Journal of Muscle Research and Cell Motility **9**, 499–515 (1988)

Functional regeneration in the hindlimb skeletal muscle of the mdx mouse

J. E. ANDERSON, B. H. BRESSLER and W. K. OVALLE

Department of Anatomy, University of British Columbia, 2177 Wesbrook Mall, Vancouver, B.C., Canada, V6T 1W5

Received 26 January 1988 and in revised form 3 June 1988

Summary

The pattern of spontaneous skeletal muscle degeneration and clinical recovery in hindlimb muscles of the mdx mutant mouse was examined for functional and metabolic confirmation of apparent structural regeneration. The contractile properties, histochemical staining and myosin light chain and parvalbumin contents of extensor digitorum longus (EDL) and soleus (Sol) muscles of mdx and age-matched control mice were studied at 3–4 and 32 weeks. Histochemical staining (myofibrillar ATPase and NADH–tetrazolium reductase) revealed no significant change in slow-twitch-oxidative (SO) or fast-twitch-oxidative-glycolytic (FOG) fibre type proportions in mdx Sol apart from the normal age-related increase in SO fibres. At 32 weeks mdx EDL, however, showed significantly smaller fast-twitch-glycolytic (FG) and larger FOG proportions than those in control EDL. These fibre type distributions were confirmed by differential staining with antibodies to myosin slow-twitch and fast-twitch heavy chain isozymes. Frequency distribution of cross-sectional area for each fibre type showed a wider than normal range of areas especially in FOG fibres of mdx Sol, and FG fibres of mdx EDL, supporting previous observations using autoradiography of myofibre regeneration. Isometric twitch and tetanic tensions in Sol were significantly less than in controls at 4 weeks, but by 32 weeks, values were not different from age-matched controls. In mdx EDL at 3 weeks, twitch and tetanus tensions were significantly less, and time-to-peak twitch tensions were significantly faster than in control EDL. By 32 weeks, mdx EDL twitch and tetanus tensions expressed relative to muscle weight continued to be significantly lower than in age-matched controls, despite normal absolute tensions. The maximum velocity of shortening in 32-week mdx EDL was significantly lower than in control EDL. Myosin light chain distribution in mdx Sol exhibited significantly less light chain 2-slow (LC2s) and more light chain 1b-slow(LC1bs) at 32 weeks than age-matched control Sol. Gels of EDL from 32-week-old mdx mice showed significantly less light chain 2-fast-phosphorylated (LC2f-P) and light chain 3-fast (LC3f) and significantly more light chain 1-fast (LC1f) and light chain 2-fast (LC2f), but normal parvalbumin content compared to age-matched controls. These observations suggest that mdx hindlimb muscles are differentially affected by the disease process as it occurs in murine models of dystrophy. However, the uniqueness of mdx Sol and to a lesser extent EDL is that they also undergo an important degree of functional regeneration which is able to compensate spontaneously for degenerative influences of genetic origin. The mdx mutant may therefore be an important model for the study of regeneration by skeletal muscle, and of the nerve–muscle interactions which enable or restrict that regeneration.

Introduction

The ultrastructural and autoradiographic characterization of hindlimb muscles in the mdx mouse (Anderson *et al.*, 1987) revealed that slow-twitch soleus (Sol) and fast-twitch extensor digitorum longus (EDL) muscles were primarily affected by early focal degeneration and necrosis. Although lesions in Sol appeared earlier and more diffusely than in EDL, by 32 weeks of age both muscles were largely populated by a normal (Sol) or above-normal (EDL) number of centrally nucleated myofibres which showed a wide range of cross-sectional areas. However, Sol and EDL did not exhibit the interstitial connective tissue fibrosis or adipose tissue infiltration seen in muscles of other mouse models of dystrophy (Harris & Slater, 1980) and in human Duchenne muscular dystrophy (Rowland & Layzer, 1979). The morphological observations of mature and maturing myofibres, single or grouped inside the external lamina, were accompanied by the finding of increased tritiated thymidine uptake by sublaminal nuclei (Anderson *et al.*, 1987). Thus the structural features of regeneration were exhibited by Sol and EDL. To elucidate the degree and efficacy of the regenerative potential of mdx skeletal muscle, a detailed comparison was made of the fibre type distribution, protein distribution and isometric contractile properties from representative fast- and slow-twitch muscles from this strain.

0412-4319/88 $03.00 + .12

Materials and methods

Breeding pairs of the mdx mutant of the C57BL/10 ScSn stain were obtained from the Agriculture Research Centre in Scotland, courtesy of Professor G. Bulfield, and mice were bred and maintained in our own animal facility. Mice of the C57BL/6J +/+ strain served as their controls. Mdx and control male mice at postnatal ages of 3–4 and 32 weeks were killed by cervical dislocation under ether anaesthesia. These ages were chosen to allow comparison with our previous work on the mdx hindlimb muscles (Anderson *et al.*, 1987) and with earlier studies of the dy^{2J}/dy^{2J} strain of dystrophic mice in this laboratory (Jasch *et al.*, 1982; Bressler *et al.*, 1983; Ovalle *et al.*, 1983).

HISTOCHEMISTRY

Whole soleus (Sol) and extensor digitorum longus (EDL) muscles with their epimysial coverings intact were quickly excised from hindlimbs and placed in gauze dampened with physiological saline. Muscles were embedded, frozen and sectioned according to previously reported methods (Ovalle *et al.*, 1983). Alternate sections were treated histochemically to detect NADH-tetrazolium reductase (NADH-TR). Myosin ATPase activity was determined (Dubowitz & Brooke, 1973) after preincubation of sections at varying pH values (Sol: 4.2, 4.6 and 9.6; EDL: 4.2, 4.6 and 10.2). For each of mdx and control, Sol and EDL at 4 and 32 weeks of age, there were five muscles sectioned per group. To corroborate histochemical fibre-typing patterns, alternate sections were also treated immunocytochemically with polyclonal antibodies to myosin fast and slow heavy-chain isoforms by using diaminobenzidine to stain the peroxidase-labelled antibody complex (Jones *et al.*, 1987). The specificities of anti-fast (raised against IIA myosin; Mascarello *et al.*, 1982) and anti-slow (raised against cat soleus type I fibres; Rowlerson *et al.*, 1981) myosin antibodies have been reported previously. Primary antibodies were applied to unfixed cryostat sections at dilutions of 1/1000 for anti-I and 1/50 for anti-IIA.

All extrafusal muscle fibres in each cross-section were classified (Peter *et al.*, 1972; Silverman & Atwood, 1980; Ovalle *et al.*, 1983) as slow-twitch-oxidative (SO), fast-twitch-oxidative-glycolytic (FOG) or fast-twitch-glycolytic (FG). A small number of fibres in both Sol and EDL which could not be classified on this basis were designated 'atypical' fibres; they stained after preincubation in both acid and alkaline pH. Each muscle cross-section was projected on to paper by a camera lucida attachment to a Leitz Orthoplan light microscope and drawn in outline at 80× magnification. The fibre type of every fibre was identified and marked on the drawing underlying the image. Absolute numbers of each fibre type in Sol and EDL control and mdx muscles were expressed as proportions of total numbers of fibres observed in the serial sections. To determine the fibre cross-sectional area, a sample of approximately 100 fibres along the longest chord perpendicular to the largest diameter of each muscle (Dubowitz & Brooke, 1973) were drawn by camera lucida projection and individual fibre types indicated. The drawings were traced on to a calibrated Zeiss MOP-graphics tablet and the samples used to determine the cross-sectional area (mean ± SE) and area distribution for each of the fibre types in Sol and EDL. Only the determination of fibre cross-sectional area was made by sampling fibres; fibre type proportions were determined from classification of all extrafusal fibres in each muscle.

CONTRACTILE PROPERTIES

Experiments were carried out *in vitro* using EDL or Sol muscles from the right leg of male mdx and control mice at the following ages (±3 days): 3 (EDL) or 4 (Sol) and 32 (EDL and Sol) weeks.

Dissection

After body weight was measured, the right hindlimb was detached, skinned and bathed in a pool of Krebs' solution (Bressler *et al.*, 1983). The Sol was isolated following removal of the gastrocnemius. Proximal and distal tendons were tied close to the myotendinous junctions with surgical silk to avoid any stray series compliance due to excessive lengths of tendon. The EDL was exposed in a similar manner from beneath tibialis anterior, tied, and dissected away from the surrounding connective tissue. Either the Sol or EDL was used from a single animal. During the experiment, muscles were maintained at room temperature (22° C) in Krebs' solution, gassed with 95% O_2/5% CO_2 at pH 7.3–7.4.

Experimental apparatus

The muscles were mounted in a muscle bath between two platnium plate electrodes placed parallel to but not in contact with the muscle. For the younger age group, the proximal muscle tendon was tied to a Cambridge model 300H servomotor, and the distal tendon to a stainless-steel wire. Tension was measured directly from the motor output with a resolution of 50 mN V^{-1}. Muscles from 32-week-old mice were tied with the proximal tendon attached to the anode pin of an RCA 5734 tension transducer (resolution 73.66 mN V^{-1}; resonant frequency, 1.2 kHz) and the distal tendon secured to a stainless-steel wire. The wire passed through a short brass tube and was rigidly connected to a light aluminium rod extending from the centre of a loudspeaker cone. These length servosystems allowed examination of the isometric contractile properties as previously reported (Bressler *et al.*, 1983; Redenbach & Bressler, 1988).

Experimental procedures

To allow comparison between muscles, they were mounted at the length at which the maximum isometric twitch tension was recorded. Stimulation consisted of supramaximal square-wave pulses, 1 ms in duration. The fusion frequency for a smooth tetanus at room temperature was determined to be 60–80 impulses s^{-1} for the Sol and 80–100 impulses s^{-1} for the EDL at a duration of 275 ms (EDL) or 350 ms (Sol). A regimen of three twitches followed by a fused tetanus, with a contraction every 90 s was used to prevent fatigue of muscles due to repeated tetani. For each

muscle, a minimum of ten twitches and four tetani was recorded. The maximum velocity of unloaded muscle shortening (V_o) was determined by the slack test method (Edman, 1979; Redenbach & Bressler, 1988). Following the V_o determination, the muscle was allowed to rest for 20 min. Post-tetanic twitch potentiation (PTP) was then measured by recording a single twitch (pre-twitch) followed in 90 s by a 1 s tetanus, and 20 s later by a second twitch (post-twitch). An oscilloscope recorded the two superimposed twitches while a second oscilloscope recorded the tetanus. Following this, the fatigue characteristics of the muscle were measured. This regime consisted of giving the muscle a 1 s tetanus at a rate of 12 min^{-1} for 5 min. At the conclusion of each experiment, the length of the muscle between the ties was recorded with fine calipers, and the muscle was removed, blotted dry and weighed.

Contractile responses were recorded on 35 mm film with an Asahi Pentax camera fitted with a macrolens, and mounted on the oscilloscope frame. In addition, analog signals from the tension transducer were digitized and stored on disc using Scopedriver software (RC Electronics, California) for the Apple IIE. A 12 ms delay between triggering of the analog-digital converter and the stimulator provided a baseline on the records. From these records a custom software analysis program was used to measure the maximum isometric twitch (P_t) and tetanus (P_o) tensions, time-to-peak twitch tension (TTP), and the time from peak tension to one-half amplitude of peak tension (½RT). Maximum velocity of unloaded shortening (V_o) and post-tetanic twitch potentiation (ratios of post-twitch to pre-twitch tensions, PTP) were determined by tracing images stored on the calibrated digital oscilloscope (Nicolet 3091) on to an *x–y* plotter (Hewlett–Packard 7035). Fatigue responses were measured from the photographed tetanus records magnified on a standard darkroom enlarger, and normalized to the height of the first tetanus. The decay of peak tetanic tension in the fatigue profile was plotted over time. In addition, both the maximum twitch (P_t) and tetanus (P_o) tensions were normalized with respect to wet muscle weight (P_t/mwt and P_o/mwt, respectively). Finally, values from each animal were averaged and the means for a minimum of six muscles per age group were statistically analysed.

PROTEIN DISTRIBUTION

The Sol and EDL muscles from one to three animals were dissected away from their tendons, and pooled to obtain a standard 11 mg of tissue to be applied to each gel. Isoelectric focusing between pI 4.5 and 7.2 on 5 mm polyacrylamide gels was carried out with homogenized muscle tissue according to Jasch *et al.* (1982). Stained gels were scanned (Guilford spectrophotometerr) at 560 nm. Gels were photographed within 24 h of scanning. Data were derived from peak heights relative to the total combined peak height of myosin light chains, which were identified according to Weeds (1976), Fitzsimons & Hoh (1983) and Jasch & Moase (1985). The peak heights in the scans from the one-dimensional isoelectric focusing gels were found to be linearly correlated with the amount of protein applied to the gels within the range used, as determined in previous reports from this laboratory (Jasch *et al.*, 1982; Davis *et al.*, 1988). Myosin light chain 1-fast (LC1f), 2-fast (LC2f), 2-fast-phosphorylated (LC2f-P), light chain 3-fast (LC3f) and parvalbumin were measured in EDL, and light chain la-slow (LC1as), 1b-slow (LC1bs), 2-slow (LC2s) and LC2f were measured in Sol. Protein content was determined as a proportion of total myosin light chains.

STATISTICAL ANALYSIS

Data for each parameter were analysed by two-way analysis of variance to determine genetic and age-related effects as well as significant interactions. Tukey's test was appled *post hoc* to determine the significance of differences between appropriate pairs of means. Differences between age-matched control and mdx fatigue profiles were determined by Student's *t*-test for each tetanus between t = 0 s and t = 300 s. In all cases a probability of $P < 0.05$ was used to reject the null hypothesis.

Results

HISTOCHEMISTRY

Sol

Normal and mdx Sol contained two main types of myofibres that could be distinguished in sections stained for NADH-TR or myosin ATPase after varying preincubations. SO fibres stained heavily around their periphery for the oxidative enzyme (Fig. 1a) and for myosin ATPase after acid preincubations (Fig. 1b). They stained lightly for myosin ATPase after pH 9.4 preincubation. In contrast, FOG fibres stained heavily across their whole cross-section for the oxidative enzyme (Fig. 1a) and were lightly stained for myosin ATPase after acid preincubations (Fig. 1b). Myosin staining with antibodies to slow (Fig. 1c) and fast (Fig. 1d) isozymes revealed that fibres were accurately assigned to slow and fast fibre types.

Comparative data on the proportions of the observed fibres exhibiting characteristic staining patterns in normal and mdx Sol at 4 and 32 weeks are presented in Fig. 2. Apart from normal age-related changes in both control and mdx Sol, there were no significant differences between control and mdx Sol fibre type proportions at either age.

EDL

In the control and mdx EDL at 4 weeks, there were three main types of fibres. FG fibres stained pale for NADH-TR (Fig. 3a) and myosin ATPase after acid preincubations (Fig. 3b), and moderately for myosin

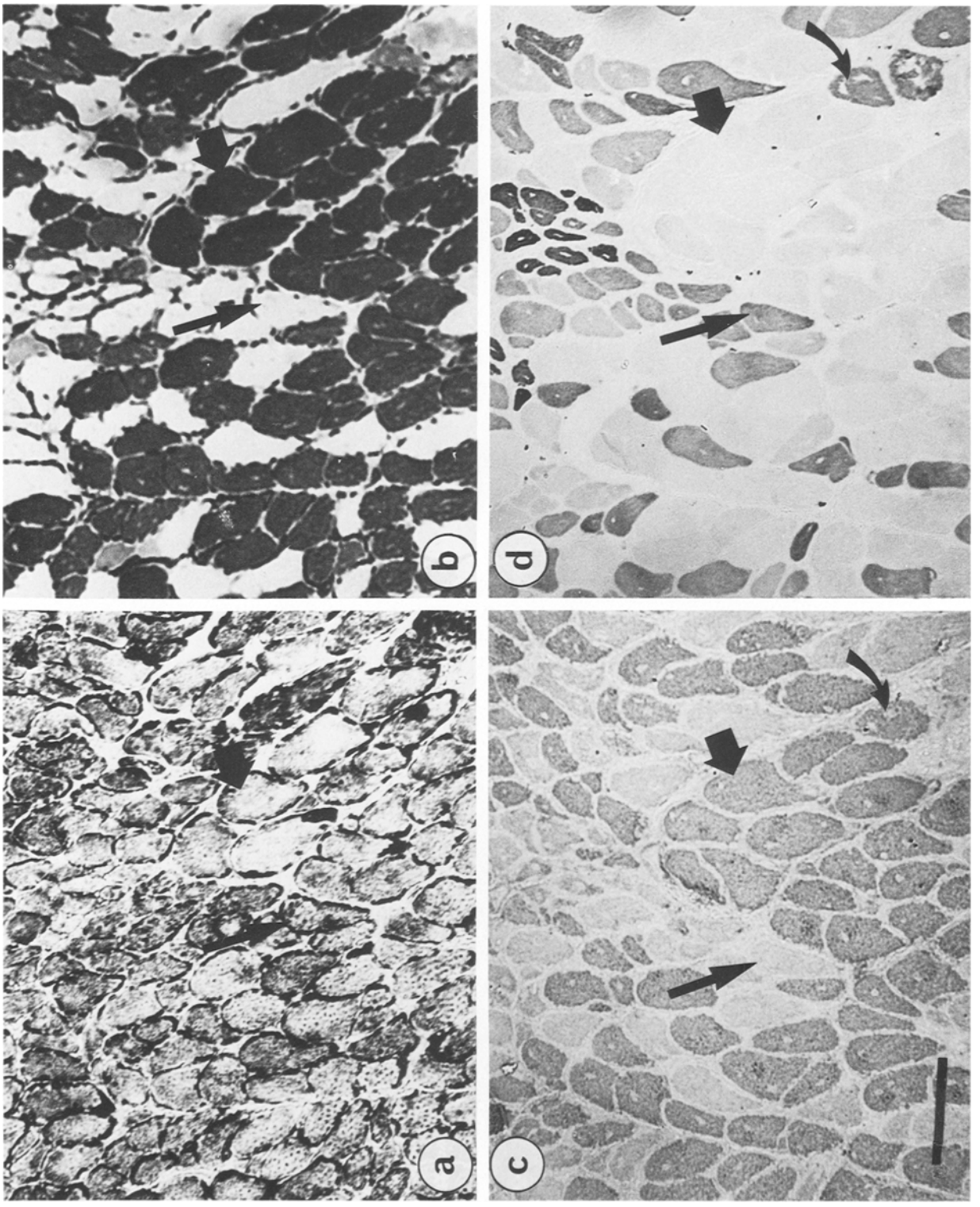

Fig. 1. Light micrographs of serial sections from a 32-week-old mdx soleus muscle stained for (a) NADH-TR and (b) myosin ATPase (pH 4.2) activities and for the presence of (c) slow and (d) fast myosin heavy chain isozymes. SO (short arrows), FOG (long arrows) and a few 'atypical' (curved arrows) fibres populate the muscle. Central nuclei are unstained. Bar = 100 μm.

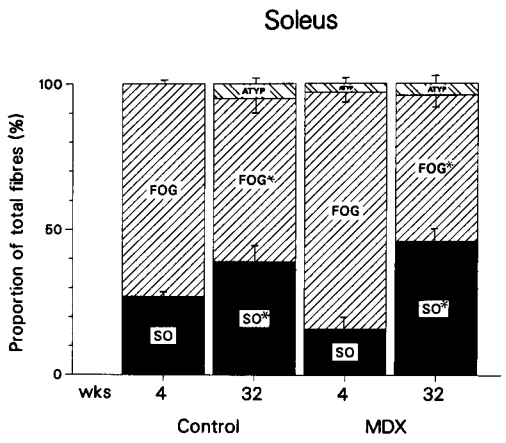

Fig. 2. Histograms of the fibre type proportions present in 4- and 32-week control and mdx soleus. The asterisks indicate a significant decrease (FOG, $P < 0.01$) or increase (SO, $P < 0.01$) proportion between 4- and 32-week groups of the same strain. There was no difference between control and mdx fibre proportions in the soleus.

ATPase after alkaline preincubation. FOG fibres stained intermediately for myosin ATPase after acid preincubation (Fig. 3b), and lightly after alkaline preincubation. SO fibres stained heavily for myosin ATPase after acid pH (Fig. 3b), and for NADH-TR (Fig. 3a), but did not stain for myosin ATPase after alkaline preincubation. Antibodies to slow (Fig. 3c) and fast (Fig. 3d) myosin heavy chain isozymes again supported fibre type assignments.

In addition to FG, FOG and SO fibres, a fourth 'atypical' staining pattern was found, particularly in 32-week-old muscles, which exhibited moderate staining for NADH-TR and for myosin ATPase at both acid and alkaline preincubations and with both anti-slow and anti-fast myosin antibodies. These fibres are similar to what some authors refer to as regenerating or type 2c fibres (Nonaka *et al.*, 1981; Mascarello *et al.*, 1984). There was no significant difference in the proportion of 'atypical' fibres between age-matched mdx and control muscles for either Sol or EDL.

The quantitative data on fibre proportions for the control and mdx EDL at 4 and 32 weeks are presented in Fig. 4. The 32-week mdx EDL had a significantly greater proportion of FOG fibres than its age-matched control. The 32-week mdx EDL had a significantly smaller proportion of FG fibres than both 32-week control EDL and 4-week mdx EDL. These changes were separate from age-related changes, which are indicated in Fig. 4.

MORPHOMETRY

Quantitative data on fibre cross-sectional area, separated by fibre type, from Sol and EDL of mdx and control mice are presented in Table 1. In the Sol, only mdx FOG fibres at 32 weeks had significantly greater mean cross-sectional area than the same fibre type in the age-matched control Sol. In the EDL, both FG and SO fibre types in the 4-week mdx EDL were statistically larger in mean cross-sectional area than their age-matched normal controls. Age-related changes are detailed on the table.

The frequency distributions of fibre cross-sectional area separated according to fibre type are illustrated for Sol in Fig. 5 and for EDL in Fig. 6. In both SOL and EDL, the rightward shift in area distributions with increased age are evident in the separate fibre types. An increased range of mdx fibre area distribution compared to their age-matched controls may be observed in the 32-week SO and FOG fibre distributions in the mdx Sol, and in the 4- and 32-week FG fibre distributions in the mdx EDL. Fibres smaller than in age-matched controls are particularly evident in the 32-week FG fibres of the mdx EDL. Large myofibres are illustrated in the distribution of FOG fibres of the 32-week mdx Sol, and in 32-week FG fibres of the mdx EDL.

CONTRACTILE PROPERTIES

The isometric tension characteristics of mdx and

Table 1. Cross-sectional area* (μm^2) of each main fibre type in Sol and EDL of control and mdx mice.

Fibre type	*Strain*	*4 weeks*	*32 weeks*
Sol			
SO	+/+	884.2 ± 24.3	2013.8 ± 89.8†
	mdx	1044.1 ± 17.6	1878.3 ± 119.9†
FOG	+/+	872.0 ± 32.9	1989.6 ± 140†
	mdx	932.3 ± 42.0	2681.9 ± 203§
EDL			
FG	+/+	800.7 ± 73.0	1703.8 ± 126.4†
	mdx	1072.3 ± 46.5‡	1784.0 ± 64.1†
FOG	+/+	491.8 ± 38.8	758.4 ± 64.0†
	mdx	471.7 ± 24.6	806.5 ± 51.4†
SO	+/+	277.9 ± 23.6	376.8 ± 75.9[a]
	mdx	412.5 ± 11.1‡‡	584.5 ± 213.5[b]

*For each value, $n = 5$, except [a] where $n = 3$, [b] where $n = 2$. Values are mean ± SEM.
†Indicates significant difference from 4-week group of same strain; $P < 0.01$.
‡Indicates significant difference from age-matched control group; ‡ $P < 0.01$; ‡‡ $P < 0.001$.
§Indicates significant difference from both age-matched control group and 4-week mdx group; $P < 0.01$.

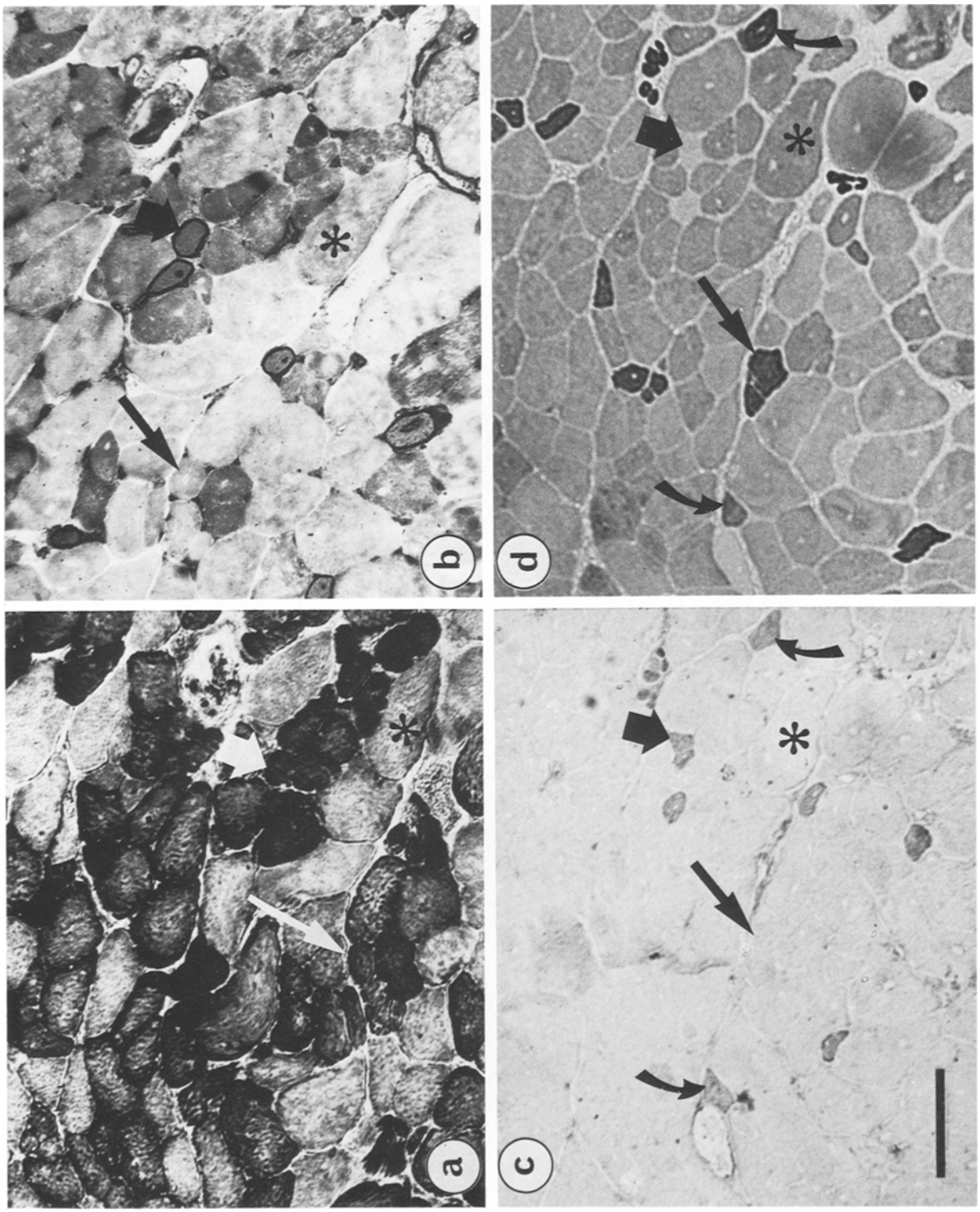

Fig. 3. Light micrographs of serial sections from a representative 32-week mdx EDL, stained for (a) NADH-TR and (b) myosin ATPase (pH 4.6) activities and for the presence of (c) slow and (d) fast myosin heavy chain isozymes. FG (asterisks), FOG (long arrows), SO (short arrows) and 'atypical' (curved arrows) fibres are present. Central nuclei are unstained. Bar = 100 μm.

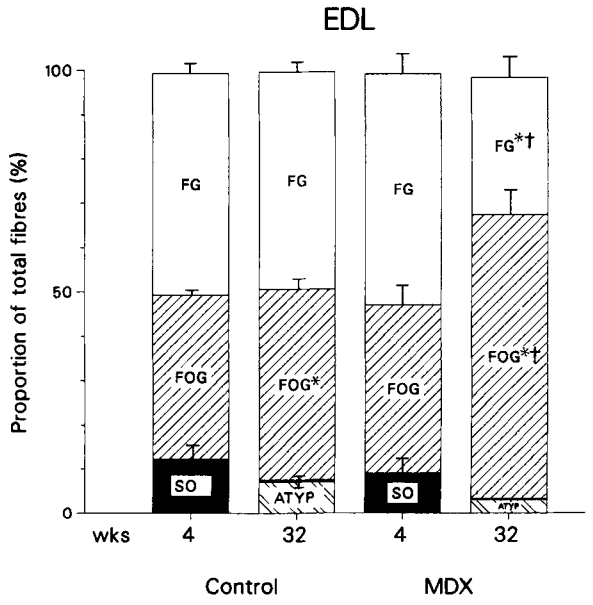

Fig. 4. Histograms of the fibre type proportions present in 4- and 32-week control and mdx EDL. Asterisks indicate a significant increase (FOG: $P < 0.05$, control; $P < 0.01$, mdx) or decrease (FG: $P < 0.01$, mdx) in proportion between 4- and 32-week groups of the same strain. Daggers indicate a significant increase (FOG, $P < 0.01$) or decrease (FG, $P < 0.01$) in proportion between the 32-week mdx EDL and its age-matched control. In addition, the SO fibre type proportion was significantly reduced in both the 32-week control and mdx groups, as compared to the 4-week groups in the same strain. There were too few (0.04%) 'atypical' fibres in the 4-week mdx EDL to draw accurately in the histogram.

control Sol and EDL are summarized in Table 2 and 3 respectively.

Sol

In the 4-week mdx Sol, absolute twitch and tetanus tensions and tetanus tension normalized to muscle weight were significantly decreased compared to age-matched controls. At 32 weeks, however, in the mdx Sol none of the contractile parameters measured were significantly altered from age-matched control values. These comparisons were separate from age-related changes as indicated in Table 2.

EDL

A comparison of contractility data for EDL from control and mdx at 3 and 32 weeks exhibits a distinctly different pattern than that observed for the Sol. At 3 weeks mdx EDL generated significantly less twitch and tetanus tension than in control EDL, both in absolute terms and when expressed relative to muscle weight. There was also a faster TTP observed in the 3-week mdx EDL compared to the 3-week control EDL.

In mdx EDL at 32-weeks, both the P_o/mwt and P_t/mwt tensions were significantly reduced from the control. In addition, V_o was also significantly decreased from that observed in the age-matched control EDL. The times of contraction (TTP) and relaxation (½RT) were unchanged from control levels in 32-week mdx EDL. These differences between control and mdx groups were separate from age-related changes, as indicated in Table 3.

Fatigue

The decline in relative isometric tetanus tension

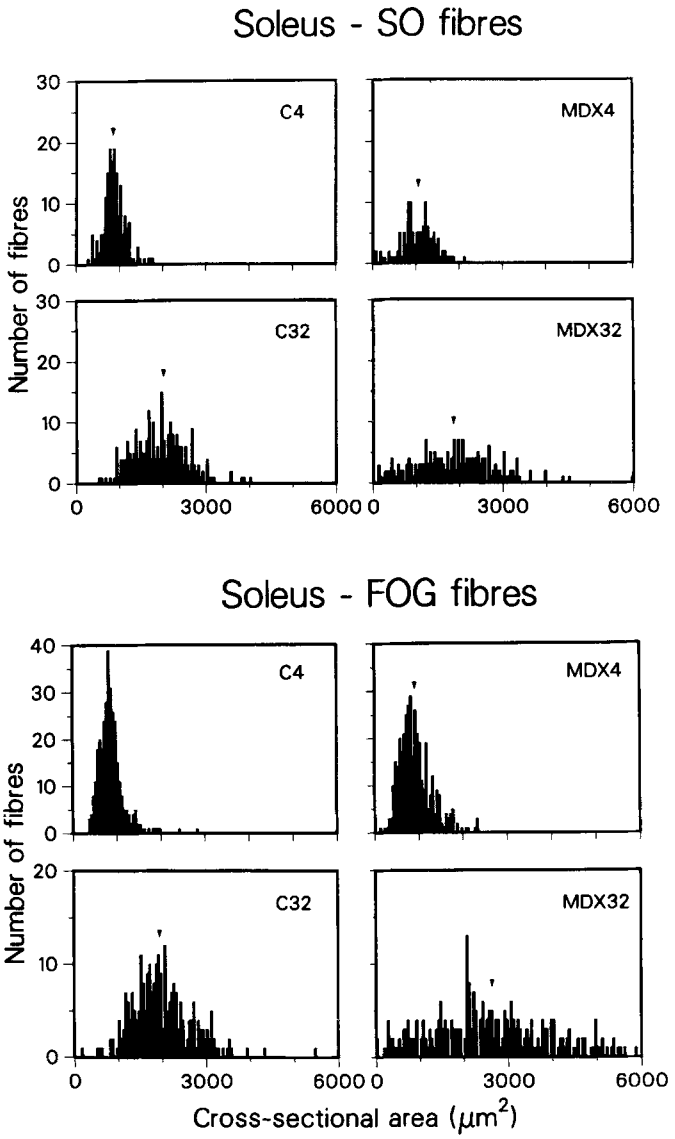

Fig. 5. Frequency distributions of fibre cross-sectional area (μm^2) for control and mdx Sol, separated into SO and FOG fibre types. Inverted arrowheads indicate mean cross-sectional area.

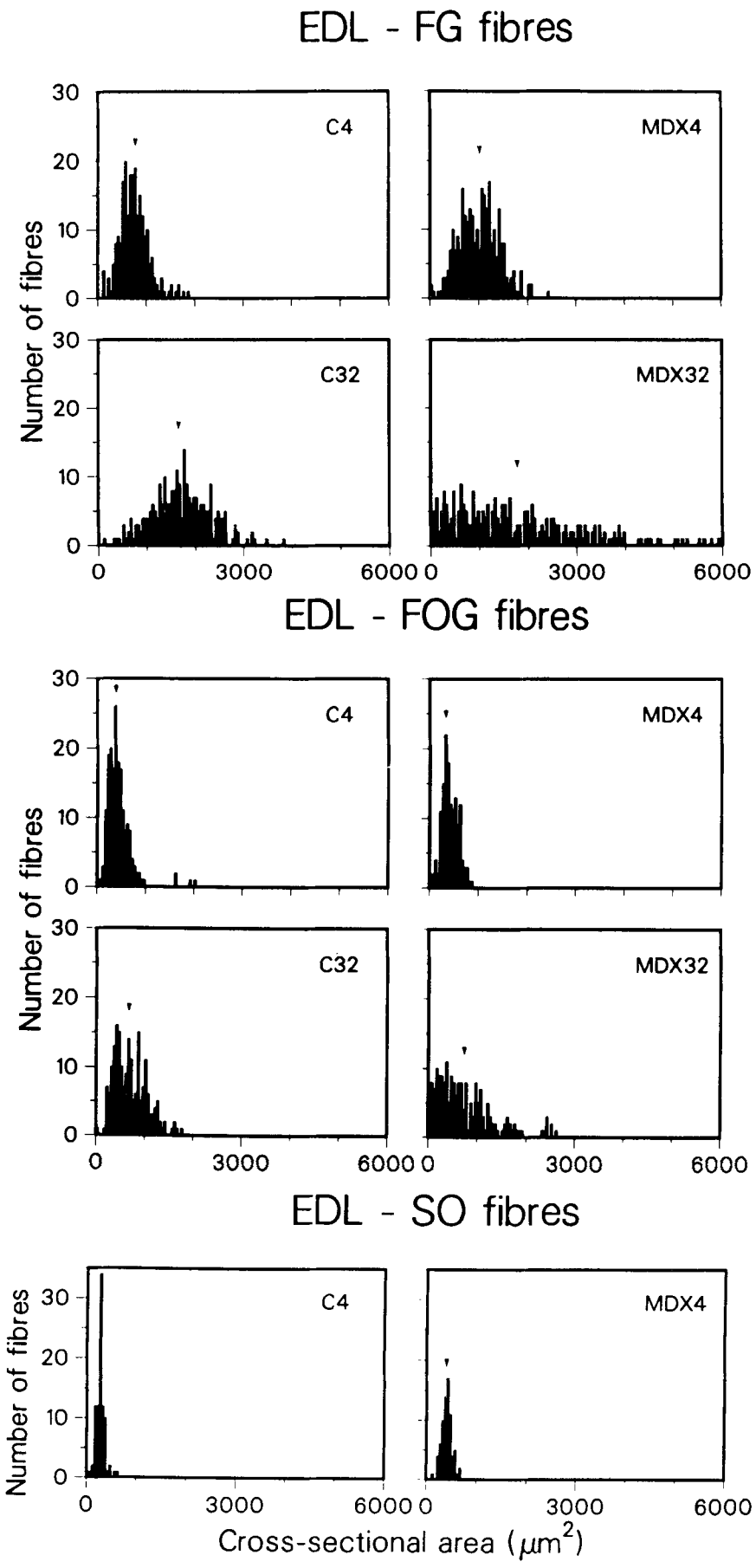

Fig. 6. Frequency distributions of fibre cross-sectional area (μm^2) for control and mdx EDL, separated into FG, FOG and SO fibre types. Inverted arrowheads indicate mean cross-sectional area.

Table 2. Contractile properties of control and mdx SOL*.

Parameter	*Strain*	*4-weeks (n)*	*32-weeks (n)*
P_o (mN)	+/+	131.6 ± 8.4 (7)	156.0 ± 27.1 (7)
	mdx	79.1 ± 9.7 (6)‡‡	156.4 ± 29.9 (6)†
P_o/mwt	+/+	21.1 ± 1.0 (7)	11.3 ± 2.1 (7)†
	mdx	15.0 ± 1.2 (6)‡‡	9.9 ± 2.0 (6)†
P_t (mN)	+/+	29.0 ± 1.3 (7)	34.2 ± 7.3 (7)
	mdx	18.9 ± 0.16 (6)‡‡	28.6 ± 5.9 (6)
P_t/mwt	+/+	4.7 ± 0.4 (7)	2.5 ± 0.6 (7)††
	mdx	3.7 ± 0.4 (6)	2.6 ± 1.1 (6)
TTP (ms)	+/+	55.14 ± 2.60 (7)	64.80 ± 6.24 (7)
	mdx	54.85 ± 3.92 (6)	78.03 ± 8.61 (6)†
½RT (ms)	+/+	70.95 ± 4.35 (7)	124.95 ± 19.02 (7)††
	mdx	67.99 ± 5.05 (6)	193.61 ± 43.91 (6)†
V_o (l_o/s)	+/+	4.90 ± 0.52 (6)	2.12 ± 0.05 (7)††
	mdx	4.77 ± 0.42 (6)	1.89 ± 0.20 (6)††
PTP	+/+	1.07 ± 0.007 (6)	0.97 ± 0.002 (7)††
	mdx	1.01 ± 0.009 (6)	0.96 ± 0.014 (6)††

*Values are means ± SEM; group size for each parameter = (*n*).
†Indicates significant difference from 4-week group of same strain; †$P < 0.05$, ††$P < 0.01$.
‡Indicates significant difference from age-matched control group; ‡‡$P < 0.01$.

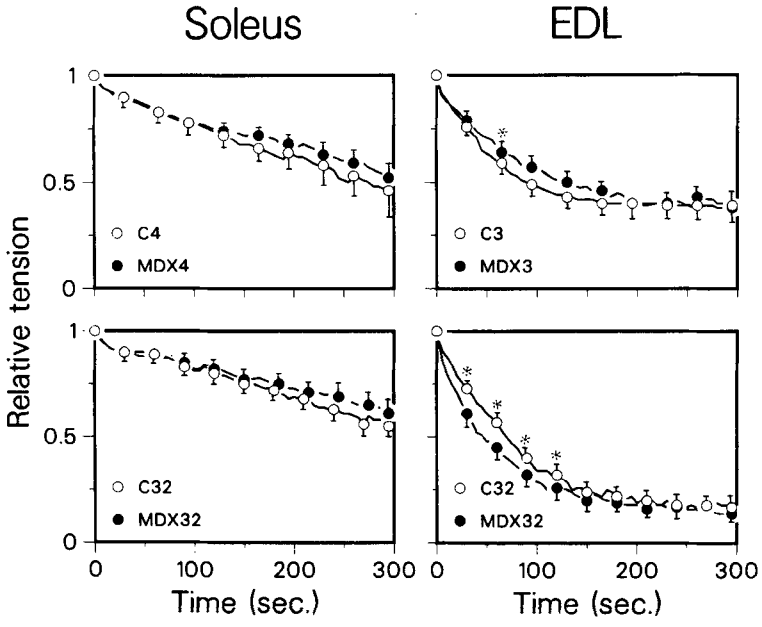

Fig. 7. Fatigue profiles showing the decline of the relative tetanus tension with time. Mdx and its age-matched control are presented in the same subplot for each of soleus (left) and EDL (right) at 3 or 4 weeks (top) and 32 weeks (bottom) of age. There was no change in response to fatigue for the control and mdx Sol. For the EDL, the control and mdx muscles became fatigued to the same relative tension, although there were intervals (asterisks) where significantly different tensions were recorded.

Table 3. Contractile properties of control and mdx EDL*.

Parameter	*Strain*	*3-weeks (n)*	*32 weeks (n)*
P_o (mN)	+/+	131.3 ± 9.8 (6)	343.6 ± 20.5 (7)††
	mdx	97.9 ± 5.6 (6)‡‡	335.3 ± 60.0 (8)††
P_o/mwt	+/+	36.6 ± 2.1 (6)	28.8 ± 2.5 (7)
	mdx	27.3 ± 1.3 (6)‡‡	17.0 ± 3.0 (8)§§
P_t (mN)	+/+	40.1 ± 1.8 (6)	93.2 ± 7.5 (7)††
	mdx	32.4 ± 2.2 (6)‡	94.2 ± 17.9 (8)††
P_t/mwt	+/+	11.2 ± 0.4 (6)	7.9 ± 0.9 (7)††
	mdx	9.1 ± 0.7 (6)‡	4.8 ± 1.0 (8)§§
TTP (ms)	+/+	29.59 ± 0.82 (6)	27.90 ± 1.85 (7)†
	mdx	26.20 ± 1.26 (6)‡	26.02 ± 1.36 (8)
½RT (ms)	+/+	38.62 ± 2.4 (6)	27.41 ± 2.42 (7)††
	mdx	34.40 ± 2.8 (6)	30.14 ± 2.31 (8)
V_o (l_o/s)	+/+	5.55 ± 0.40 (6)	3.33 ± 0.17 (6)††
	mdx	5.14 ± 0.49 (5)	2.12 ± 0.26 (7)§§
PTP	+/+	1.12 ± 0.03 (6)	1.17 ± 0.02 (6)†
	mdx	1.06 ± 0.05 (6)	1.16 ± 0.04 (6)

*Values are means ± SEM; group size for each parameter = (*n*).
†Indicates significant difference from 3-week group of same strain; †$P < 0.05$, ††$P < 0.01$.
‡Indicates significant difference from age-matched control group; ‡$P < 0.05$, ‡‡$P < 0.01$.
§Indicates significant difference from both 3-week mdx group and age-matched control group; §§$P < 0.01$.

during the fatigue regime of repetitive stimulation is presented graphically for the control and mdx Sol and EDL at 3–4 and 32 weeks of age (Fig. 7). For the mdx Sol responses, there were no significant changes in their resistance to fatigue at both 4 and 32 weeks compared to control. While there were significant differences in response to fatigue of the 3- and 32-week mdx EDL, in both age groups, the muscles reached the same tension level as age-matched control EDL at the end of the fatigue regime.

PROTEIN DISTRIBUTION

Quantitative data from one-dimensional gels are presented in Tables 4 (Sol) and 5 (EDL). Representative gels and their respective scans from each group are shown in Fig 8 (Sol) and 9 (EDL).

In the mdx Sol at both 4 and 32 weeks of age, the LC1bs proportion of total light chains content was significantly higher than in controls. The LC2s proportion was significantly lower than in controls. All Sol light chain proportions in mdx Sol exhibited the same significant age-related changes as observed for control Sol.

In mdx EDL at 4 weeks, the LC1f proportion of light chains was significantly higher than the control value, and the LC2f-P proportion was significantly less than the control level. In the 32-week mdx EDL, both LC1f and LC2f proportions were significantly higher than control values, whereas both LC2f and LC3f proportions were significantly less than control values. These changes were separate from the age-related changes in the proportions of light chains, as given in Table 5. Parvalbumin content in the mdx EDL, expressed as a proportion of total light chains, was not different from control content at either 4 or 32 weeks of age.

Table 4. Myosin light chain content* of control and mdx soleus at 4 and 32 weeks of age.

Protein	*Strain*	*4 weeks (n)*	*32 weeks (n)*
LC1as	+/+	0.104 ± 0.007 (7)	0.224 ± 0.01 (8)††
	mdx	0.101 ± 0.013 (9)	0.194 ± 0.008 (8)††
LC1bs	+/+	0.397 ± 0.016 (7)	0.230 ± 0.007 (8)††
	mdx	0.446 ± 0.011 (9)‡	0.342 ± 0.01 (8)§
LC2s	+/+	0.205 ± 0.01 (7)	0.314 ± 0.007 (8)††
	mdx	0.136 ± 0.009 (9)‡‡	0.209 ± 0.019 (8)§
LC2f	+/+	0.286 ± 0.008 (7)	0.231 ± 0.006 (8)†
	mdx	0.316 ± 0.014 (9)	0.248 ± 0.012 (8)††

*Mean ± SE, determined as a proportion of total light chain content.
†Indicates significant difference from 4-week group of same strain; †$P < 0.05$, ††$P < 0.01$.
‡Indicates significant difference from age-matched control group; ‡$P < 0.05$, ‡‡$P < 0.01$.
§Indicates significant difference from both 4-week mdx group and age-matched control group, $P < 0.01$.

Table 5. Myosin light chain content* of control and mdx EDL at 4 and 32 weeks of age.

Protein	*Strain*	*4 weeks (n)*	*32 weeks (n)*
LC1f	+/+	0.256 ± 0.008 (10)	0.247 ± 0.01 (9)
	mdx	0.291 ± 0.006 (10)‡	0.302 ± 0.01 (9)‡‡
LC2f	+/+	0.410 ± 0.016 (10)	0.242 ± 0.005 (9)†
	mdx	0.449 ± 0.014 (10)	0.290 ± 0.007 (9)†§
LC2f-P	+/+	0.146 ± 0.009 (10)	0.217 ± 0.008 (9)†
	mdx	0.090 ± 0.005 (10)‡‡	0.171 ± 0.020 (9)†§
LC3f	+/+	0.189 ± 0.01 (10)	0.294 ± 0.001 (9)†
	mdx	0.175 ± 0.01 (10)	0.229 ± 0.009 (9)§§
Parv.¶	+/+	0.896 ± 0.042 (10)	0.839 ± 0.038 (9)
	mdx	0.804 ± 0.04 (10)	0.764 ± 0.032 (9)

*Mean ± SE, determined as a proportion of total light chain content.
†Indicates significant difference from 4-week group of same strain; $P < 0.01$.
‡Indicates significant difference from age-matched control; ‡$P < 0.05$, ‡‡$P < 0.01$.
§Indicates significant difference from both 4-week mdx group and age-matched control group; §$P < 0.05$, §§$P < 0.01$.
¶Parvalbumin.

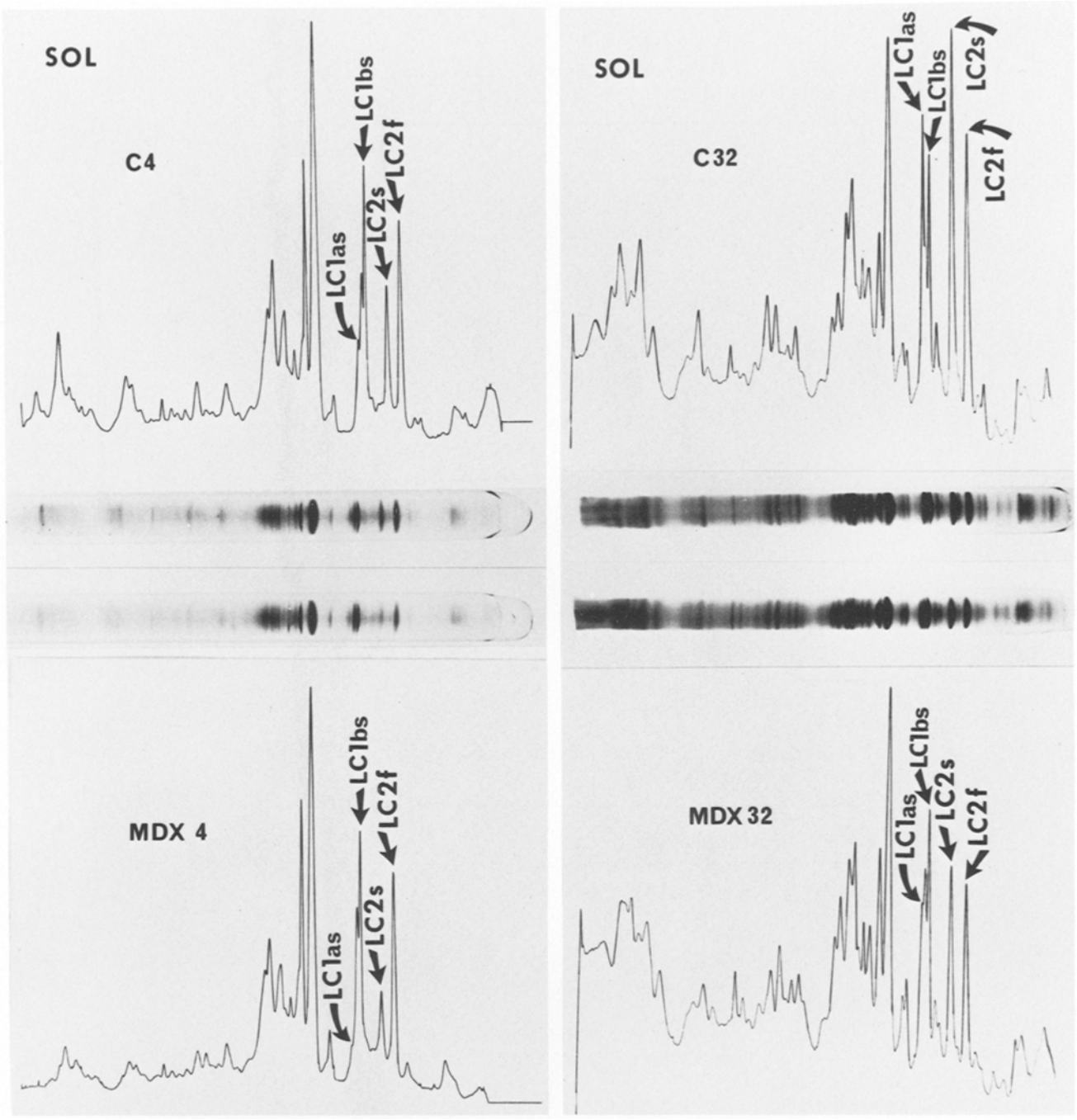

Fig. 8. Representative gels of soleus at 4 and 32 weeks of age, with their respective scans, showing LC1as, LC1bs, LC2s and LC2f peaks.

Discussion

The most striking feature of the mdx strain of mouse is the distinctive functional recovery of both the slow-twitch Sol and fast-twitch EDL muscles, with the retention of typical slow- and fast-twitch character and with a clearly higher potential for regenerative capacity in the slow-twitch skeletal muscle. Specifically for the mdx Sol, this was observed in its ability to generate normal tensions at normal speeds of contraction and relaxation, and to resist fatigue, as well as in its recovery of normal fibre type proportions and mean fibre cross-sectional areas after early degeneration. With respect to the mdx EDL, recovery was observed in the ability to generate twitch and tetanus tensions, although not on a

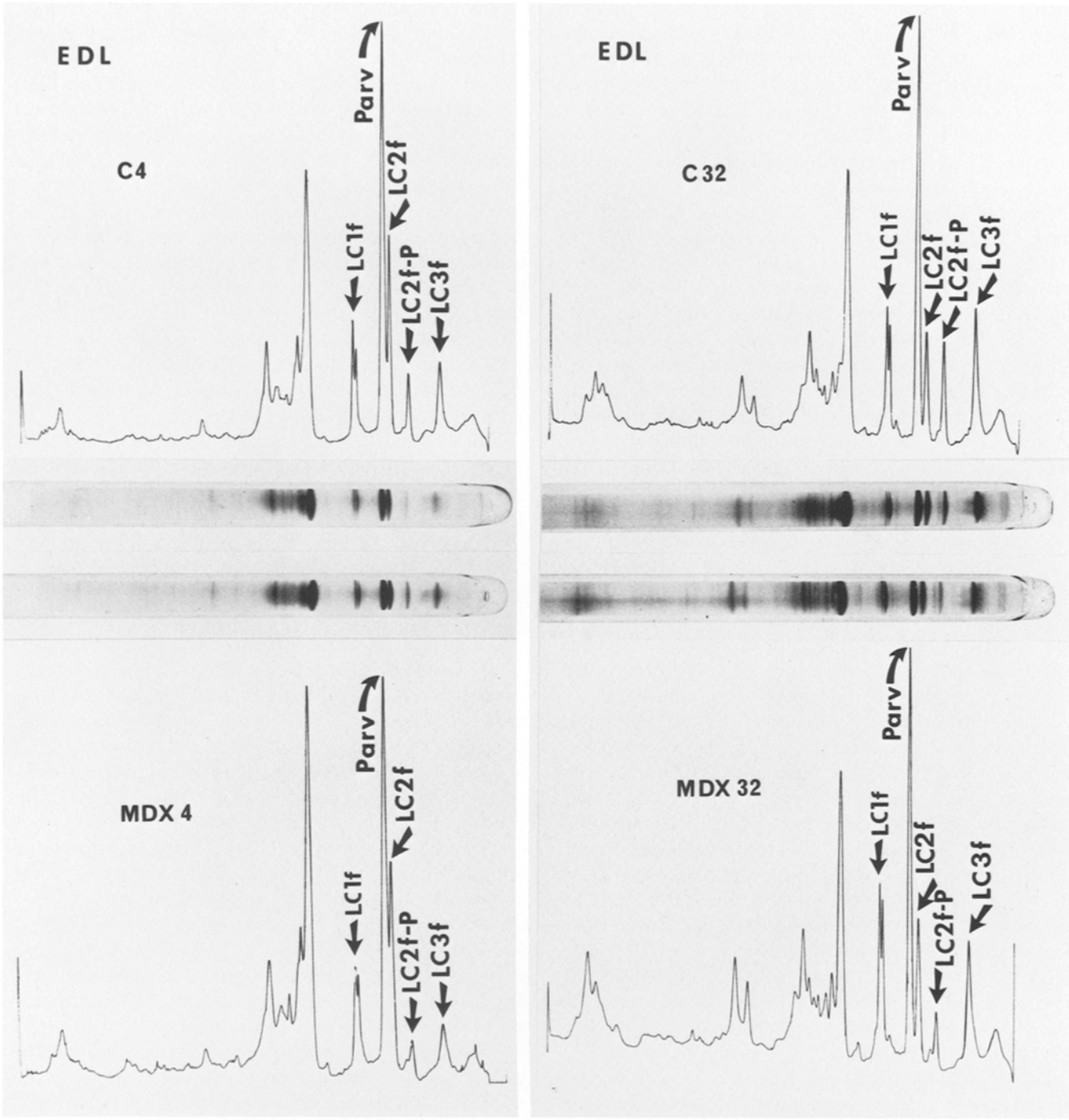

Fig. 9. Representative gels of control and mdx EDL at 4 and 32 weeks of age, with their respective scans. LC1f, LC2f, LC2f-P, LC3f and parvalbumin peaks are indicated.

weight-normalized basis. The TTP also returned to the control values in the 32-week mdx EDL, while the ½RT never varied significantly between mdx and control muscles at either age. The mdx EDL was also fatigued to the same level of relative tension as the control EDL. However, the maximum velocity of shortening in the 32-week mdx EDL was only 64% of the normal speed. In addition, both Sol and EDL had significantly altered myosin light chain content at 32 weeks of age.

The above observations reflect our previous morphological findings of normal (Sol) or above normal

(EDL) numbers of fibres in 32-week mdx muscles (Anderson *et al.*, 1987). In that study, muscles exhibited early focal inflammation and segmental fibre degeneration, from which a spontaneous recovery occurred. By 32 weeks of age, both Sol and EDL weighed more than normal, and were largely populated by regenerated fibres with large central nuclei, but muscles did not contain any notable increase in adipose or connective tissue. The ability of mdx skeletal muscle to recover from injury was clearly demonstrated by using autoradiography. Uptake of tritiated thymidine and labelling of sublaminal nuclei were dramatically higher in mdx muscles than normal at both 4 and 32 weeks. These findings indicated the operation of an ongoing process of fibre regeneration by satellite cell proliferation rather than by fibre recovery and transformation of fibre type. The latter might be deduced from observation of 'atypical' fibres, which have been reported to indicate both fibre regeneration and fibre-type transformation in different pathological (Mastaglia & Kakulas, 1969; Nonaka *et al.*, 1981) and adaptive (Bauman *et al.*, 1987) states. However, as the proportion of 'atypical' fibres was not significantly different between mdx and control for either Sol or EDL, our combined autoradiographic and histochemical studies of mdx muscles precisely identify the distinctive regenerative capacity of mdx muscles.

The ability of the mdx Sol and EDL to regenerate structure and recover function is in sharp contrast to previous reports on other murine dystrophic strains, and on the human Duchenne-type of muscular dystrophy (DMD). In the dy^{2J}/dy^{2J} dystrophic mutant, the Sol generated less tension and exhibited slower speeds of contraction and relaxation in 8 and 12 week age groups, and was less resistant to fatigue than its normal controls (Bressler *et al.*, 1983). These contractile parameters coincided with a lower SO/FOG fibre proportion, in concert with reductions in muscle weight, total fibre number and increased connective tissue content compared to controls (Ovalle *et al.*, 1983).

The dystrophic (dy^{2J}/dy^{2J}) EDL was also abnormal in function in that it generated increased tension normalized to muscle weight but with slowed TTP and ½RT, and was more resistant to fatigue by 32 weeks of age (Bressler *et al.*, 1983). An increased FOG/FG fibre type ratio has been reported for another fast-twitch muscle, the gastrocnemius crown (Silverman & Atwood, 1980).

The dy/dy dystrophic mouse mutant has been shown in experiments *in situ* to have reduced isometric twitch and tetanus tensions for both EDL and Sol, as well as an increase in ½RT in the EDL (Douglas & Baskin, 1971). There was no change in maximum velocity of unloaded shortening of the dystrophic muscle at 35–37° C apart from normal developmental changes from 1 to 12 weeks of age, but loaded shortening was slower in the dy/dy muscles.

Skeletal muscle in DMD is weaker and slower than normal muscle, both clinically *in vivo* (McComas *et al.*, 1971; Edwards *et al.*, 1987) and in single-fibre experiments *in vitro* (Wood *et al.*, 1978). Although there are centrally nucleated myofibres and 'atypically' staining type 2c fibres characteristic of regeneration in human dystrophic muscle, their numbers are unable to keep pace with the ongoing fibre degeneration and accumulation of adipose and connective tissue (Mastaglia & Kakulas, 1969; Mastaglia *et al.*, 1970). This defective recovery is despite a normal proliferative capacity of DMD myoblasts (Hurko *et al.*, 1969) and above normal numbers of myosatellite cells (Wakayama, 1976).

The comparison of mdx skeletal structure and function with that of the dystrophic murine models and with human DMD muscle is made necessary by the relatively recent discovery of the mdx mutant (Bulfield *et al.*, 1984). While descriptions of muscle pathology (Bridges, 1986; Tanabe *et al.*, 1986; Anderson *et al.*, 1987; Torres & Duchen, 1987) generally concur, there is one report of increased SO fibre type proportion in the 26-week Sol (Carnwath & Shotton, 1987), which contradicts observations of fibre mosaics similar to the normal pattern in tibialis anticus (TA), gastrocnemius and Sol in the adult mdx mouse (Dangain & Vrbova, 1984). Torres & Duchen (1987) showed normal numbers, cross-sectional area and myelination of axons in motor nerves of the mdx mouse. Very early (1-day) Z-band streaming and shallow sparse synaptic clefts at motor end-plates were also noted. The only other report of mdx contractile properties (Dangain & Vrbova, 1984) measured *in situ* in TA, showed an early weakness and prolongation of ½RT at 3-4 weeks of age, followed in the adult by normal tension outputs, speeds of contraction and relaxation. Their conclusion of complete recovery of function may, however, by questioned due to the use of a small adult mdx group ($n=3$) with a very large age range (2–12 months) which would involve muscles in various stages of a continuing process of regeneration (Anderson *et al.*, 1987; Tanabe *et al.*, 1986). However, there is a general agreement between their results and those of the present investigation on the large regenerative capacity, both structural and functional, of mdx skeletal muscle.

The reduced phosphorylation of LC2f in the mdx EDL with no change in PTP is in contrast to the temporal association between the time course of twitch potentiation and phosphate incorporation into the P-light chain (LC2f) of fast myosin found in human fast-twitch fibres (Houston *et al.*, 1985). It also varies from the association of decreased PTP and

reduced LC2f-P content in denervated hindlimb muscle (Davis *et al.*, 1988). Our data do agree with findings of Westwood and co-workers (1984) who showed no correlation of PTP and the time course of dephosphorylation in rabbit fast-twitch muscle. However, our assay of myosin light chains in resting muscles would not necessarily be expected to show a correlation between phosphorylation and PTP.

The alteration of myosin light chain proportions in mdx Sol and EDL and the lack of change in parvalbumin content in mdx EDL differ from previous findings on the dy^{2J}/dy^{2J} dystrophic mutant in three ways. First, although LC2s in mdx Sol and LC2f-P and LC3f in mdx EDL were also decreased from normal levels in the dystrophic Sol and EDL respectively (Jasch *et al.*, 1982), that difference was much greater than between mdx and control muscles. Furthermore, the difference is not due to our determination of protein content as proportions of total myosin light chains rather than as proportions relative to total LC2f content (Jasch *et al.*, 1982). Second, dystrophic muscles failed to show normal age-related increases in light chain content, which were observed in mdx Sol and EDL. Third, the lack of a difference in parvalbumin content between mdx and control muscles is in marked contrast to its decrease in the dystrophic EDL, and abnormal appearance in the dystrophic Sol (Jasch *et al.*, 1982; Jasch & Moase, 1985).

The precise function of the myosin light chains is unknown, but their amounts have been taken to indicate a developmental state (Schaub *et al.*, 1986). Thus the reduced contents of particular light chains in mdx Sol and EDL, and the predicted complementary increase from the normal content of another light chain (Syrovy, 1979), may be a marker of some immaturity of structure during regeneration (Strohman & Matsuda, 1983). This immaturity may not affect contractile speed or tension-generating capacity, particularly in the EDL (Julian *et al.*, 1981), although developmental changes in the force/pCa relationship have been reported to occur in mammalian muscle (Kerrick *et al.*, 1976). In addition, the changes in myosin light chain and parvalbumin contents in mdx muscles compared to age-matched controls would seem to exclude denervation effects, seen as reduced parvalbumin and increased ½RT (Muntener *et al.*, 1985; Davis *et al.*, 1988) on mdx muscle pathology.

The decrease in maximum velocity of unloaded shortening in the 32-week mdx EDL could reflect its increased FOG fibre proportion. In that the V_o measured in a whole muscle is a response of the fibres with the highest ATPase activity and lowest phosphorylation of myosin light chains (Fitzsimons & Hoh, 1983; Franks *et al.*, 1984; England, 1984), the slower speed of shortening may also indicate that the regenerated FG fibres, reduced in proportion in the mdx EDL, are not functionally typical, and have an abnormal distribution of myosin light chains.

The fatigue sensitivity of the fast-twitch EDL in control muscles has been maintained by the mdx EDL, as has the fatigue resistance of the mdx Sol. It might be expected that the increase in oxidative fibre proportion in the mdx EDL would increase resistance to fatigue in this muscle. However, the TTP and ½ RT were within the normal fast-twitch range, and parvalbumin content was unchanged, indicating that the mdx EDL maintained its fast-twitch character after recovery.

In conclusion, mdx muscles have been found to recover many contractile features in the context of marked structural regeneration. An increased number of myosatellite cells has been reported in DMD muscle (Wakayama, 1976) and in muscle denervated for 5 weeks (Snow, 1983). As well, the mitotic activity of sublaminal satellite cell nuclei in mdx muscle is greater than normal in both young and old mice (Anderson *et al.*, 1987). Although the number of satellite cells in mdx muscle has not been determined to date, a non-specific increase in satellite cell populations would not adequately explain why mdx muscle is able to regenerate but DMD muscle is not. Indeed, the mdx muscle recovery is remarkable, and is in contrast to the progressive pathology in murine and human muscular dystrophy. This is all the more remarkable in the light of recent reports that the mdx mutant possesses the same protein defect, absence of dystrophin, as does DMD muscle (Hoffman *et al.*, 1987). Two possibilities may explain this apparent discrepancy. First, dystrophin, thought to be the primary product of the gene which is deleted in DMD (Hoffman *et al.*, 1987), may not have the same function in mouse muscle as in human muscle, because the mdx muscles also lack dystrophin but can recover from spontaneous injury. Alternatively, the mdx mutant may have developed a compensatory mechanism which allows it to overcome the defect, and to regenerate muscle tissue, while DMD muscle cannot. It is this second possibility which requires further study, and which makes this mouse model so unique.

Acknowledgements

The authors gratefully acknowledge the expertise of Dr Anthea Rowlerson, and her gift of antibodies to myosin slow and fast isoforms for immunocytochemical fibre typing. We also wish to thank Miss J. McLeod and Mr B. Dales for their excellent technical assistance, and Miss Z. Pound for typing the manuscript. B. H. Bressler holds a grant from the Medical Research Council of Canada and J. E. Anderson is a M.R.C. Postdoctoral Fellow.

References

ANDERSON, J. E., OVALLE, W. K. & BRESSLER, B. H. (1987) Electron microscopic and autoradiographic characterization of hindlimb muscle regeneration in the mdx mouse. *Anat. Rec.* **219**, 243–57.

BAUMANN, H., JAGGI, M., SOLAND, F., HOWALD, H. & SCHAUB, M. C. (1987) Exercise training induces transitions of myosin isoform subunits within histochemically typed human muscle fibres. *Pflügers Arch.* **409**, 349–360.

BRESSLER, B. H., JASCH, L. G., OVALLE, W. K. & SLONECKER, C. E. (1983) Changes in isometric contractile properties of fast-twitch and slow-twitch skeletal muscle of C57BL/6J dy^{2J}/dy^{2J} dystrophic mice during postnatal development. *Expl. Neurol.* **80**, 457–70.

BRIDGES, L. R. (1986) The association of cardiac muscle necrosis and inflammation with the degenerative and persistent myopathy of mdx mice. *J. Neurol. Sci.* **72**, 147–57.

BULFIELD, G., SILLER, W. G., WIGHT, P. A. L. & MOORE, K. J. (1984) X Chromosome-linked muscular dystrophy (mdx) in the mouse. *Proc. natn. Acad. Sci. U.S.A.*, **81**, 1189–92.

CARNWATH, J. W. & SHOTTON, D. M. (1987) Muscular Dystrophy in the mdx mouse: histopathology of the soleus and extensor digitorum longus muscles. *J. Neurol. Sci.* **80**, 39–54.

DANGAIN, J. & VRBOVA, G. (1984) Muscle development in mdx mutant mice. *Muscle Nerve* **7**, 700–4.

DAVIS, H. L., BRESSLER, B. H. & JASCH, L. G. (1988) Myotrophic effects on denervated fast-twitch muscles of mice: correlation of physiological, biochemical and morphological findings. *Expl. Neurol.* **99**, 474–89.

DOUGLAS, W. B. JR & BASKIN, R. J. (1971) Contractile properties of developing mouse dystrophic muscle. *Am. J. Physiol.* **220**, 1344–54.

DUBOWITZ, V. & BROOKE, M. H. (1973) *Muscle Biopsy: A Modern Approach.* Philadelphia: W. B. Saunders.

EDMAN, K. A. P. (1979) The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol.* **291**, 143–59.

EDWARDS, R. H. T., CHAPMAN, S. J., NEWMAN, D. J. & JONES D. A. (1987) Practical analysis of variability of muscle function measurements in Duchenne muscular dystrophy. *Muscle Nerve* **10**, 6–14.

ENGLAND, P. J. (1984) The significance of phosphorylation of myosin light chains in heart. *J. molec. Cell Cardiol.* **16**, 591–5.

FITZSIMONS, R. B. & HOH, J. F. Y. (1983) Myosin isoenzymes in fast-twitch and slow-twitch muscles of normal and dystrophic mice. *J. Physiol.* **343**, 539–50.

FRANKS, K., COOKE, R. & STULL, J. T. (1984) Myosin phosphorylation decreases the ATPase activity of cardiac myofibrills. *J. molec. Cell. Cardiol.* **16**, 597–604.

HARRIS, J. B. & SLATER, C. R. (1980) Animal models: What is their relevance to the pathogenesis of human muscular dystrophy? *Br. Med. Bull.* **36**, 193–7.

HOFFMAN, E. P., KUNDSON, C. M., CAMPELL, K. P. & KUNKEL, L. M. (1987) Subcellular fractionation of dystrophin to the triads of skeletal muscle. *Nature Lond.* **330**, 754–7.

HOUSTON, M. E., GREEN, H. J. & STULL, J. T. (1985) Myosin light chain phosphorylation and isometric twitch potentiation in intact human muscle. *Pflügers Arch.* **403**, 348–52.

HURKO, O., MCKEE, L., ZUURVELD, J. G. E. M., WALSH, F. S. & SWICK, H. M. (1986) Proliferative capacity of Duchenne and wild-type myoblasts derived from a DMD-G6PD double heterozygote. In *Molecular Biology of Muscle Development*, pp. 921–8. New York: Alan R. Liss.

JASCH, L. G., BRESSLER, B. H., OVALLE, W. K. & SLONECKER, C. E. (1982) Abnormal distribution of proteins in the soleus and extensor digitorum longus of dystrophic mice. *Muscle Nerve* **5**, 462–70.

JASCH, L. G. & MOASE, E. H. (1985) Evidence for the identities of five proteins decreased in skeletal muscle of dystrophic mice. *Muscle Nerve* **8**, 389–401.

JONES, S. P., RIDGE, R. M. A. P. & ROWLERSON, A. (1987) The non-selective innervation of muscle fibres and mixed composition of motor units in a muscle of neonatal rat. *J. Physiol.* **386**, 377–94.

JULIAN, F. J., MOSS, R. L. & WALLER, G. S. (1981) Mechanical properties and myosin light chain composition of skinned muscle fibres from adult and new born rabbits. *J. Physiol.* **311**, 201–18.

KERRICK, W. G. L., SECRIST, D., COBY, R. & LUCAS, S. (1976) Development of difference between red and white muscles in sensitivity to Ca^{2+} in the rabbit from embryo to adult. *Nature, Lond.* **260**, 440–1.

MCCOMAS, A. J., SICA, R. E. P. & CURRIE, S. (1971) An electrophysiological study of Duchenne dystrophy. *J. Neurol. Neurosurg. Psychol.* **34**, 461–8.

MASCARELLO, F., CARPENE, E., VEGGETTI, A., ROWLERSON, A. & JENNY, E. (1982) The tensor tympani muscle of cat and dog contains IIM and slow-tonic fibres: an unusual combination of fibre types. *J. Musc. Res. Cell Motility* **3**, 363–74.

MASCARELLO, F., ROWLERSON, A. & SCAPOLO, P. A. (1984) The fibre type composition of the striated muscle of the oesophagus in ruminants and carnivores. *Histochemistry* **80**, 277–88.

MASTAGLIA, F. L. & KAKULAS, B. A. (1969) Regeneration in Duchenne muscular dystrophy: a histological and histochemical study. *Brain* **92**, 809–18.

MASTAGLIA, F. L., PAPADIMITRIOU, J. M. & KAKULAS, B. A. (1970) Regeneration of muscle in Duchenne muscular dystrophy: an electron microscope study. *J. Neurol. Sci.* **11**, 425–44.

MUNTENER, M., BERCHTOLD, M. W. & HEIZMANN, C. W. (1985) Parvalbumin in cross-reinnervated and denervated muscles. *Muscle Nerve* **8**, 132–7.

NONAKA, I., TAKAGI, A. & SUGITA, H. (1981) The significance of type 2c muscle fibres in Duchenne muscular dystrophy. *Muscle Nerve* **4**, 326–33.

OVALLE, W. K., BRESSLER, B. H., JASCH, L. G. & SLONECKER, C. E. (1983) Abnormal distribution of fiber types in the slow-twitch soleus muscle of the

C57BL/6J dy^{2J}/dy^{2J} dystrophic mouse during postnatal development. *Am. J. Anat.* **168**, 291–304.

PETER, J. B., BARNARD, R. J., EDGERTON, V. R., GILLESPIE, C. A. & STEMPEL, K. E. (1972) Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **11**, 2627–33.

REDENBACH, D. M. & BRESSLER, B. H. (1988) Alterations in contractile properties of extensor digitorum longus muscle from C57BL/6J mice following neonatal denervation at one day of age. *Expl Neurol.* **100**, 542–55.

ROWLAND, L. P. & LAYZER, R. B. (1979) X-linked muscular dystrophies. In *Handbook of Clinical Neurology. Diseases of Muscle* (edited by VINKEN, P. J. & BRUYN, G. W.), Vol. 46, pp. 349–414. New York: North-Holland.

ROWLERSON, A., POPE, B., MURRAY, J., WHALEN, R. B. & WEEDS, A. G. (1981) A novel myosin present in the cat jaw-closing muscles. *J. Musc. Res. Cell Motility* **2**, 415–38.

SCHAUB, M. C., JAUCH, A., WALZTHOENY, D. & WALLIMANN, T. (1986) Myosin light chain functions. *Biomed. Biochim. Acta* **45**, S39–S44.

SILVERMAN, H. & ATWOOD, H. L. (1980) Increase in oxidative capacity of muscle fibres in dystrophic mice and correlation with overactivity in these fibers. *Expl Neurol.* **68**, 97–113.

SNOW, M. H. (1983) A quantitative ultrastructural analysis of satellite cells in denervated fast and slow muscles of the mouse. *Anat. Rec.* **207**, 593–604.

STROHMAN, R. C. & MATSUDA, R. (1983) Myosin expression during regeneration and in denervated skeletal muscle. *Adv. Exp. Med. Biol.* **182**, 259–63.

SYROVY, I. (1979) Changes in light chains of myosin during animal development. *Int. J. Biochem.* **10**, 223–7.

TANABE, Y., ESAKI, K. & NOMURA, T. (1986) Skeletal muscle pathology in X-chromosome-linked muscular dystrophy (mdx) mouse. *Acta Neuropathol. (Berlin)* **69**, 91–5.

TORRES, L. F. B. & DUCHEN, L. W. (1987) The mutant *mdx*: Inherited myopathy in the mouse. *Brain* **110**, 269–99.

WAKAYAMA, Y. (1976) Electron microscopic study on the satellite cell in the muscle of Duchenne muscular dystrophy. *J. Neuropathol. Expl. Neurol.* **35**, 532–40.

WEEDS, A. G. (1976) Light chains from slow-twitch muscle myosin. *Eur. J. Biochem.* **66**, 157–73.

WESTWOOD, S. A., HUDLICKA, O. & PERRY, S. V. (1984) Phosphorylation *in vivo* of the light chain of myosin in rabbit fast and slow skeletal muscles. *Biochem. J.* **218**, 841–7.

WOOD, D. S., SORENSON, M. M., EASTWOOD, A. B., CHARASH, W. E. & REUBEN, J. P. (1978) Duchenne dystrophy: Abnormal generation of tension and Ca^{++} regulation in single skinned fibres. *Neurology* **28**, 447–57.