which were used to study tetanic force

and heat production in 10 s tetani near 0 °C. The dry weight per unit length at a resting sarcomere length of 2·2 μm varied from 3·46 to 9·06 μg mm^{-1}. Tetanic force, normalized for the dry weight per millimetre, varied between fibres from 0·387 to 0·968 N m (g dry wt)$^{-1}$, with a coefficient of variation of 21 %.

An example of an experimental recording of force and temperature change is shown in Fig. 7*A*. The time course of heat production, obtained after correction

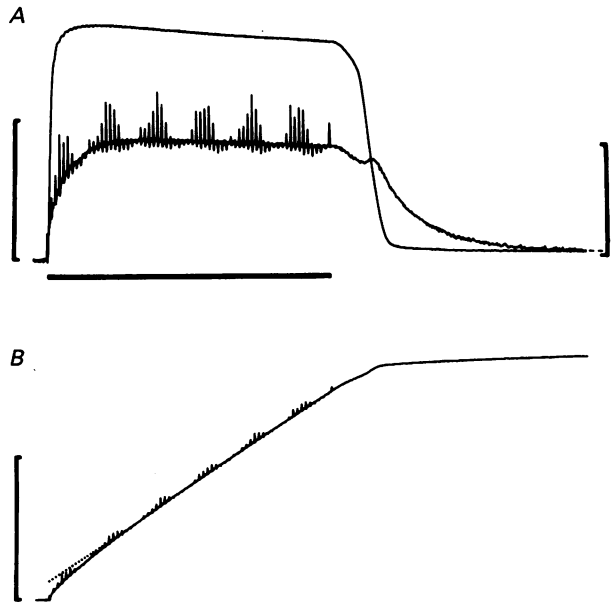

Fig. 7. Force and heat production in a single isolated fibre at 0·4 °C. *A*, force (upper trace) during a 10 s fixed-end tetanus and the accompanying rise in temperature (lower trace). The spikes on the temperature record are artifacts of stimulus pick-up. Calibration bars: horizontal, 10 s; vertical, left, 0·55 N m (g dry wt)$^{-1}$, right, 2 mK. *B*, heat production calculated from the temperature rise in *A* after correction for heat loss. The dotted line is tangential to the record in its later stages and illustrates the decline in the rate of production evident in the first few seconds. Calibration bar: 1 J (g dry wt)$^{-1}$.

for heat loss and subsequent calibration, is shown in Fig. 7*B*. It can be seen that heat is produced more rapidly at first but that the rate of production falls continuously to become steady after about 3 s. The steady slope corresponds to the stable heat rate (Aubert, 1956). Stable heat rate varied between fibres from 55 to 152 mW (g dry wt)$^{-1}$ with a mean of 96 mW (g dry wt)$^{-1}$ and was significantly correlated ($r = 0{\cdot}677$, $P < 0{\cdot}001$) with normalized tetanic force (Fig. 8). Assuming that the ATP splitting (at full filament overlap) required for calcium cycling is a constant fraction of the stable heat rate (Rall, 1979), this would indicate that higher forces were associated with higher rates of myofibrillar ATP hydrolysis. This does not support the idea that the variations in force could be principally due to the presence in some fibres of an internal force opposing that recorded externally, although this remains a possible contributory factor.

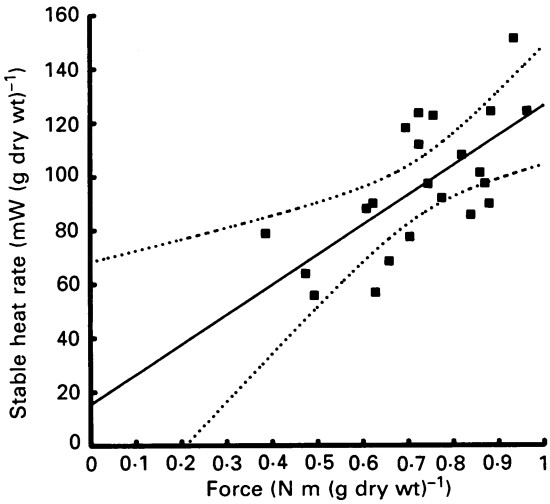

Fig. 8. The relationship between the stable heat rate and isometric tetanic force near 0 °C. The continuous line is the regression of heat rate on force. The dotted lines mark the 95% confidence band for the regression line. There is a clear positive correlation (see text).

DISCUSSION

We show in this paper that normalized tetanic force varies among isolated frog muscle fibres more than can be explained by errors in measurement of cross-sectional area, or by variation in histochemical fibre type. The excellent correlation of our preferred normalizing factor, dry weight per unit length, with the cross-sectional area, and other considerations, show that the variation in normalized force is not due to errors in measuring weight or length. Similarly, the good correlation between normalized force and stable heat rate suggests that failure to record externally the force developed internally is also unlikely to be more than a part of the explanation. This correlation does, however, indicate that differences in the number (or properties) of active cross-bridges per unit volume during tetanic contraction are related to the force variation.

Myosin heads of different myosin isozymes form cross-bridges during contraction which have quite distinct kinetic properties (Ferenczi & Stienen, 1988). Fibres which do not contain the same isozymes, such as the five types of fibre which can be isolated from the iliofibularis muscle of *Xenopus* (Lännergren, 1987), differ in mechanical behaviour. A wide range of twitch : tetanus ratios is found, and V_0 (Lännergren, 1987) and myofibrillar ATPase values (van der Laarse, Diegenbach & Hemminga, 1986) vary by a factor of about 10. The fact that the fibres studied here have the same myosin isozymes is indicated by our histochemical analysis (Fig. 6), the lack of variation in V_0 and the lack of correlation between normalized force and either twitch : tetanus ratio or V_0. The cross-bridge properties of the fibres are therefore likely to be the same. The variations in the stable heat rate are therefore probably caused by differences in the number of active cross-bridges and the calcium pumping activity.

Tetanic force normalized by cross-sectional area is strongly correlated with the ratio of dry weight per unit length to cross-sectional area, which we have called 'dry density' (Fig. 3*A*). Such variations in water content as are seen may reflect differences in the tonicity of the frogs' extracellular fluids relative to our Ringer solution. Blinks (1965) demonstrated an inverse dependence of volume on osmolarity in single fibres of frog anterior tibialis muscle exposed to solutions of varying tonicity. From these measurements, our values for the 'dry density' of the fibres would be consistent with a spread of initial tonicities of about twofold, if all the variation in the measured density were due to osmotic effects. Blinks (1965) also found that fibres became more circular when osmotically swollen, and less circular when shrunk. However, there was no correlation between our index of circularity and the 'dry density' (see Results). The relation between force normalized by cross-sectional area and 'dry density' is consistent with the water in the fibres forming a variable fraction of the cross-section. This variability is not expressed if force is normalized by the dry weight per unit length.

Surprisingly, our studies reveal that the factor which is most strongly correlated with the force normalized by either factor is the shape of the fibre cross-section, here quantified as an index of circularity (d_a/d_p). The majority of the variance of force per unit cross-sectional area and the variance of force per unit (dry weight/length) can be 'explained' by differences in cross-sectional profile. As noted above, these effects are apparently independent of any influence of water content (on the force normalized for cross-sectional area).

It is not clear why the cross-sectional shape of a fibre of a given area should influence its capacity to produce force. One might suggest that myofibrils at the periphery of a fibre might produce less force than their counterparts in the fibre core. Certainly, non-circular fibres will have a greater surface area:volume ratio than circular fibres with the same cross-sectional area. However, this ratio will also increase as fibre cross-section decreases, and no significant correlation between normalized force and fibre cross-section was found in this study for either method of determining size. This makes the hypothesis of weaker myofibrils near the surface unlikely.

The inward spread of activation might be somehow impaired or less uniform in non-circular fibres, but it is difficult to understand why this would prevent full activation of all the myofibrils in a tetanus of any but the briefest duration, since calcium would have sufficient time to diffuse throughout the cross-section. Nevertheless, the possibility that the impairment of force production is a membrane-related phenomenon should be investigated by comparing the variability of normalized force in intact and demembranated frog fibres (the latter maximally activated with calcium), if possible in the same fibres. If there were a reduction in the variability in force produced by the demembranated fibres compared with the intact fibres, this would also be consistent with the presence of a diffusible substance or substances acting to modulate force in the intact fibre. Winegrad & Weisberg (1987) have provided evidence for the latter possibility in cardiac muscle. If the correlation between circularity and normalized tetanic force is not a causal phenomenon, it may be a reflection of a common dependence of these properties on other variables, such as the age or state of development of the fibre. If such were the case, one might

expect frog muscle in states of atrophy, hypertrophy and regeneration to show parallel changes in mean fibre circularity and normalized force production. To interpret such studies correctly, one would need to allow for possible changes in the proportion of fibre types.

Further studies are therefore required to clarify the sources of, and reasons for, variation in the isometric tetanic force of isolated frog muscle fibres. Although this quantity is very familiar to workers in muscle physiology and has been much measured, it is evidently not adequately understood.

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