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ISOMETRIC FORCE PRODUCTION BEFORE AND AFTER CHEMICAL SKINNING IN ISOLATED MUSCLE FIBRES OF THE FROG *RANA TEMPORARIA*

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SUMMARY

1. The force produced in single fibres isolated from the anterior tibialis muscle of the frog *Rana temporaria* has been measured in tetani near 4 °C, and then in calcium-activated contractures of segments of the same fibres after chemical demembranation. All measurements were made at a sarcomere length of 2·3 μm. Force was normalized for fibre cross-section by the dry weight per unit length of the segments, which is proportional to cross-sectional area (Elzinga, Howarth, Rall, Wilson & Woledge, 1989).

2. The ratio of the force developed by the skinned segments to that produced by the intact fibres was inversely related to segment cross-section (dry weight per unit length), falling from approximately 1·0 for the thinnest segments to 0·6 for the thickest segments.

3. It is calculated that the accumulation of orthophosphate ion within contracting segments can account for a significant part of the decline in relative force in thicker segments.

4. The absolute forces in intact fibres and their derived segments were strongly correlated, but normalization by segment cross-section removed the correlation.

5. It is concluded that the sources of the approximately twofold variation in normalized force in both intact and skinned preparations are different. The existence of diffusible, force-modulating factors in intact fibres, which may be removed during skinning, is considered.

INTRODUCTION

This paper examines the relationship between the force produced in a fused isometric tetanus of a frog skeletal muscle fibre and a maximal calcium contracture of one or more chemically skinned (demembranated) segments from the same fibre. This is of interest for two reasons. Firstly, demembranated segments of fibres from frog and other animals are now extensively used in studies of muscle contraction. Although the contractile behaviour of these segments may be similar to that from intact muscle, in only one case (Moss, 1979; five fibres) has any direct comparison using the same fibres been reported. Moreover, a comparison of the force-generating

capacity of different intact and skinned muscle fibres from previous studies is difficult because of the radial swelling of fibres after skinning and the difficulties of measuring cross-sectional area accurately. This is therefore the first systematic study of the way in which skinning affects force production.

Secondly, the capacity of frog muscle fibres to produce force, as measured by the tetanic force normalized by the fibre cross-sectional area, is known to vary by a factor of two or more (Elzinga, Howarth, Wilson & Woledge, 1985; Elzinga, Howarth, Rall, Wilson & Woledge, 1989). This variation is related to the cross-sectional shape of the intact fibres (Elzinga *et al.* 1989). If the cause is the presence in varying amounts of a soluble sarcoplasmic factor which modulates force production, either directly or indirectly (as suggested by Winegrad & Weisberg (1987) for cardiac muscle), or is dependent on the plasma membrane, then chemical skinning might remove this source of variation in force and hence reduce the coefficient of variation of normalized force. For comparative studies such as this, it is desirable to use the same fibres for the measurement of force before and after skinning.

A brief account of some of these results has been presented previously (Elzinga, Stienen & Wilson, 1988).

METHODS

Intact fibres

Single fibres were dissected from the anterior tibialis muscle of the frog *Rana temporaria* in Ringer solution which contained (concentrations in mM): NaCl, 116·5; KCl, 2·0; $CaCl_2$, 1·9; EGTA, 0·1; NaH_2PO_4, 2·0; adjusted to pH 7·0 with NaOH. Small hooks made of 50 μm platinum wire were tied to the trimmed tendons with nylon monofilament thread. The maximum and minimum diameters were measured for some fibres with an eyepiece graticule in the dissecting microscope (Zeiss), and cross-sectional areas were calculated, assuming elliptical profile. The fibre was transferred to the experimental chamber, where the hooks were passed through small holes in extensions made to a force transducer (Sensonor, model AE801) and a length-control motor (which was used only in isometric mode in these experiments). Ringer solution was circulated through the chamber during the measurements. The temperatures in different experiments were in the range 2·0–5·5 °C. The sarcomere length, measured by helium–neon laser light diffraction from the resting fibre, was set to 2·3 μm. Stimulation was through platinum foil electrodes which ran parallel to the fibre. Suprathreshold current pulses of 0·2 ms duration were applied for 1 s. The frequency of stimulation was increased in successive tetani (separated by 1 min) until a fused tetanus of maximal amplitude was obtained. The fibre was then removed from the chamber and held at approximately slack length in Ringer solution in a dissecting dish by passing small pins through the tendons. The skinning procedure described below was then followed.

Skinned fibres

The Ringer solution was removed from the dissecting dish by suction, leaving a thin film just sufficient to moisten the fibre. The fibre was then covered with a small volume of relaxing solution (see below) and left at room temperature for 5–10 min. The fibre was then cut from the tendons and divided into segments of approximately equal length, usually two segments, but sometimes three, each about 3 mm long. A segment was then transferred in contact with a smooth glass rod to a dish containing relaxing solution maintained at about 5 °C. T-shaped aluminium clips were then crimped on either end of the segment, and holes in these clips were passed over hooks connected to another force transducer–motor assembly. A non-ionic detergent (Triton X-100, 0·5 % v/v) was added to the solution to solubilize the membrane. The segment was left in this solution for at least 30 min.

Three bathing solutions were used with the segments: a relaxing solution, a pre-activating solution with a low concentration of the calcium chelator EGTA, and an activating solution. The composition of the bathing solutions was calculated with a computer program similar to that of

Fabiato & Fabiato (1978), using the equilibrium constants given by Godt & Lindley (1982). The relaxing, pre-activating and activating solutions respectively contained (all concentrations in mM): Na_2ATP, 5·49, 5·49, 5·59; phosphocreatine, 26·2, 26·2, 26·2; EGTA, 20, 0·5, 20; HDTA, 0, 19·5, 0; $CaCl_2$, 0, 0, 20; imidazole, 60, 60, 60; M_gCl_2, 7·04, 6·66, 6·51; KCl 39·14, 40·15, 0. The pH was adjusted to 7·1 with potassium hydroxide solution. The ionic strength of the solutions was calculated to be 200 mM. The calculated pCa of the activating solution was 4·4, and the calculated free magnesium and magnesium ATP concentrations were 1 and 5 mM respectively. Each solution contained 200 units ml^{-1} creatine kinase. The solutions were contained in wells in a temperature-controlled block, which could be moved rapidly to change the solution bathing the segment.

After the initial transfer from skinning solution to relaxing solution, the sarcomere length of the segment was adjusted to 2·3 μm. It was left for at least 5 min and then transferred to pre-activating solution for at least 5 min. The segment was then transferred to activating solution, and a calcium contracture developed. This was usually complete within 10 s at 2·0–5·5 °C, the range of temperatures in these experiments. If a steady force was not attained, or if visible damage occurred, the segment was discarded. Segments giving a satisfactory contracture were transferred back to relaxing solution, and the cycle was repeated. If the force produced in the second contracture was less than 90 % of that in the first, the segment was discarded and the first contracture was not used in our analysis. The higher force in the two contractures (almost invariably the first) was used for comparison with the intact force.

As for the intact fibres (see above), the cross-sectional area of some of the segments was estimated. The fibres swelled radially on skinning, the diameters typically increasing by 20–25 %.

Analysis

The factor used to normalize force production was dry weight per unit length, which is proportional to cross-sectional area in intact fibres from frog anterior tibialis muscle (Elzinga *et al.* 1989). To measure this, the segment was withdrawn from the relaxing solution while still attached to the apparatus, and allowed to dry in air at the preset length. It was then removed from the apparatus, and the clips were cut off. The length of the segment was then determined under a dissecting microscope, before transferring it to an electrobalance (Cahn Instruments, Model 29) and weighed.

We obtained satisfactory contractures from segments derived from twenty-four fibres. Of these, eleven fibres yielded two satisfactory segments and the rest one. Where two segments from the same fibre gave satisfactory contractures, we used the higher force for comparison with the tetanus of the intact fibre. In these cases, the average segment weight per unit length was used for normalization. Since the measurements were not all made at precisely the same temperature, as noted above, we corrected all forces to 0 °C using a Q_{10} for force production of 1·24 (Edman, 1979).

The reliability of our method for estimating fibre size, by measuring the dry weight per unit length of the segments, was assessed by comparing two segments from the same fibre, where these had given contractions judged satisfactory as described above. The results (shown in Fig. 3*A*) indicate that errors in these measurements are small and thus do not contribute significantly to the variation in estimated cross-section among different fibres. If error in the weighing was appreciable, then the scatter about the line of identity in Fig. 3*A* should be greatest for the smallest segments. In fact, the converse is true.

RESULTS

The time course of a 1 s tetanus in an intact fibre is shown in Fig. 1*A*, and Fig. 1*B* shows a contracture of a segment of the same fibre after skinning. Since the cross-sectional area of the segments increased on average by about 50 % after skinning, we have used dry weight per unit length (see Methods) to quantify fibre size and enable a more direct comparison of intact and skinned preparations. The contracture in the skinned segment develops much more slowly than isometric tetanic force in the intact fibre, due principally to the greater distance (and therefore time taken) for calcium diffusion (Moisescu & Thieleczek, 1978). This can further be shown by demonstrating that thicker segments develop force more slowly. This tendency is

illustrated in Fig. 2, where the time taken to develop 90% of the maximum force in a contracture of the segment is plotted against the dry weight per unit length. The relation is scattered, but fairly well described by a straight line. From the slope, an estimate of the apparent diffusion constant of calcium (actually the calcium–EGTA complex) in the fibre may be obtained, using in addition the relationship between dry weight per unit length and cross-sectional area given by Elzinga *et al.* (1988), and assuming a circular cross-section. The value thus obtained is $(3{\cdot}3 \pm 0{\cdot}5) \times 10^{-10}$ m^2 s^{-1} (mean and standard error of estimate). This is similar to the figure for the diffusion

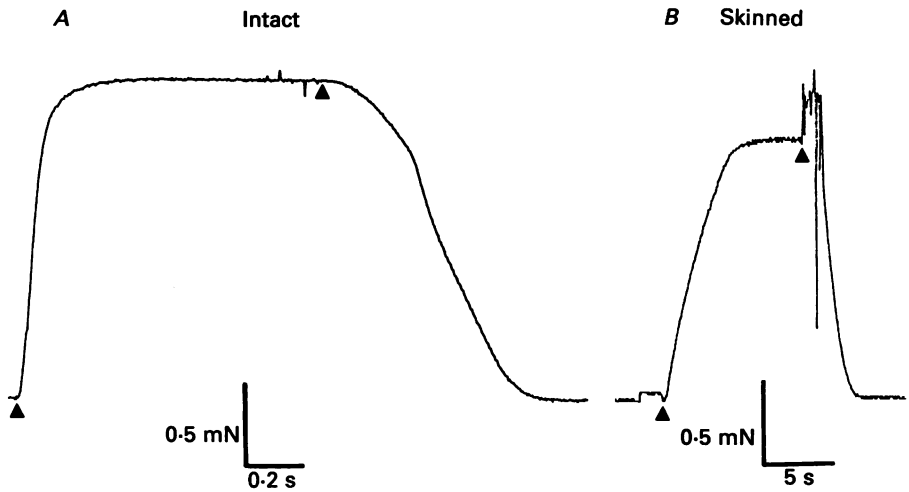

Fig. 1. Isometric contractions of an isolated fibre before and after chemical skinning. *A*, a 1 s tetanus showing complete fusion. Stimulation at 25 Hz begins and ends at times indicated by the arrows. *B*, a segment of the same fibre undergoing a calcium contracture in activating solution. The segment was immersed in activating solution, and transferred back to a low-calcium relaxing solution, at the times indicated by the arrows. The short period of elevated force after the second arrow occurred while the fibre was in air. The sarcomere length in both cases was 2·3 μm and the temperature was 3·9 °C.

constant of EGTA ($4{\cdot}6 \times 10^{-10}$ m^2 s^{-1}) given by Moisescu & Thielecek (1978). However, our measurements were made near 4 °C rather than 20–25 °C, and the binding of calcium ions to sites in the myoplasmic space would also be expected to retard diffusion. Factors which might cause us to underestimate the apparent rate of diffusion are possible non-circularity of the fibre cross-sections, and the fact that our pre-activating solution contained a low (0·5 mM) concentration of EGTA. It can be seen that one of the segments showed a much slower development of force than that expected for its thickness. The reason for this is not known, but may reflect incomplete skinning of the segment. This segment produced a rather low force for its thickness, but satisfied our criteria for reproducibility of contraction. It is denoted with an open symbol in this and other figures, and we have not used it in our calculations.

In the eleven fibres in which two satisfactory segments were obtained, we could compare the amount of force produced by each. Because the segments were skinned independently the amount of variation introduced by the skinning procedure could

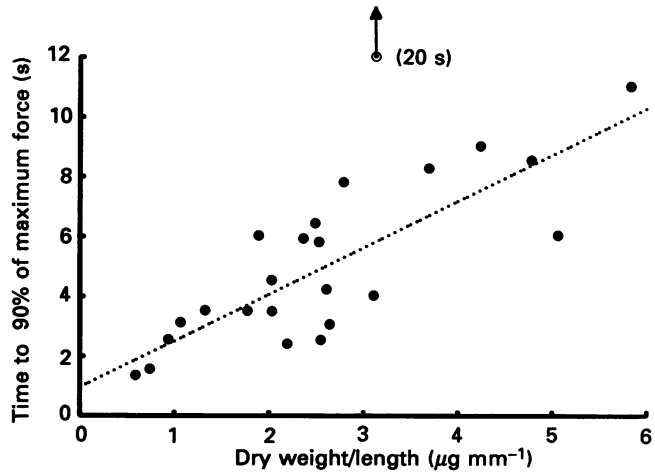

Fig. 2. The dependence of the time taken for skinned segments to produce 90% of maximum isometric force on their dry weight per unit length. At constant density, dry weight/length will be proportional to the segment cross-sectional area. The dotted line shows the regression of the time to 90% force on dry weight/length. From the slope, an estimate of the apparent diffusion constant of calcium in the segments may be made (see text). One segment (○) contracted unexpectedly slowly (20 s to reach 90% maximum force). It is similarly denoted in later figures.

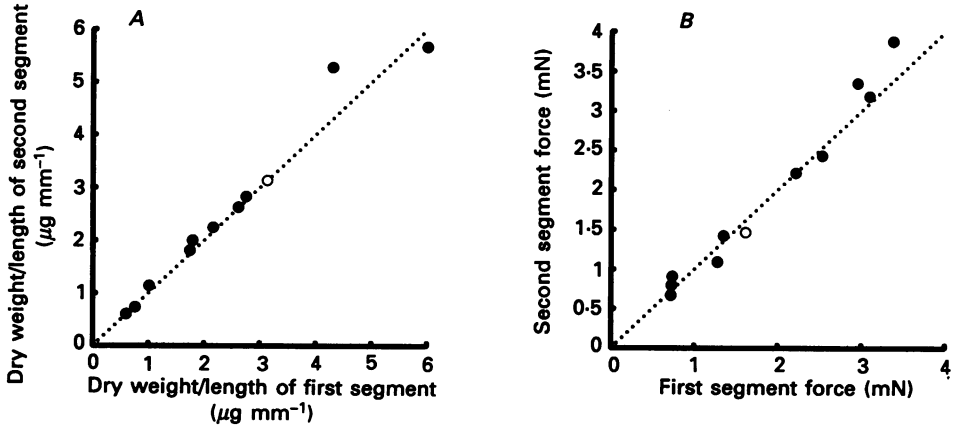

Fig. 3. *A*, the dry weights per unit length of pairs of skinned segments prepared from isolated muscle fibres. Each point shows the value for two segments derived from the same fibre. The line of identity is dotted. The consistency of the measurements indicates that errors of determination of dry weight and segment length are small. *B*, the forces produced by pairs of independently skinned segments derived from the same isolated, intact fibres. The line of identity is shown dotted. The data show a high degree of reproducibility, indicating that changes introduced by the skinning process show small variability.

be assessed. These results are shown in Fig. 3*B*. The correlation coefficient is high ($r = 0{\cdot}987$), showing that very little variation is introduced by the skinning procedure. As already noted, the factor used to normalize force – the dry weight per unit length – is extremely reproducible (Fig. 3*A*). The choice of which segment to use first was based on the appearance in the dissecting microscope. When there was any

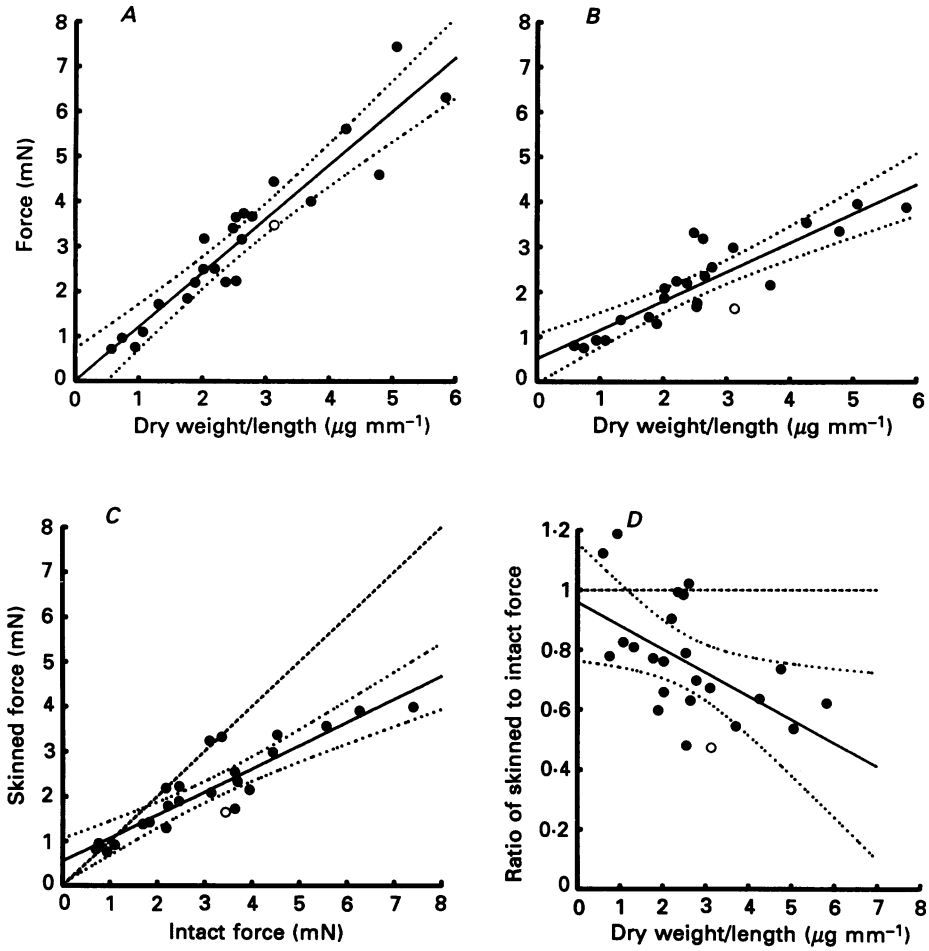

Fig. 4. The dependence of the force produced by intact fibres (*A*) and skinned segments (*B*) of these fibres on their dry weight per unit length. The regression of force on dry weight/length is shown as a continuous line. *C*, force produced by skinned segments plotted against the force produced by the intact fibres. The regression of skinned on intact force is shown as a continuous line, and the line of identity is dotted. *D*, the fraction of the intact force produced by skinned segments plotted as a function of dry weight per unit length. The continuous line is the regression of the ratio on dry weight per unit length. The 95% confidence bands of the population regression lines are dotted.

difference, the segment judged to be more uniform was selected first. There is no indication from the results that the first segment of a pair consistently produced more force. It therefore appears that the criteria used to select reliable segments were satisfactory. As expected, the amount of force developed depends on the fibre size. This is shown in Fig. 4, parts *A* and *B*: both intact and skinned force clearly rise with increasing dry weight per unit length (cross-section), but considerable scatter is evident in both cases. For the intact fibres, the intercept of the regression line of force on dry weight per unit length is not significantly different from zero ($0{\cdot}01 \pm 0{\cdot}27$ mN) and the slope is 1·20 N m (g dry wt)$^{-1}$, which would correspond to a force per unit

cross-sectional area of approximately 250 kPa, based on the relationship between dry weight per unit length and area found by Elzinga *et al.* (1989).

However, for the skinned segments there *is* a significant intercept (0·51 ± 0·21 mN); the slope is 0·65 N m (g dry wt)$^{-1}$, equivalent to about 135 kPa. The non-zero intercept indicates that the skinned force, normalized for the dry weight per unit length, decreases as the cross-section increases. A direct comparison of the amount of force produced in fibres before and after skinning is shown in Fig. 4*C*. The dashed line in this figure is the line of identity, around which the points would scatter if the forces before and after skinning were equal. The results clearly tend to fall below this

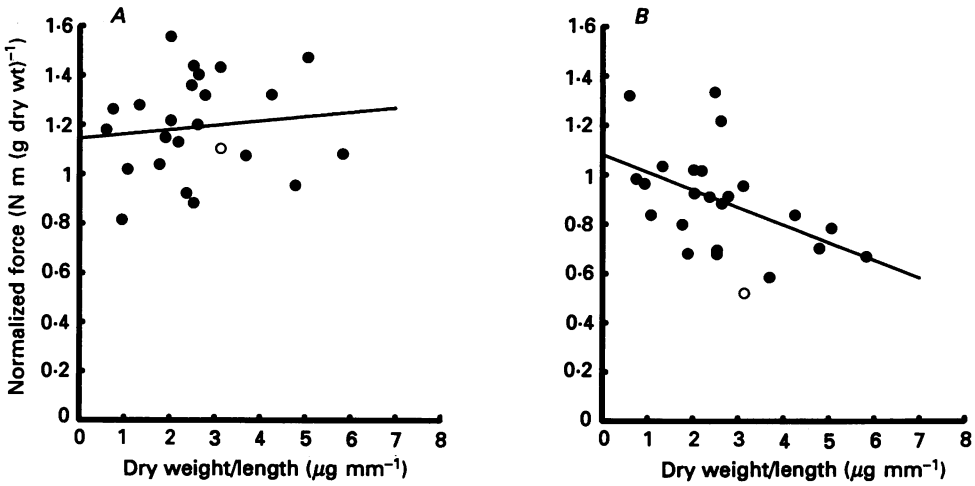

Fig. 5. Force normalized for dry weight per unit length in intact fibres (*A*) and skinned segments (*B*) derived from these fibres. The decline in normalized force in thicker skinned segments is statistically significant (see text).

line. Here, segment thickness is an implicit rather than an explicit variable. Again, the intercept is significantly greater than zero. In Fig. 4*D*, the fraction of the intact force attained by the skinned segment is shown, plotted as a function of dry weight per unit length. Only in the thinner segments does this ratio approximate unity; in the thicker segments it decreased to values of 0·6 or less.

Figure 5 shows the forces in the intact and skinned preparations normalized for dry weight per unit length and plotted against the normalizing factor. The variability in the normalized intact force, which has previously been noted to have an approximately twofold range (Elzinga *et al.* 1985, 1989), is again illustrated in Fig. 5*A*; the highest and lowest values have a ratio of 1·9 (coefficient of variation 17%). It might be expected that the extra handling and treatment would make the variation in normalized force in the skinned segments even greater. Figure 5*B* shows that this is apparently so, although to a limited extent; the forces here vary by a factor of up to 2·5 (coefficient of variation 22%). However, since the correlation between normalized skinned force and dry weight per unit length is significant (Fig. 6*B*; $r = -0{\cdot}492$, $P < 0{\cdot}05$), this means that part of the variation in the skinned

normalized force can be attributed to size-related factors rather than the method of preparation.

If the variables determining normalized force are the same in intact fibres and their derived skinned segments, then a correlation between the normalized force before and after skinning should be seen. As Fig. 6 illustrates, the normalized forces are in fact uncorrelated ($r = 0{\cdot}257$, $n = 23$, $P > 0{\cdot}2$). In other words, after removing the controlling effect of cross-sectional size, which causes the strong correlation between the absolute forces (Fig. 4*C*), the intact and skinned normalized forces vary essentially independently.

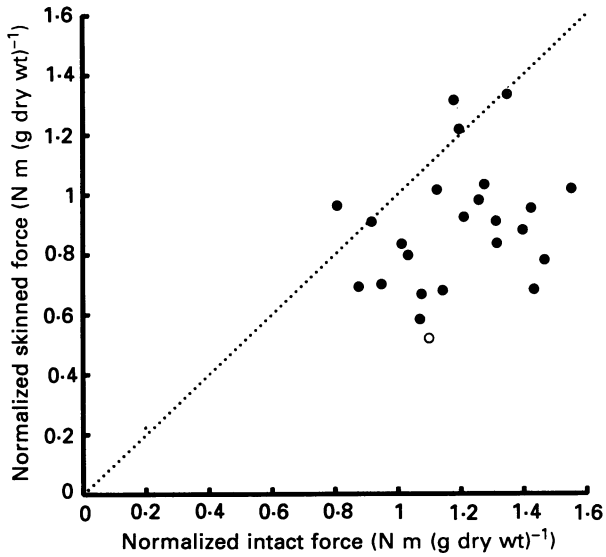

Fig. 6. The normalized forces in twenty-four intact fibres and their derived skinned segments. The line of identity is dotted. The correlation coefficient is not statistically significant (see text).

DISCUSSION

In comparing the amounts of force produced by intact fibres and skinned segments derived from them, it is evident that the relationship between force and dry weight per unit length is more scattered for the skinned segments than for intact fibres (Fig. 4*A* and *B*). For the twenty-three fibres in this study which yielded one or more satisfactory skinned segments, the correlations between force and cross-section were 0·943 (intact) and 0·892 (skinned). From this it can be calculated that 89% of the variation in intact force, and 80% of the variation in skinned force, is 'explained' by cross-section. We should note, however, that these large correlation coefficients are the consequences of the deliberate use of a wide range of fibre sizes in this work and that fibres of similar size can produce forces differing almost twofold.

The average force produced by the segments was 77 ± 4% (mean $\pm$ S.E.M., $n = 23$) of that produced by the intact fibres; this is significantly lower ($P < 0{\cdot}001$, Student's paired t test). Thus, force production is impaired after skinning. This value is in fact

the same as that reported by Moss (1979), but this is coincidental. Moss (1979) used conditions of partial calcium activation (producing about 90% of the maximal force), and it is not clear what the fibre diameters were, nor what selection criteria were applied. The swelling of fibres after skinning which takes place in solutions similar to ours (Matsubara & Elliott, 1972; Godt & Maughan, 1977) probably does not contribute to this force decrease. Goldman & Simmons (1986) show that it may in fact lead to an *increase* of about 3% in the force expressed relative to the force produced by segments osmotically compressed to approximately their original cross-sectional area. Our measurements of fibre and segment diameters before and after skinning indicated that the cross-sectional areas increased by about 50%, and that the diameter increased in the same proportion in every direction. The shape of the intact fibre, which is related to the force it produces through the circularity of its cross-section (Elzinga *et al.* 1989), is thus apparently unchanged by skinning.

Effect of orthophosphate accumulation in skinned segments

The fall in the percentage of intact force attained by skinned fibres with increasing thickness (Fig. 4*D*) may indicate the presence during activation of greater amounts in larger segments of a substance depressing force production. Orthophosphate ions in the millimolar range are known to depress force in skinned segments of frog muscle fibres (Stienen, Güth & Rüegg, 1983; Lacktis & Homsher, 1986), and the phenomenon has been reported in muscle from several other sources (Nosek, Fender & Godt, 1987, and references therein). If orthophosphate were to accumulate significantly during contractures, it might therefore contribute to a reduction in force. The plausibility of this line of argument depends on whether the rate of phosphate production during contraction is sufficiently high, and the rate of escape by diffusion is sufficiently low, to allow the concentration to rise to values commensurate with the observed fall of force.

Curtin & Woledge (1979) found a concentration of orthophosphate plus hexose monophosphate (a small fraction of the total) of 20·88 μmol (g dry wt)$^{-1}$, which rose by 22·17 μmol (g dry wt)$^{-1}$ in a 10 s tetanic contraction. Since 1 g of dried matter is associated with approximately 4 g of water in fibres from frog anterior tibialis muscle (Elzinga *et al.* 1989), these figures correspond to a resting phosphate concentration of 5·2 mM, and a rate of phosphate production of 0·55 mM s^{-1}. A similar estimate of the rate of phosphate production is provided by the stable heat rates measured by Elzinga *et al.* (1989), who found an average steady rate of energy liberation of 96 mW (g dry wt)$^{-1}$ at 0 °C. Making the appropriate conversion to cell water volume and taking the enthalpy of hydrolysis of phosphocreatine as 34 kJ mol^{-1} under these conditions (Woledge & Reilly, 1988) yields a rate of phosphocreatine breakdown (or phosphate production) of 0·56 mM s^{-1}. Woledge, Curtin & Homsher (1985) have noted that the ATPase rate of skinned frog fibres measured by Levy, Umazume & Kushmerick (1976) is similar.

The average temperature for the measurements of force in the skinned fibre segments was 3·6 °C. The Q_{10} of the stable heat rate in intact fibres near 0 °C has been estimated to be 4·06 (Curtin, Howarth, Rall, Wilson & Woledge, 1986): the average ATPase rate in intact fibres at the temperature used in this study is thus calculated to be about 0·9 mM s^{-1}. Since the skinned fibre segments are swollen by about 50%,

the ATPase rate in them is estimated to be 0·6 mM s^{-1}. This rate of production must be balanced against the diffusional escape of phosphate into the medium. In the Appendix, we calculate the average phosphate concentration and expected depression of force reached in the steady state during contractions of skinned segments of varying cross-section. In this calculation, the phosphate gradient across the fibre and the non-linear relationship between steady-state force and phosphate are taken into account. The results of this analysis are shown in Fig. 7.

Our experimental results show (Fig. 5*B*) that the normalized force in the thickest segments used in this study – with dry weights per unit length approaching 6 $\mu g/mm^{-1}$ – was about 40% less than that in the thinnest segments. To investigate if such a fall in force could be caused by the accumulation of orthophosphate alone, we compared our model calculations with experimental reports. Lacktis & Homsher (1986) have published equations describing the dependence of the force depression in intact and skinned frog muscle on orthophosphate. Other workers (e.g. Altringham & Johnson, 1985; Cooke & Pate, 1985; Kentish, 1986) have made similar studies in fish, mammalian and cardiac muscle. From this work, it appears that the limiting depression of force at high phosphate concentrations is expected to be about 50%, with a half-saturating phosphate concentration of 2 mM or more. In terms of our calculations, this fixes the quantity $\Delta F/F_0$, the maximum fractional fall in force, to be about 0·5.

The curves in Fig. 7 are actually plotted in terms of the *fraction* of $\Delta F/F_0$. A fall of 40% from the maximum, as found here, would therefore reduce the force to a value equivalent to $(1–0·4/0·5) = 0·2$. In order to do this, it is clear that the parameter τ (the time taken to raise the phosphate concentration to half the saturating value, if no loss into the medium occurred) must be 1 s or less. Since we define τ (see Appendix) as K/A, where K is the half-saturating concentration and A the ATPase rate, our earlier estimate of 0·6 mM s^{-1} for the ATPase rate leads to an estimate for K of 0·6 mM.

This value is smaller that previous estimates of K. There is some evidence (Kentish, 1986) that thicker preparations show a lessened sensitivity to phosphate (a higher K). This is consistent with the accumulation of phosphate inside the preparations at low external concentrations, which would cause an underestimation of the true extent of the depressant effect of phosphate and an overestimation of K. Until measurements of the dependence of force on phosphate have been made in skinned segments of single frog fibres of different thickness, it is not clear whether the accumulation of phosphate is by itself a sufficiently strong effect to account for the decline in force noted here. However, it seems clear that such an accumulation can explain a significant fraction of the effect.

Other causes of force depression in skinned segments

A comparison of the variability of the normalized force in intact and skinned preparations reveals that the coefficient of variation of the normalized skinned force (22·3%) is only slightly greater than that of the intact skinned force (17·0%). The correlation between dry weight per unit length and normalized skinned force is sufficient to explain this difference. It is clear (Fig. 6) that the respective normalized forces vary essentially independently ($r = 0·257$, $n = 23$, $P > 0·2$). The principal

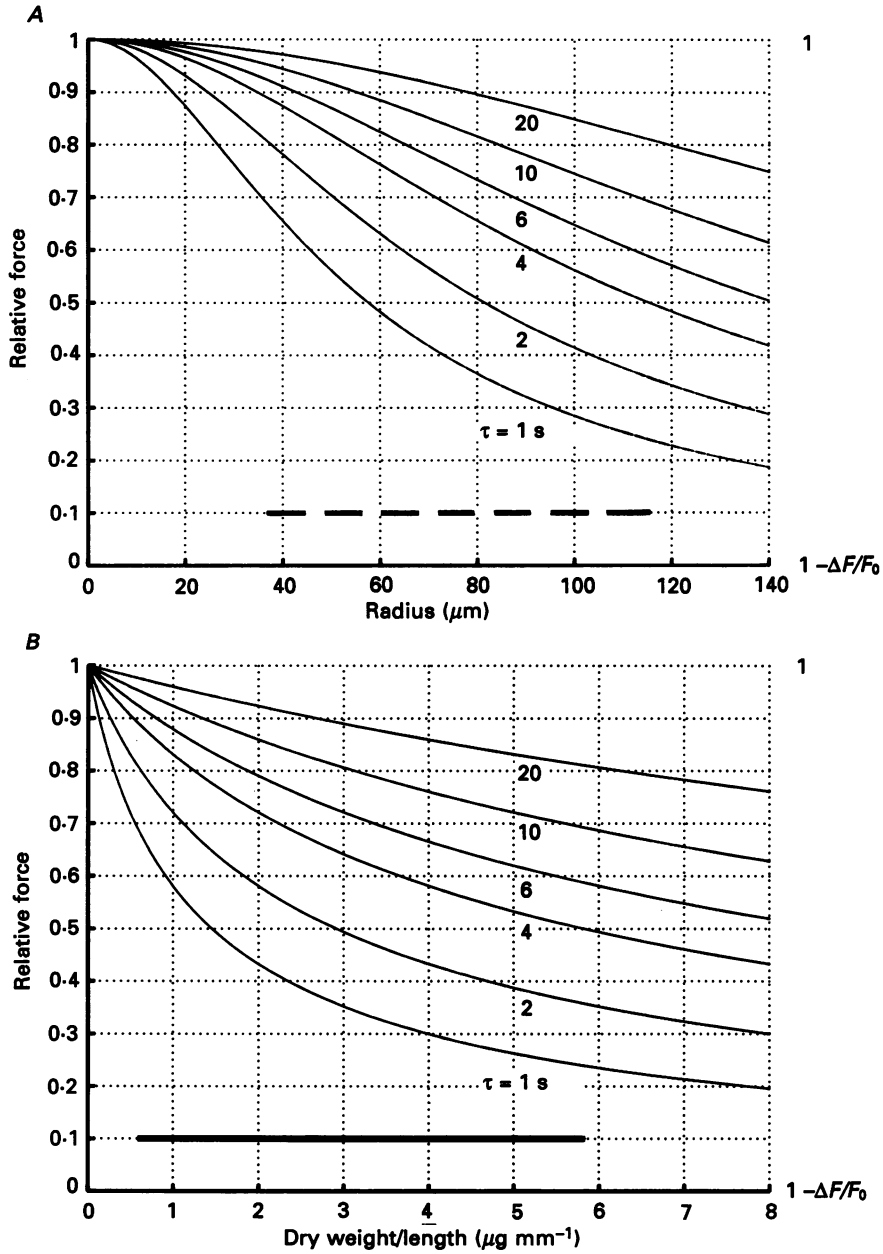

Fig. 7. The expected decrease in the component of force affected by phosphate as a function of the radius (*A*, dashed horizontal bar indicates the approximate range of segment radii in these experiments) and the dry weight per unit length of skinned fibre segments (*B*, continuous horizontal bar shows the range in these experiments). Calculated from eqn (5) with $\Delta F/F_0 = 1$ and D as in the text, for various values of K/A. $\Delta F/F_0$ is the fraction of force 'sensitive' to phosphate, D the diffusion constant for phosphate in frog muscle and K/A the time taken to produce the concentration of phosphate giving a half-saturating depression of force. Dry weight per unit length is here related to radius through the dependence on area found by Elzinga *et al.* (1989) in intact fibres, allowing for an increase in area of 50% on skinning.

sources of the variability in normalized force are therefore different in intact and skinned fibres. In the preceding paper, Elzinga *et al.* (1989) report that the shape of the cross-section of intact fibres is related to the force-producing capacity. If this was the case in skinned segments, evident correlation would remain, but it does not. It therefore appears that shape is not correlated with normalized force in skinned fibres. This suggests that the correlation between shape and normalized force in intact frog fibres is dependent on the integrity of the plasma membrane, and is not a consequence of shape *per se*.

What, then, *is* responsible for the variability of normalized force in skinned fibre segments? We have already noted that fibre size, possibly through the mechanism of orthophosphate build-up, is negatively correlated with normalized skinned force. However, the correlation with size is sufficient to 'explain' only 24% of the variation: the major part must arise differently. If it is not present in intact fibres, then it might arise as a consequence of the skinning procedure, either as some unspecified form of damage, or as the removal to varying degrees of a factor or factors instrumental in affecting force production.

We can evaluate the contribution made by skinning to the variation in normalized skinned force by considering those fibres from which two viable segments were obtained and performing an analysis of variance. This shows that the ratio of the variance between segments of the same fibre to that within segments of different fibres is (0·0001/0·0639), with 1 and 20 degrees of freedom respectively. It is clear from this that variation between segments of the same fibre is very small compared to that among segments of different fibres. This shows that the variation is not produced by the skinning procedure, but reflects differences which were pre-existent in the fibres at the time of skinning. Since, however, we have already noted that most of the variation in the normalized force produced by the skinned segments is uncorrelated with that in the intact fibres, those differences which we deduce to exist in intact fibres must be largely ineffective in altering the force-producing capacity until after skinning.

It is clear that segments of frog skeletal muscle fibres skinned as described are capable of producing as much force (if not more) as the intact fibres from which they were derived. This is only seen with thin segments (less that about 120 μm equivalent diameter), possibly because of accumulation of orthophosphate ion. A similar degree of variability in the maximum force normalized by dry weight per unit length (cross-section) is seen in both intact and skinned fibres, but the respective variations are independent. The reasons for the variation of the normalized force both in intact frog fibres and in skinned preparations therefore remain at least partially unclear.

APPENDIX

The accumulation of orthophosphate in contracting skinned fibres

In a contracting skinned fibre segment, the rise in orthophosphate produced by ATP splitting will be offset to some extent by diffusional loss into the surrounding medium. We wish to calculate the steady-state orthophosphate concentration during contraction in phosphate-free solutions, and to examine the possible consequences

for force production. For simplicity, we assume that the medium phosphate concentration does not rise significantly, neglect the (insignificant or small) effect of phosphate on the ATPase activity of skinned fibres reported in fish (Altringham & Johnston, 1985) and rabbit (Webb, Hibberd, Goldman & Trentham, 1986; Kawai, Güth, Winnekes, Haist & Rüegg, 1987), possible unstirred layers and assume that the segments are of circular cross-section. In such a case, the steady-state concentration $P(r)$ of orthophosphate at a radial distance r from the axis of a fibre will be (e.g. Carslaw & Jaeger, 1959)

$$P(r) = \frac{A(a^2-r^2)}{4D}, \tag{1}$$

where A is the rate of phosphate production (the steady-state ATPase rate), a is the fibre radius and D is the diffusion constant of phosphate in the fibre. The average phosphate concentration in the fibre will therefore be

$$\bar{P} = \int_0^a rP(r)\,\mathrm{d}r \Big/ \int_0^a r\,\mathrm{d}r = Aa^2/8D. \tag{2}$$

The steady-state radial distribution of orthophosphate will be approximated for times τ (since the start of contraction) such that $D\tau/a^2 > 0{\cdot}6$ (Carslaw & Jaeger, 1959; their eqn 7·9 (1) and Fig. 26). Taking the value of D in frog muscle as $3{\cdot}3 \times 10^{-10}$ m^2 s^{-1} (Yoshizaki, Seo, Nishikawa & Morimoto, 1982), this will occur for times greater than 6·5 s for segments in this study of average radius ($a \approx 60\ \mu$m), or 14·7 s for the thickest segments ($a \approx 90\ \mu$m). As Fig. 3 indicates, these times are similar to those taken to develop 90% of the maximum force. This means that the orthophosphate distribution in the fibre closely matches the steady-state distribution at the time when the measurement of maximum force is made.

To calculate the effect of phosphate accumulation on force production, we describe the steady-state relationship between force (F) and phosphate concentration (P) by

$$F = F_0 - \Delta FP/(K+P), \tag{3}$$

where F_0 is the force produced in the absence of any phosphate, ΔF is the maximum decrease in force produced by elevating phosphate, and K is a constant with units of concentration which represents the phosphate concentration producing a decrease in force of $\Delta F/2$. This relation, which describes a saturating decrease in force with phosphate, is in reasonably good agreement with studies on skeletal (e.g. Altringham & Johnston, 1985; Nosek, Fender & Godt, 1987) and cardiac muscle (e.g. Kentish, 1986). Recent attempts to explain the effects of phosphate on force production (Cooke & Pate, 1985; Hibberd, Dantzig, Trentham & Goldman, 1985) are compatible with a decrease of this general form. Since the phosphate concentration is a function of r, the mean steady-state force per cross-bridge will depend on r also. Substituting eqn (1) into eqn (3), we calculate the observed (average) steady-state force to be

$$\bar{F} = \int_0^a rF(r)\,\mathrm{d}r \Big/ \int_0^a r\,\mathrm{d}r = \frac{2}{a^2}\int_0^a \left[F_0 r - \Delta F \frac{r(a^2-r^2)}{c+a^2-r^2}\right]\mathrm{d}r, \tag{4}$$

where $c = 4DK/A$ and is a constant with dimensions of (length)^2.

For convenience, we further define the constant $\tau = K/A$. Here, τ would be the time taken for the ATPase activity to raise the phosphate concentration to the half-saturating value for force depression if no loss into the medium occurred.

Evaluating the integral and rearranging yields

$$\frac{\bar{F}}{F_0} = 1 - \frac{\Delta F}{F_0}\left[1 + \frac{c}{a^2}\ln\left(\frac{c}{c+a^2}\right)\right], \tag{5}$$

which describes the force relative to the maximum (at zero phosphate) as a function of the fibre radius. Figure 7 shows various possible solutions to eqn (5) for the case where $\Delta F/F_0 = 1$, that is, sufficiently high concentrations of phosphate can abolish all force. (If $\Delta F/F_0 < 1$, as is probably the case, these curves would represent the extent of the component of force which is 'sensitive' to phosphate.) Each curve is drawn for a different value of τ. This facilitates comparison with other muscle types where the ATPase rate or sensitivity to phosphate may be different, although differences in the diffusion constant should also be taken into account.

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