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CALCIUM AND STRONTIUM ACTIVATION OF SINGLE SKINNED MUSCLE FIBRES OF NORMAL AND DYSTROPHIC MICE

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SUMMARY

1. Differences in contractile activation by Ca^{2+} and Sr^{2+} between various types of normal and dystrophic murine muscle fibres were investigated using mechanically skinned fibres derived from soleus and extensor digitorum longus (e.d.l.) muscles of normal and dystrophic mice of strain 129ReJ.

2. In terms of contractile activation, the normal e.d.l. muscle was found to consist of one relatively homogeneous population of muscle fibres characterized by steep force–pCa and force–pSr curves, low sensitivity to Ca^{2+} and very low sensitivity to Sr^{2+}.

3. Normal soleus muscles contained two fibre populations of similar size which could be distinguished on the basis of their contractile activation properties. The first fibre population was characterized mainly by its shallow force–pCa and force–pSr curves, high Ca^{2+} sensitivity, high Sr^{2+} sensitivity and the occurrence of large, slow force oscillations of myofibrillar origin. The second fibre population was characterized by force–pCa and force–pSr curves of steepness intermediate between those of normal e.d.l. and those of the first fibre population of normal soleus, by faster myofibrillar force oscillations and by low sensitivity to Ca^{2+} and Sr^{2+}.

4. The dystrophic e.d.l. fibre population had contractile characteristics which were distinct from those of the three types of normal fibre populations. However, some characteristics of the dystrophic e.d.l. fibres were very similar to those of the normal e.d.l. fibre population. Of all the fibre types investigated, dystrophic e.d.l. fibres were the least sensitive to Ca^{2+}.

5. Dystrophic soleus muscle contained a single homogeneous population of fibres which shared some common contractile activation characteristics with both of the fibre populations present in normal soleus muscle. However, of all fibre types investigated, the dystrophic soleus fibres were the most sensitive to Ca^{2+}. Because of this characteristic, these fibres formed a distinct population.

6. The maximum tensions induced by Ca^{2+} and Sr^{2+} were usually smaller in dystrophic fibres than in normal fibres obtained from equivalent muscles.

7. In conclusion, various normal murine muscle fibre types can be identified on

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the basis of differences in the mechanism of force activation by Ca^{2+} and Sr^{2+}. Furthermore, it is possible to detect significant physiological differences in the mechanism of force activation brought about by murine muscular dystrophy.

INTRODUCTION

The dystrophy which occurs in mice of strain 129ReJ resembles the Duchenne and Erb's type muscular dystrophy in man in a number of clinical, histological and physiological characteristics (Michelson, Russell & Harman, 1955; Sandow & Brust, 1958; Meier & Southard, 1970). Thus, dystrophic mice of this strain have been used as animal models in many studies to detect and characterize the changes that take place in dystrophic muscle. In all previous studies of the contractile modifications that occur in this type of dystrophy, whole muscles were used (e.g. Sandow & Brust, 1958, 1962; Brust, 1966; Douglas & Baskin, 1971; Harris & Wilson, 1971; John, 1974, 1976; Fitzsimons & Hoh, 1983). However, such results are difficult to interpret because both normal and dystrophic muscles contain populations of different types of fibres (Fitzsimons & Hoh, 1983), which have different contractile characteristics. Recently, physiological techniques have been developed which permit the study of the contractile properties of single, skinned mammalian muscle fibres of different types (Kerrick, Secrist, Coby & Lucas, 1976; Takagi & Endo, 1977; Stephenson & Williams, 1981, 1982). No such studies have been undertaken to characterize the basic differences in the process of contractile activation between the various normal and dystrophic murine muscle fibre types. Such an investigation could contribute important information about a major question in muscular dystrophy: whether all murine dystrophic muscle fibres have abnormal contractile properties compared to normal fibres, or whether in murine dystrophy there is only a change in the proportion of different fibre types in a particular muscle. The latter explanation can be inferred from the recent paper of Fitzsimons & Hoh (1983). The aim of the present study was, therefore, to use mechanically skinned single fibre preparations to characterize murine muscle in terms of the contractile activation properties of the fibres present in e.d.l. and soleus muscles of normal and dystrophic mice of strain 129ReJ, and to correlate the results with recent histochemical and biochemical observations on the same strain of mice (see Fitzsimons & Hoh, 1983).

A preliminary account of some of the results described here has been presented to the Australian Physiological and Pharmacological Society (Fink, Williams & Stephenson, 1982).

METHODS

Preparation

Normal and dystrophic mice of strain 129ReJ were obtained from colonies maintained in the animal house at Monash University, Clayton, Melbourne. The breeding nuclei originated from the Jackson Laboratories, Bar Harbor, U.S.A. The mice were killed by cervical dislocation. Single muscle fibres were isolated from the extensor digitorum longus (e.d.l.) and soleus muscles of normal mature (+/?) and dystrophic (*dy/dy*) mice aged between 6 and 12 weeks. Some experiments were also carried out with e.d.l. and soleus muscles from hooded Wistar rats (see Fink, Stephenson & Williams, 1986, for details). Dissection and mechanical skinning of the fibres were performed in

paraffin oil (Ajax Chemicals) under a dissecting miscroscope and the procedures and apparatus were described in detail elsewhere (Moisescu & Thieleczek, 1978; Stephenson & Williams, 1981; Fink *et al.* 1986).

Solutions

The solutions for the activation of the skinned muscle fibres were similar in composition to those used in previous studies on mammalian muscle (Stephenson & Williams, 1981). All solutions contained (mM): K^+, 117; Na^+, 36; 2-(2-hydroxy-1, 1-bis(hydroxymethyl)ethyl)aminoethane sulphonic acid, (TES) 60; (TES^-, 17); Mg^{2+}, 1; total adenosine 5′-triphosphate (ATP), 8; creatine phosphate, 10; creatine phosphokinase, 150 u. ml^{-1}; caffeine, 10. The main anions in solutions (50 mM) were either the ethyleneglycol-bis-(β-aminoethylether)*N,N,N′,N′*-tetraacetic acid ions ($EGTA^{2-}$) + Ca $EGTA^{2-}$ (or Sr $EGTA^{2-}$) or the hexamethylenediamine-*N,N,N′,N′*-tetraacetic acid ions ($HDTA^{2-}$). The pH of all solutions was adjusted to $7{\cdot}10 \pm 0{\cdot}01$ at room temperature (21–23 °C). The apparent affinity constants (K_{app}) of the various cations to ligands in solution were determined experimentally (Moisescu & Thieleczek, 1978; Stephenson & Williams, 1981). The K_{app} of Ca^{2+} and Sr^{2+} to EGTA at pH 7·10 and in the presence of 1 mM-Mg^{2+} were calculated to be $4{\cdot}78 \times 10^6$ M^{-1} and $1{\cdot}91 \times 10^4$ M^{-1} respectively. Balanced sets of solutions of different concentrations of ionized Ca^{2+} ($pCa = -\log [Ca^{2+}] \geqslant 5$) and Sr^{2+} ($pSr = -\log [Sr^{2+}] \geqslant 3{\cdot}7$) were obtained by mixing a stock solution containing 50 mM-$EGTA^{2-}$ in various proportions with either a stock solution containing about 50 mM-Ca EGTA (EGTA was in excess over Ca^{2+} by about 0·5–1 mM to avoid a change in pH following mixing) or a stock solution containing 40 mM-Sr EGTA + 10 mM-$EGTA^{2-}$ respectively (Ashley & Moisescu, 1977; Moisescu & Thieleczek, 1978). Solutions of $pCa < 5{\cdot}0$ and $pSr < 3{\cdot}7$ were prepared separately. At the start of an experiment the stock solutions were titrated with $CaCl_2$ to determine the amount of excess EGTA present. This was necessary in order to prepare solutions with known pCa values. At the end of an experiment all solutions were titrated with $CaCl_2$ or $SrCl_2$ to allow an accurate determination of the pCA and pSr levels (Ashley & Moisescu, 1977; Stephenson & Williams, 1981; Miller & Smith, 1984).

Measurements and analysis of data

The fibre dimensions (length, diameter) were measured under a dissecting microscope (× 40) while the skinned fibre was still in paraffin oil (saturated with water). The average sarcomere length was measured using a He–Ne laser (Stephenson & Williams, 1981) while the skinned fibre preparation was in a relaxing solution containing 50 mM-EGTA. Sarcomere length was measured at the beginning of and during an experiment. The preparations were rapidly activated by Ca^{2+} and Sr^{2+} using the technique of Moisescu (1976). The diameter of the preparations ranged between 18 and 45 μm (in oil) and the fibre length between 0·6 and 2 mm. All experiments were performed at room temperature (21–23 °C). The relative steady-state isometric force responses were corrected for time-dependent force deterioration by normalizing to interpolated control contractions using the method of Julian (1971). When the fibres were activated by both Ca^{2+} and Sr^{2+}, the fibres were randomly activated first by Ca^{2+} then by Sr^{2+} and again by Ca^{2+} (see e.g. Fig. 1). The Student's *t* test was used to compare results statistically.

The most common method of presenting the effect of Ca^{2+} and Sr^{2+} on the force activation is a graphical representation of relative force (P_r) as a function of the pCa and pSr. A necessary restriction in this study was to obtain complete force–pCa and force–pSr curves for as many preparations as possible since the force–pCa (pSr) curves differ between various fibre types. Previous work with skinned mammalian muscle fibres indicated that slow-twitch fibres have contractile characteristics which are clearly distinct from those of fast-twitch fibres. For example, slow-twitch fibres have a relatively high Sr^{2+} sensitivity (Kerrick *et al.* 1976; Takagi & Endo, 1977) and a relatively shallow force–pCa (pSr) curve (Stephenson & Williams, 1981, 1982; Fink *et al.* 1986), whereas fast-twitch fibres are less sensitive to Sr^{2+} and have very steep force–pCa (pSr) curves.

There are several quantitative characteristics which may be derived from a set of force–pCa (pSr) curves. The pCa_{50} and pSr_{50} values represent the pCa and pSr corresponding to 50 % maximum Ca^{2+}- and Sr^{2+}-activated force responses respectively. These two characteristics describe the sensitivity of the contractile apparatus to Ca^{2+} and Sr^{2+} respectively, while the difference $pCa_{50} - pSr_{50}$ refers to the relative sensitivity of a particular fibre to Ca^{2+} and Sr^{2+}. A second set of characteristics, pCa_{10} and pSr_{10}, gives an indication of the activator's 'threshold' for contraction and represents the pCa and pSr corresponding to a relative force value of 0·1. A third set of characteristics are the associated

Hill coefficients n_{Ca} and n_{Sr} which represent the numbers n in the Hill equation $P_r = K[X^{2+}]^n/(1+K[X^{2+}]^n)$ which provide the closest fit to the experimental points. $[X^{2+}]$ is either the $[Ca^{2+}]$ or the $[Sr^{2+}]$ and K, a constant, is related to pCa_{50} and pSr_{50} by the following expression: $\log_{10}K = n_{Ca}pCa_{50}$ ($n_{Sr}pSr_{50}$). The Hill coefficients give an indication of the maximum steepness of the sigmoidal curve relating P_r to pCa and pSR. A further set of characteristics, T_0^{Ca}, T_0^{Sr}, refers to the maximum Ca^{2+}- and Sr^{2+}-activated tension responses respectively. The maximum tensions T_0^{Ca} and T_0^{Sr} were calculated from the initial maximum Ca^{2+}- and Sr^{2+}-activated force responses and the apparent diameter of the skinned fibres measured in paraffin oil at the beginning of an experiment. In addition to these characteristics describing the steady-state force responses, we have also analysed the frequency and the amplitude of oscillatory processes of myofibrillar origin (Fabiato & Fabiato, 1978; Stephenson & Williams, 1981) observed on the force traces of some fibre types. These force oscillations show characteristic differences between different mammalian fibre types (Stephenson & Williams, 1981; Fink *et al.* 1986).

Results which we obtained from single skinned rat muscle fibres of known fibre type (Close, 1972; see also Stephenson & Williams, 1981) showed that the major fibre populations, i.e. slow-twitch, fast-twitch and intermediate fibres, could be clearly distinguished when the following criteria were used under our experimental conditions (Table 1): presence of myofibrillar oscillations of low frequency (< 0·4 Hz), n_{Ca}, $n_{Sr} < 2{\cdot}5$, $pCa_{50} - pSr_{50} < 0{\cdot}6$ for slow-twitch fibres and n_{Ca}, $n_{Sr} > 2{\cdot}5$, $pCa_{50} - pSr_{50} > 1{\cdot}0$ and no force oscillations or faster force oscillations (frequency > 0·8 Hz) for fast-twitch and intermediate fibres. These criteria enabled us to distinguish between two fibre populations in the normal murine soleus muscle with no overlap occurring between the populations. No clear criteria could be formulated from our data to subdivide the fibre populations in normal and dystrophic murine e.d.l. muscle and dystrophic soleus murine muscle. Therefore we have treated these muscles as containing single fibre populations.

RESULTS

Data for normal murine muscle are presented first, followed by data for dystrophic muscle. A comparison is then made between normal and dystrophic muscle fibres.

Normal muscle fibre activation characteristics

Typical force–pCa and force–pSr curves obtained with the same e.d.l. skinned fibre are shown in Fig. 1*A*. These curves are relatively steep and can be fitted using the Hill equation with $n_{Ca} = n_{Sr} = 4$. The sensitivity to Sr^{2+} ($pSr_{50} = 4{\cdot}7$) was about 12 times lower than the sensitivity to Ca^{2+} ($pCa_{50} = 5{\cdot}8$). All the e.d.l. fibre preparations from normal mice had characteristics similar to those of the fibre in Fig. 1*A* and therefore formed a relatively homogeneous group. The results obtained with the normal murine e.d.l. fibres are summarized in Table 1.

In contrast to the e.d.l. fibres, the skinned muscle fibres obtained from the normal soleus muscle can be subdivided into two major groups on the basis of their contractile activation characteristics (for criteria see Methods). The force–pCa and force–pSr relationships characteristic of each group are shown in Fig. 1*B*, *C*. The first group (see Fig. 1*B*), population 1, had a significantly lower threshold for Ca^{2+} and Sr^{2+} activation than the e.d.l. fibres and the force–pCa and force–pSr curves were significantly less steep, with an associated Hill coefficient of about 2. Fibres from this group were markedly more sensitive to Sr^{2+} than are e.d.l. fibres, and they showed only a relatively small difference in their sensitivity to Ca^{2+} and Sr^{2+}. The results from the analysis of the various characteristics of all fibres of soleus population 1 are given in Table 1. Another interesting feature of the fibres belonging to this group was the occurrence of large force oscillations when the fibres were activated submaximally by either Ca^{2+} or Sr^{2+}. A typical example of this is shown in Fig. 2*A*. The large force

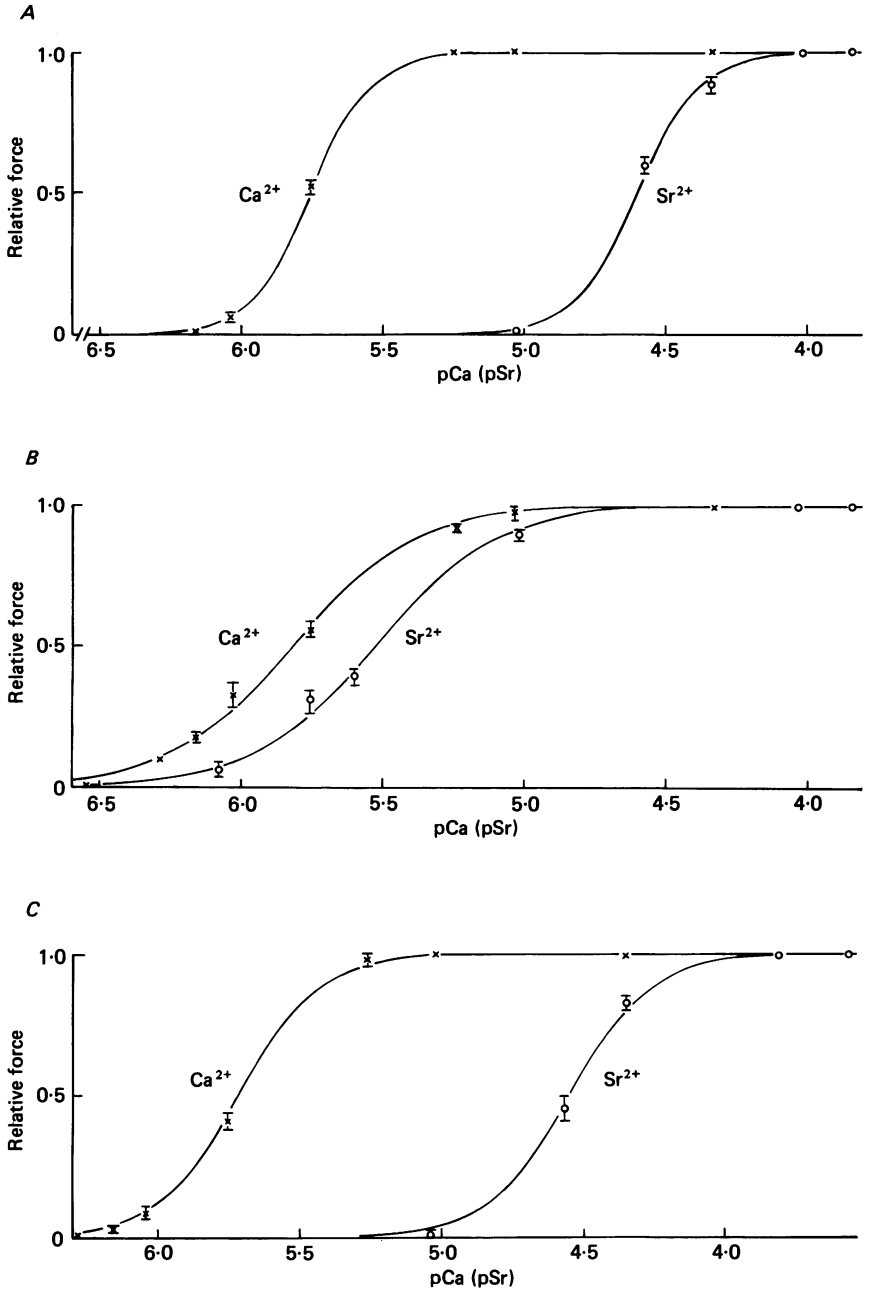

Fig. 1. Steady-state isometric force–pCa (×) and force–pSr (○) relationships of single skinned fibres from normal mice. Both Ca^{2+} and Sr^{2+} activation of force were obtained with the same single fibre of each type. Vertical bars show the range of the results of two to five activations. *A*, representative fibre from the e.d.l. muscle; *B*, representative soleus fibre of population 1; *C*, representative soleus fibre of population 2. The sigmoidal curves are predictions from the Hill equation with $n_{Ca} = 4{\cdot}0$, $n_{Sr} = 4{\cdot}0$ for *A*, $n_{Ca} = 2{\cdot}0$, $n_{Sr} = 2{\cdot}0$ for *B* and $n_{Ca} = 3{\cdot}0$, $n_{Sr} = 3{\cdot}0$ for *C*. Sarcomere length (μm): 2·75, 2·70, 2·70, for *A*, *B* and *C* respectively.

oscillations showed marked damping and were similar to those described earlier for slow-twitch rat soleus fibres (Stephenson & Williams, 1981). The frequency of the oscillations observed at activation levels between 10 and 50 % maximum Ca^{2+} or Sr^{2+} activated force, P_0, was low (about 0·3 Hz) and was independent of the nature of the activating ion, whether Ca^{2+} or Sr^{2+}.

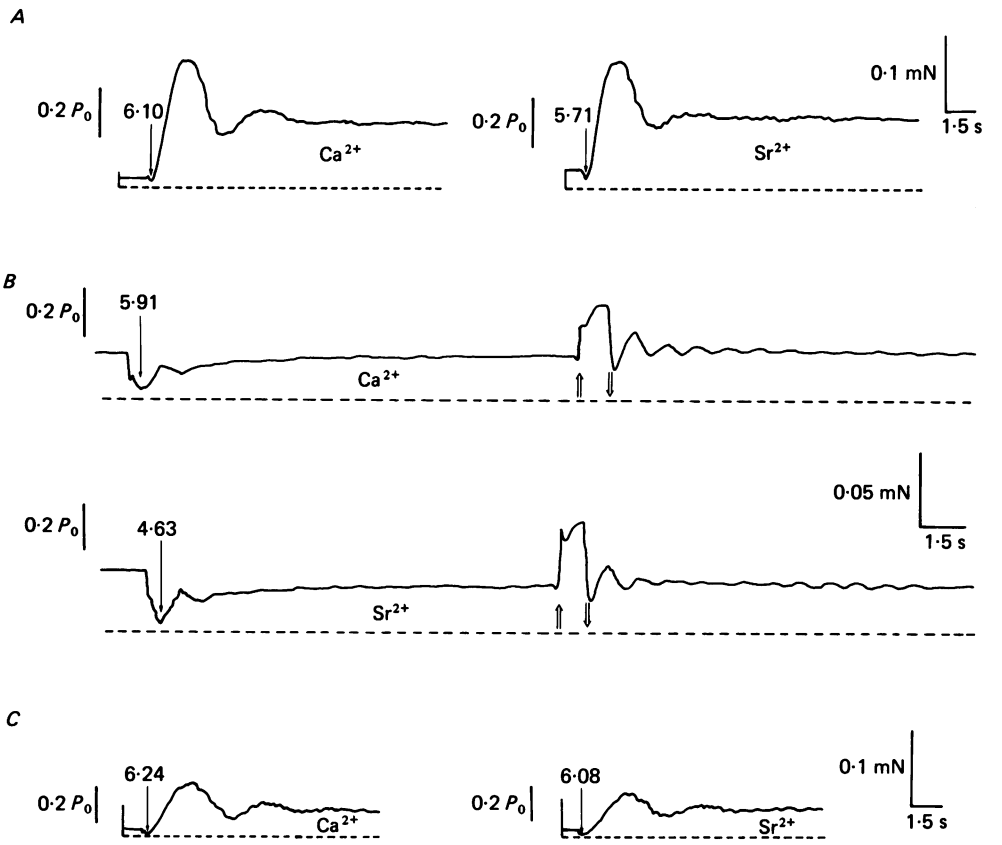

Fig. 2. Typical force oscillations of myofibrillar origin in murine skinned fibres. The fibres were suddenly activated by either Ca^{2+} or Sr^{2+} and the pCa or pSr values in the activating solutions are shown above arrows. The double parallel arrows in *B* for Ca^{2+} and Sr^{2+} indicate the transfer of the preparation through the solution–air and air–solution interfaces respectively. *A*, representative response with a soleus fibre of population 1; *B*, representative response with a soleus fibre of population 2; *C*, representative response with a dystrophic soleus fibre. Dimensions of preparations (length, sarcomere length, diameter): 1·2 mm, 2·7 μm, 45 μm, for *A*; 2·0 mm, 2·7 μm, 25 μm, for *B*; 1·0 mm, 2·7 μm, 45 μm, for *C*.

The second group of fibres from the murine soleus muscle, population 2, showed some Ca^{2+} and Sr^{2+} activation characteristics intermediate between the e.d.l. and soleus fibres of population 1. Force–pCa and force–pSr relationships typical of these fibres are shown in Fig. 1*C*. The force–pCa and force–pSr curves for the fibres of soleus population 2 were less steep than those for the e.d.l. fibres, but steeper than those for the fibres of soleus population 1. The Hill coefficient of the fibres of population

2 was close to 3. Force oscillations in fibres of population 2 could be observed when they were activated submaximally between 10 and 50 % P_0. An example of oscillatory responses obtained with Ca^{2+} and Sr^{2+} is shown in Fig. 2*B*. The force oscillations in these fibres did not reach the large amplitudes observed in the soleus fibres of

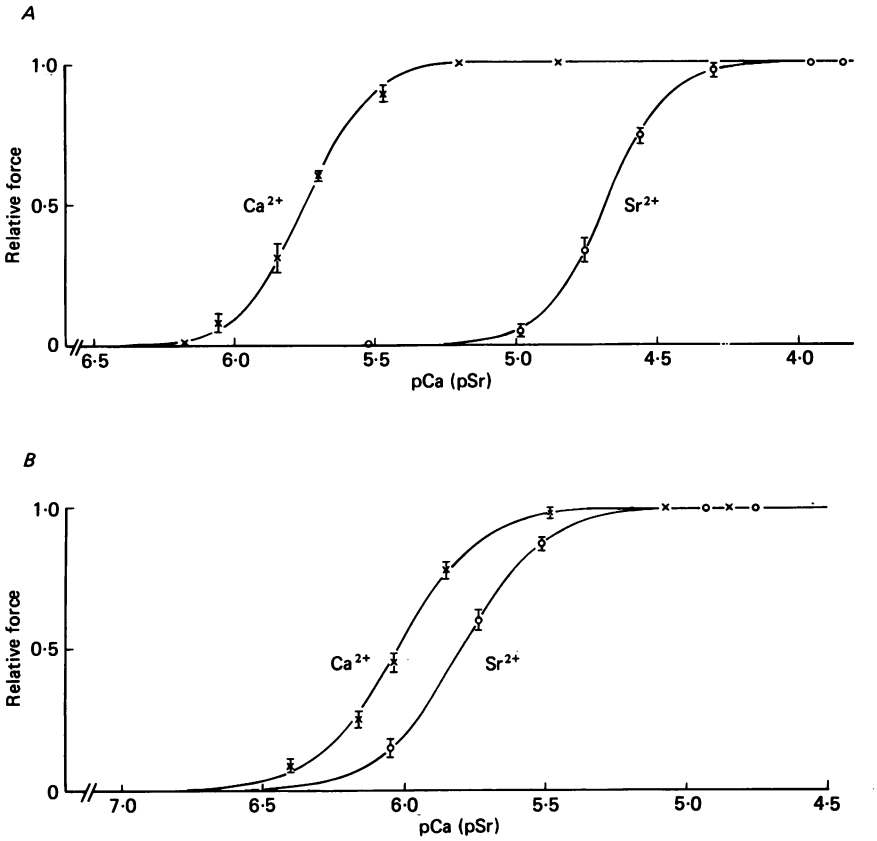

Fig. 3. Representative force–pCa and force–pSr relationships of single murine fibres from dystrophic animals. *A*, mechanically skinned e.d.l. fibre, n_{Ca}, n_{Sr} = 4·0, sarcomere length 2·65 μm; *B*, mechanically skinned soleus fibre, n_{Ca}, n_{Sr} = 3·0, sarcomere length 2·65 μm.

population 1 and were characterized by a frequency of about 1·0 Hz which was insensitive to the type of ion used for activation and to the level of force activation. These highly damped force oscillations could be easily restarted by simply moving the preparations through the solution–air–solution interface as shown in Fig. 2*B*. A summary of the results obtained with the soleus fibres of population 2 is presented in Table 1.

Dystrophic muscle fibre characteristics

A Hill curve similar to that for normal e.d.l. fibres (i.e. similar n_{Ca}, n_{Sr}) could be fitted through the steady-state force–pCa and force–pSr data points for e.d.l. dystrophic fibres; see Fig. 3*A* and Table 1. However, the range for the pCa_{50} in

dystrophic e.d.l. fibres was considerably wider (5·39–5·92) than that for normal e.d.l. fibres (5·67–5·95), being displaced to lower pCa values ($P < 0·07$ for average pCa_{50}). The difference in the sensitivities to Ca^{2+} and Sr^{2+} was also less and the $pCa_{50} - pSr_{50}$ average values were significantly different from dystrophic and normal e.d.l. fibres ($P < 0·01$). In common with normal e.d.l. fibres, no force oscillations of myofibrillar origin could be detected for dystophic e.d.l. fibres.

Dystrophic soleus fibres showed characteristics distinct from those of normal soleus muscle fibres and no clear critieria were found to allow further subdivision (see Methods). We therefore regard soleus dystrophic fibres as forming a homogeneous group. The shapes of the force–pCa and force–pSr curves of the dystrophic soleus fibres were similar to those of normal soleus fibres of population 2 (Fig. 3*B* and Table 1). However, the dystrophic soleus fibres were more sensitive to Sr^{2+} than any other fibre type investigated in this study. The average values for $pCa_{50} - pSr_{50}$ were also the smallest of all the fibre types investigated ($P < 0·01$). The dystrophic soleus fibres showed force oscillations when activated submaximally with either Ca^{2+} or Sr^{2+} (Fig. 2*C*). The frequency of the large oscillations was similar to that shown by the soleus fibres of population 1.

Maximum tensions induced by Ca^{2+} and Sr^{2+} in normal and dystrophic fibres

The maximum calculated tensions induced by Ca^{2+} (T_0^{Ca}) and Sr^{2+} (T_0^{Sr}) for the various normal murine fibre types at 2·7–2·9 μm sarcomere length are shown in Fig. 4. There were no significant differences between T_0^{Ca} and T_0^{Sr} in any of the normal fibre types examined. Dystrophic e.d.l. and soleus fibres developed smaller tensions than their normal counterparts (Fig. 4). The average T_0^{Ca}, T_0^{Sr} values for dystrophic e.d.l. fibres were approximately 80 % of the respective values for normal muscle and the T_0^{Ca}, T_0^{Sr} values for dystrophic soleus fibres were approximately 60 % of the values for the two normal soleus fibre populations. However, only the results for soleus muscle are significantly different at the 5 % level (dystrophic soleus fibres compared to normal soleus fibres) owing to the large scatter in the data (see also Table 1).

The calculated tension values (T_0^{Ca}) for the normal e.d.l. murine fibres were only approximately 60 % of those for the rat e.d.l. fibres obtained under similar, but not identical conditions (Stephenson & Williams, 1981). As it is important to know whether the ability of the murine fibres to develop maximum tension is different from that of the rat muscle fibres, we have also maximally activated rat e.d.l. and soleus skinned fibres with Ca^{2+} and Sr^{2+} under identical conditions. The results for rat fibres at sarcomere length 2·7–2·9 μm are also included in Fig. 4. Rat fibres usually developed greater maximum tensions than their murine counterparts, but only the maximum tensions for the e.d.l. fibres were significantly higher in the rat.

Comparison of normal and dystrophic murine fibres

There is little doubt that there are basic differences in the Ca^{2+} and Sr^{2+} activation process of force development between normal e.d.l. fibres and the soleus fibres of population 1 (Table 1). Normal e.d.l. fibres are more closely related to the soleus fibres of population 2, which comprise about 50 % of the murine soleus fibre population, than to the soleus fibres of population 1. The average values for pCa_{10}, pSr_{10}, pCa_{50}, pSr_{50} and $pCa_{50} - pSr_{50}$ are very similar in the normal e.d.l. fibres and soleus fibres

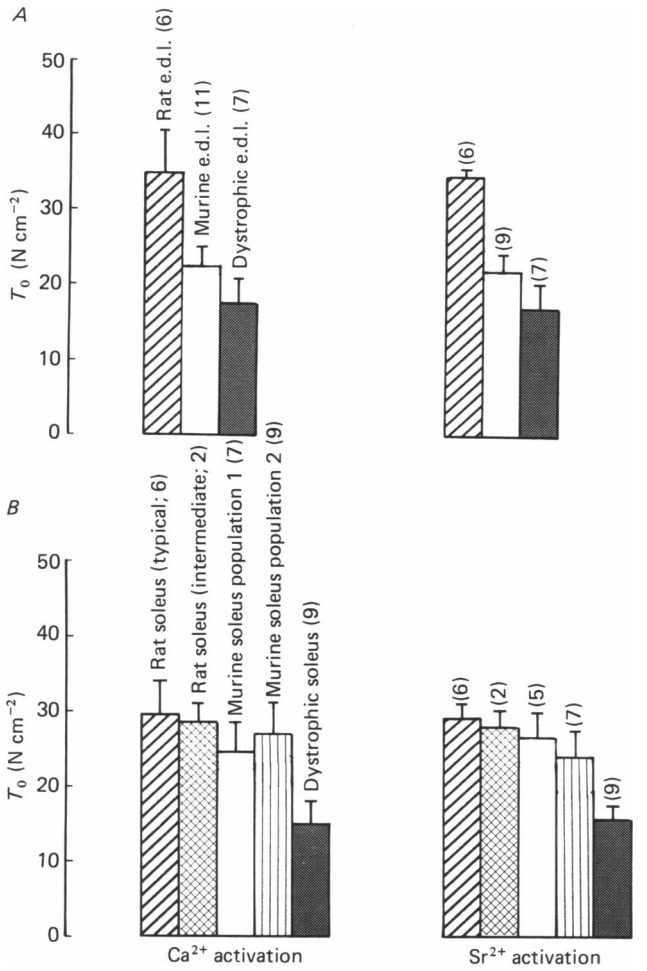

Fig. 4. Average maximum tensions induced by Ca^{2+} (left) and Sr^{2+} (right) in murine and rat skinned muscle fibres of different types. The vertical bars show the S.E. of mean, number of fibres in parentheses.

of population 2. A statistically significant difference was noticed for n_{Ca} ($P < 0{\cdot}02$) while for n_{Sr}, $P < 0{\cdot}1$. Furthermore, the soleus fibres of population 2 respond with force oscillations when suddenly activated by Ca^{2+} and Sr^{2+} (Fig. 2*B*), unlike the normal murine e.d.l. fibres. The soleus fibres of population 2 resemble the 'intermediate' fibres from soleus muscle of the rat in their main characteristics (Table 1). The average values of all characteristics of soleus fibres of population 1 were significantly different from those of soleus fibres of population 2.

The differences in the average values for pCa_{50} and $pCa_{50} - pSr_{50}$ between normal and dystrophic e.d.l. fibres appear rather small in absolute terms but they can have a marked effect on the Ca^{2+}-activated force responses, owing to the steep force–pCa curves for these fibres. For example, if the ionized Ca^{2+} in the sarcoplasm is such as to lead to 50 % P_0 in the average normal e.d.l. fibre, the same Ca^{2+} level could induce not more than 25 % P_0 in the average dystrophic e.d.l. fibre. This calculation was made

TABLE 1. Characteristics* of Ca^{2+}- and Sr^{2+}-activated force in normal and dystrophic fibres

Muscle fibre type		Sarcomere length (μm)	pCa_{10}	pCa_{50}	n_{Ca}	pSr_{10}	pSr_{50}	n_{Sr}	$pCa_{50}-pSr_{50}$†	Frequency myofibrillar oscillations (Hz)
Normal mice										
E.d.l.		2·70–2·90	6·04±0·03 (9)	5·80±0·03 (9)	4·02±0·15 (9)	4·79±0·03 (6)	4·53±0·03 (6)	3·78±0·18 (6)	1·24±0·03 (6)	N.a.
Soleus	population 1	2·70–2·90	6·47±0·08 (7)	6·00±0·08 (7)	2·16±0·15 (7)	6·05±0·10 (4)	5·63±0·06 (4)	2·02±0·14 (4)	0·46±0·06 (4)	0·3
	population 2	2·70–2·90	6·08±0·04 (7)	5·78±0·03 (7)	3·36±0·21 (7)	4·88±0·08 (5)	4·55±0·05 (5)	3·18±0·27 (5)	1·26±0·04 (5)	1·0
Dystrophic mice										
E.d.l.		2·50–2·90	5·91±0·07 (8)	5·67±0·06 (8)	4·05±0·17 (8)	4·82±0·07 (5)	4·57±0·06 (5)	4·10±0·24 (5)	1·02±0·06 (5)	N.a.
Soleus		2·60–2·90	6·30±0·03 (9)	5·98±0·03 (9)	3·03±0·04 (9)	6·10±0·04 (5)	5·78±0·04 (5)	3·04±0·07 (5)	0·23±0·02 (5)	0·3
Normal rat										
E.d.l.	typical	2·60–2·80		5·90±0·04 (6)	4·22±0·10 (6)		4·51±0·04 (6)	4·17±0·10 (6)	1·39±0·05 (6)	N.a.
Soleus	typical	2·60–2·80		6·17±0·12 (6)	1·94±0·10 (6)		5·70±0·09 (6)	2·00±0·10 (6)	0·47±0·07 (6)	0·28
	intermediate	2·65–2·70		5·85±0·03 (2)	2·90±0·08 (2)		4·68±0·08 (2)	2·99±0·10 (2)	1·17±0·03 (2)	1·0

Statistically significant differences were observed between normal murine e.d.l. fibres and soleus fibres of population 1: $P < 0·001$ for n_{Ca}, n_{Sr}, pCa_{10}, pSr_{10}, pSr_{50}, $pCa_{50}-pSr_{50}$; $P < 0·02$ for pCa_{50}; also absence or presence of force oscillations (low frequency). Normal murine e.d.l. fibres differed from soleus fibres of population 2 in n_{Ca}, $P < 0·02$ (n_{Sr}, $P < 0·1$), and absence or presence of force oscillations (higher frequency). All characteristics were significantly different between the fibres of soleus populations 1 and 2 with $P < 0·01$, except for pCa_{50} when $P < 0·05$. Dystrophic e.d.l. fibres differed from normal murine e.d.l. fibres in $pCa_{50}-pSr_{50}$, $P < 0·01$, and pCa_{50}, $P < 0·07$ (pCa_{10}, $P < 0·10$), and from soleus fibres of population 1 in all characteristics, $P < 0·001$; $P < 0·01$ for pCa_{50}. When comparing dystrophic e.d.l. fibres with normal soleus fibres of population 2 there are differences with respect to the absence or presence of force oscillations, $P < 0·02$ for $pCa_{50}-pSr_{50}$, $P < 0·05$ for n_{Ca}, n_{Sr}, and $P < 0·08$ for pCa_{10}. Dystrophic soleus fibres differ (i) from normal soleus fibres of population 1 with $P < 0·01$ for n_{Ca}, n_{Sr}, $pCa_{50}-pSr_{50}$; $P < 0·08$ for T_0^{Ca}, $P < 0·05$ for T_0^{Sr}, pCa_{10}; $P < 0·07$ for pSr_{50}, and (ii) from normal soleus fibres of population 2 with respect to frequency of oscillations; $P < 0·001$ for pCa_{10}, pCa_{50}, pSr_{50}, $pCa_{50}-pSr_{50}$; $P < 0·05$ for T_0^{Ca}, $P < 0·08$ for T_0^{Sr}. There are also significant differences between dystrophic soleus fibres and normal murine e.d.l. fibres with $P < 0·001$ for n_{Ca}, pCa_{50}, pSr_{50}, $pCa_{50}-pSr_{50}$, pCa_{10}, pSr_{10} and $P < 0·01$ for n_{Sr}. Dystrophic e.d.l. fibres also show statistically significant differences compared with dystrophic soleus fibres with $P < 0·001$ for pCa_{10}, pCa_{50}, n_{Ca}, pSr_{10}, pSr_{50}, $pCa_{50}-pSr_{50}$; $P < 0·01$ for n_{Sr}, and dystrophic e.d.l. fibres did not show force oscillations in contrast to the dystrophic soleus fibres.

* The values for the parameters analysed are given as means ±S.E. of mean obtained with (n) fibres.

† Values for paired results for the same fibre.

considering a difference in pCa_{50} of 0·13 log units between the average normal and dystrophic fibre and an average Hill coefficient of 4 for both fibres.

The contractile characteristics analysed for the dystrophic soleus fibres show that there are significant differences between dystrophic soleus fibres and normal soleus fibres of populations 1 and 2, and also normal e.d.l. fibres. Hence, there is good evidence that dystrophic soleus fibres are quite distinct from the three characteristic types of normal murine muscle fibres. Dystrophic soleus fibres also show statistically significant differences compared with dystrophic e.d.l. fibres with respect to all contractile characteristics analysed in Table 1. In addition, there are pronounced force oscillations in dystrophic soleus fibres (Fig. 3*C*) in contrast to the dystrophic e.d.l. fibres.

DISCUSSION

An important conclusion of this study is that by using physiological techniques, it is possible to identify three types of fibres in normal murine muscles. Some of the criteria used for this physiological classification (maximum steepness of the force–pCa curve, sensitivity to Ca^{2+}, force oscillations of myofibrillar origin) were used extensively in previous studies from this laboratory to characterize fast- and slow-twitch fibres of the rat (Stephenson & Forrest, 1980; Stephenson & Williams, 1981, 1982; Fink *et al.* 1986). Other characteristics of the contractile activation by Sr^{2+}, e.g. $pCa_{50} - pSr_{50}$, were also used in this study to identify and classify the murine and rat fibre types. These characteristics have been shown to differ in the two major fibre types of rabbit (Kerrick *et al.* 1976) and guinea-pig (Takagi & Endo, 1977).

The fibre populations described in our study can be clearly correlated with the fibre populations described by Fitzsimons & Hoh (1983) in a recent biochemical and histological study on the same muscles from the same strain of mice. This is because the normal e.d.l. muscle of the 129ReJ strain of mice consists almost entirely of type II B fibres, whereas the normal murine soleus muscle consists of two fibre populations of similar size containing type II A and type I fibres (Fitzsimons & Hoh, 1983). Fibre population 1 of the normal murine soleus muscle has characteristics (see Table 1) which are similar to those of the type I fibre population from the rat soleus muscle. In the rat soleus muscle, type I fibre is the predominant type of fibre ('typical' soleus slow-twitch type, Close, 1972; Stephenson & Williams, 1981). Therefore, the characteristics of the normal e.d.l. murine fibres in Table 1 are typical of type II B fibres and the characteristics of the populations 1 and 2 of the normal soleus fibres are typical of type I and type II A fibres respectively.

The differences observed in the process of activation of contraction by Ca^{2+} and Sr^{2+} indicate that there are differences in the regulatory system(s) in different types of mammalian fibres which permit fibre type identification without resorting to destructive biochemical procedures. However, we cannot decide here whether the differences observed in the process of activation are a direct reflexion of the myosin differences observed by Fitzsimons & Hoh (1983), or whether the troponin–tropomyosin system, which is known to be different in type I and type II fibres (see e.g. John, 1976), is also different in fibres of type II A and type II B.

The average maximum tensions generated in skinned murine fibres from normal

e.d.l. and soleus muscle, fully activated with Ca^{2+} and Sr^{2+}, were in the range 22–27 N cm^{-2}. These values are close to those for normalized tetanic tensions measured in intact murine soleus and e.d.l. muscles at similar sarcomere lengths and temperature (21–25 N cm^{-2}, Luff, 1981). Average maximum tensions in mechanically skinned dystrophic muscles were lower in fibres from both soleus and e.d.l. muscles compared to their counterparts from normal muscles (Fig. 4). Statistically significant differences were, however, recorded only for the soleus fibres. These results can explain the lower tetanic tensions obtained in dystrophic muscles of this strain of mice (Sandow & Brust, 1958; Brust, 1966; Douglas & Baskin, 1971).

In this study we have observed significant differences between normal and dystrophic e.d.l. fibres, the latter being less sensitive to Ca^{2+} (but not to Sr^{2+}). This indicates that there are also differences in the mechanism of contractile activation by Ca^{2+} between normal and dystrophic e.d.l. fibres. This observation can be correlated with the changes observed in the Mg-ATPase activity of predominantly fast-twitch dystrophic fibres (John, 1976) and with the distribution of fast isomyosins in the normal and dystrophic e.d.l. and muscle (Fitzsimons & Hoh, 1983). Fitzsimons & Hoh (1983) observed a pronounced shift towards the slow myosin in dystrophic soleus muscle compared to normal soleus muscle, the dystrophic soleus muscle containing almost exclusively slow myosin. This is consistent with our observations of dystrophic soleus fibres which are apparently a homogeneous population and share important characteristics with slow-twitch fibres of the normal soleus muscle (soleus population 1; see Results). However, our studies indicate that dystrophic soleus fibres are not identical to normal slow-twitch soleus fibres, since they differ significantly from soleus fibres of population 1 with respect to several important contractile characteristics: n_{Ca}, n_{Sr}, $pCa_{50} - pSr_{50}$, pCa_{10}, T_0^{Ca}, and T_0^{Sr} (see Results). Some of these contractile characteristics (n_{Ca}, n_{Sr}) are similar to those of the soleus fibres of population 2. This indicates that while the dystrophic soleus and the normal slow-twitch soleus fibres may contain the same isomyosin (Fitzsimons & Hoh, 1983), other contractile and regulatory proteins are likely to be different (e.g. exist in different polymorphic forms) in the dystrophic soleus fibres and the normal soleus fibres of population 1. It is interesting that Fitzsimons & Hoh (1983) found that individual dystrophic soleus fibres stained with intensities intermediate between type I (soleus fibres of population 1) and type II A (soleus fibres of population 2).

Thus, on the one hand our results show that there are differences in the mechanism of force regulation by Ca^{2+} and Sr^{2+} between individual normal and dystrophic murine fibres which do not support the hypothesis that dystrophy simply changes the proportion of otherwise 'normal' fibre types in muscles. However, on the other hand, there is no strong evidence to assume that myofibrillar components are different in dystrophic as compared to normal fibres (Fitzsimons & Hoh, 1983). Therefore, a possible simple explanation for our results and those of others could be that dystrophic murine fibres are the result of the expression of genes which leads to anomalous (inefficient) combinations of otherwise normal contractile and regulatory isoproteins.

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REFERENCES

ASHLEY, C. C. & MOISESCU, D. G. (1977). Effect of changing the composition of the bathing solutions upon the isometric tension–pCa relationship in bundles of crustacean myofibrils. *Journal of Physiology* **270**, 627–652.

BRUST, M. (1966). Relative resistance to dystrophy of slow skeletal muscle of the mouse. *American Journal of Physiology* **210**, 445–451.

CLOSE, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* **52**, 129–197.

DOUGLAS, W. & BASKIN, R. J. (1971). Contractile properties of developing mouse dystrophic muscle. *American Journal of Physiology* **220**, 1344–1354.

FABIATO, A. & FABIATO, F. (1978). Myofilament-generated tension oscillations during partial calcium activation and activation dependence of the sarcomere length–tension of skinned cardiac cells. *Journal of General Physiology* **72**, 667–699.

FINK, R. H. A., STEPHENSON, D. G. & WILLIAMS, D. A. (1986). Potassium and ionic strength effects on the isometric force of skinned twitch muscle fibres of the rat and toad. *Journal of Physiology* **370**, 317–337.

FINK, R. H. A., WILLIAMS, D. A. & STEPHENSON, D. G. (1982). Force–pCa relations in normal and dystrophic mammalian skinned muscle fibres. *Proceedings of the Australian Physiological and Pharmacological Society* **13**, 22p.

FITZSIMONS, R. B. & HOH, J. F. Y. (1983). Myosin isoenzymes in fast-twitch and slow-twitch muscles of normal and dystrophic mice. *Journal of Physiology* **343** 539–550.

HARRIS, J. B. & WILSON, P. (1971). Mechanical properties of dystrophic mouse muscle. *Journal of Neurological and Neurosurgical Psychiatry* **34**, 512–520.

JOHN, H. A. (1974). The myosin of developing and dystrophic skeletal muscle. *Federation of European Biochemical Societies Letters* **39**, 278–282.

JOHN, H. A. (1976). Myofibrillar proteins of developing and dystrophic skeletal muscle. *Federation of European Biochemical Societies Letters*, **64**, 116–121.

JULIAN, F. J. (1971). The effect of calcium on the force–velocity relation of briefly glycerinated frog muscle fibres. *Journal of Physiology* **218**, 117–145.

KERRICK, W. G. L., SECRIST, D., COBY, R. & LUCAS, S. (1976). Development of differences between red and white muscles in sensitivity to Ca in the rabbit from embryo to adult. *Nature* **260**, 440–441.

LUFF, A. R. (1981). Dynamic properties of the inferior rectus, extensor digitorum longus, diaphragm and soleus muscles of the mouse. *Journal of Physiology* **313** 161–171.

MEIER, H. & SOUTHARD, J. L. (1970). Muscular dystrophy in the mouse caused by an allele at the *dy*-locus. *Life Sciences* **9**, 137–144.

MICHELSON, A., RUSSELL, E. S. & HARMAN, P. J. (1955). Dystrophia muscularis – a hereditary myopathy in the house mouse. *Proceedings of the National Academy of Sciences of the U.S.A.* **41**, 1079–1084.

MILLER, D. J. & SMITH, G. L. (1984). EGTA purity and the buffering of calcium ions in physiological solutions. *American Journal of Physiology* **246**, C160–166.

MOISESCU, D. G. (1976). Kinetics of reaction in Ca-activated skinned muscle fibres. *Nature* **262**, 610–613.

MOISESCU, D. G. & THIELECZEK, R. (1978). Calcium and strontium concentration changes within skinned muscle preparations following a change in the external bathing solution. *Journal of Physiology* **275**, 241–262.

SANDOW, A. & BRUST, M. (1958). Contractility of dystrophic mouse muscle. *American Journal of Physiology* **184**, 557–563.

SANDOW, A. & BRUST, M. (1962). Effects of activity on contraction of normal and dystrophic mouse muscle. *American Journal of Physiology* **202**, 815–820.

STEPHENSON, D. G. & FORREST, Q. G. (1980). Different isometric force–[Ca^{2+}] relationships in slow and fast twitch skinned muscle fibres of the rat. *Biochimica et biophysica acta* **589**, 358–362.

STEPHENSON, D. G. & WILLIAMS, D. A. (1981). Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. *Journal of Physiology* **317**, 281–302.

STEPHENSON, D. G. & WILLIAMS, D. A. (1982). Effects of sarcomere length on the force–pCa relation in fast- and slow-twitch skinned muscle fibres from the rat. *Journal of Physiology* **333**, 637–653.

TAKAGI, A. & ENDO, M. (1977). Guinea pig soleus and extensor digitorum longus; A study on single-skinned fibres. *Experimental Neurology* **55**, 95–101.