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Skeletal Muscle Pathology in X Chromosome-linked Muscular Dystrophy (*mdx*) Mouse

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Summary. Histological, histochemical, and morphometric analyses were performed chronologically on muscles from mutant mice with X chromosome-linked muscular dystrophy (*mdx*), and the findings were compared with those in nondystrophic control animals (C57BL/10ScSn). Massive grouped muscle fiber destruction, followed by complete regeneration, occurred abruptly at 20 days of age. There were no preceding changes in body weight, the number and mean diameter of fibers, and fiber type differentiation before the initial episode of muscle fiber necrosis. Muscle fiber necrosis decreased in intensity after 60 days of age. Even after repeated muscle fiber necrosis and regeneration, the most striking finding was that interstitial fibrosis and adipose tissue replacement were minimal, and there was no apparent fiber loss. Since the necrosis was probably well compensated by the active regenerative process, the *mdx* mice developed no obvious muscle weakness and thus differed from human and other animal muscular dystrophies with the exception of the dystrophic hamster.

Key words: *mdx* Mouse – Muscle pathology – Muscular dystrophy – Regeneration

Introduction

To date numerous descriptions of the pathology of human muscular dystrophy have appeared. However, the ultimate pathogenesis and effective treatment of this disease are not yet established. Several experimental dystrophic animals, such as the mouse (Michelson et al. 1955), chicken (Asmundson et al. 1966), and hamster (Homburger et al. 1962) have provided possible models for the study of muscular dystrophy.

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Recently, a new mutant mouse inherited through an X linked-recessive trait (*mdx* mice) was discovered in the C57BL/10ScSn strain (Bulfield et al. 1984). Since even female homozygotes and male hemizygotes have minimal muscle weakness and an ability to mate, both male and female dystrophic offsprings can be obtained with no difficulty from dystrophic parents. In these mice, the skeletal muscle pathology was documented to consist of degenerative and regenerative changes accompanied by numerous centronucleated fibers, similar to those in human muscular dystrophy (Bulfield et al. 1984).

Later on, Dangain and Vrbova (1984) reported that massive muscle degeneration occurred abruptly in 3–4 week-old animals and was followed by complete regeneration. However, it remained unclear whether abnormal muscle fiber growth and type differentiation as seen in the dystrophic chicken (Ashmore and Doerr 1971; Nonaka and Nakamura 1982) and mouse (Wirtz et al. 1983), preceded the necrotic episode in these mutant mice.

We, therefore, recorded the body weight, clinical symptoms, and skeletal muscle histopathology of the mice in a chronologic sequence.

Materials and Methods

For each observation of the body weight and clinical symptoms, ten *mdx* and ten control (C57BL/10ScSn) mice were studied at 0, 5, 10, 15, 20, 30, 60, 90, 120, and 180 days, respectively, after birth.

For histological evaluation, both *mdx* and control mice were killed by i.p. pentobarbital administration, and bilateral extensor digitorum longus (EDL), soleus and tibialis anterior muscles were taken from the hind limbs. At 30 and 180 days, additional muscles, including rectus femoris, biceps brachii, pectoralis and diaphragm muscles, were examined. Muscles from the right limbs were frozen immediately in isopentane cooled by liquid nitrogen and serial transverse sections were stained by hematoxylin and eosin (HE), modified Gomori trichrome, and a battery of histochemical techniques (Dubowitz and Brooke 1973).

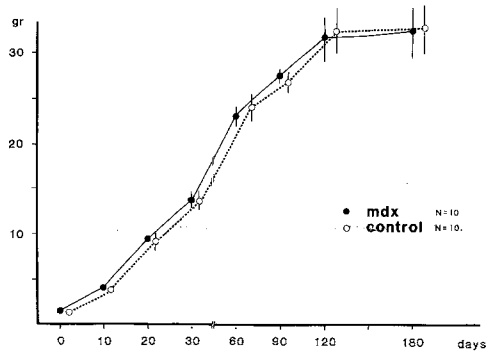

Fig. 1. Body weight in *mdx* and control mice at different ages

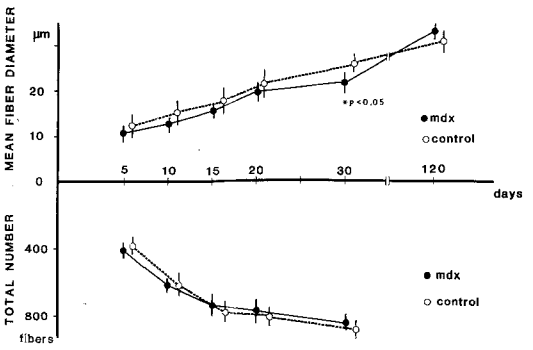

Fig. 2. Morphometric analysis of soleus muscle during early development. The *vertical bars* represent the SE (mean)

The entire left soleus muscle was fixed in cacodylate-buffered glutaraldehyde solution, rinsed in the same buffer solution, postfixed in s-collidine-buffered osmium tetroxide containing lanthanum nitrate, dehydrated in ethanol, and embedded in epoxy resin. From each animal 1-μm-thick sections were cut and stained with toluidine blue, and photographed at a final magnification of ×1,000. The total number and diameter of muscle fibers in the mid-portion of soleus muscle were measured with a MOP-AMO3 semi-automatic image analyzer (Kontron Co., Munich, FRG). The incidence of the centronucleated muscle fibers was estimated from 300–400 muscle fibers in both soleus and EDL muscles stained with HE.

Results

Body Weight and Clinical Symptoms

The *mdx* mice gained body weight at equal tempo to the controls with no statistical difference from birth to 180 days (Fig. 1). Up to 180 days, *mdx* mice moved around in the same manner as did the controls with no visible muscle weakness and atrophy.

Morphometric Analysis in Soleus Muscle in Early Developmental Stage

In the mid-portion of the control soleus muscles, the total number of muscle fibers of 390 ± 68 (mean ± SD) at 5 days of age were gradually increased to 779 ± 116 at 30 days of age. The number of muscle fibers in the *mdx* mice increased equally to that in controls with no statistical difference (Fig. 2). Although the size of muscle fibers in *mdx* mice did not differ from the control up to 20 days of age, the muscle fibers in the former were smaller in caliber (21.8 ± 0.8 μm) (mean ± SD) as compared with controls (24.1 ± 0.9 μm) at 30 days of age ($p < 0.05$).

Pathologic Alterations in Dystrophic Muscles with Development

From birth to 5 days of age, muscle fibers appeared to be immature: there was no clear fiber type differentiation on NADH-TR and ATPase staining, and there were clusters of fibers with vesicular nuclei and basophilic cytoplasm with no distinct cellular border. However, these findings were not different from those seen in control animals.

At 10–15 days, the muscle fibers began to differentiate into type 1 and 2 fibers in both dystrophic and control animals. On ATPase staining, the soleus consisted of both type 1 and 2 fibers and EDL mostly type 2 fibers with only a few type 1 fibers. There was no difference in fiber type distribution between dystrophic and control muscles. Up to 15 days of age, no apparent fiber necrosis was seen in either soleus or EDL muscles, except for one soleus muscle where some muscle fibers underwent necrosis.

At 20 days, muscle fiber necrosis was found in both the soleus and EDL muscles in all *mdx* mice. Clusters of tens to hundreds of muscle fibers underwent necrosis simultaneously. In the early necrotic areas, there were scattered opaque (hypercontracted) fibers which were occasionally stained positively with GBHA to demonstrate calcium ions. In the advanced necrotic areas, the fibers were invaded by acid-phosphatase positive phagocytes showing massive mononuclear cell infiltration in large areas (Fig. 3). Apart from the areas of fiber necrosis, the muscle fibers appeared normal with no variation in fiber size, no centronucleated fiber and normal distribution of well differentiated muscle fiber types.

At 30 days, the muscles showed far advanced morphological changes. There were small to large groups of regenerating fibers which had basophilic cytoplasm, centrally placed vesicular nuclei with occasional prominent nucleoli, and were positive to acid-phosphatase. Soleus and EDL muscles were almost equally involved, and there was no sexual difference in the severity of muscle involvement.

At 60 days, more than half of the muscle fibers had centrally placed nuclei. Scattered fibers appeared hypertrophic and some showed fiber splitting. The findings seen at 90 days were quite similar to those at 60 days.

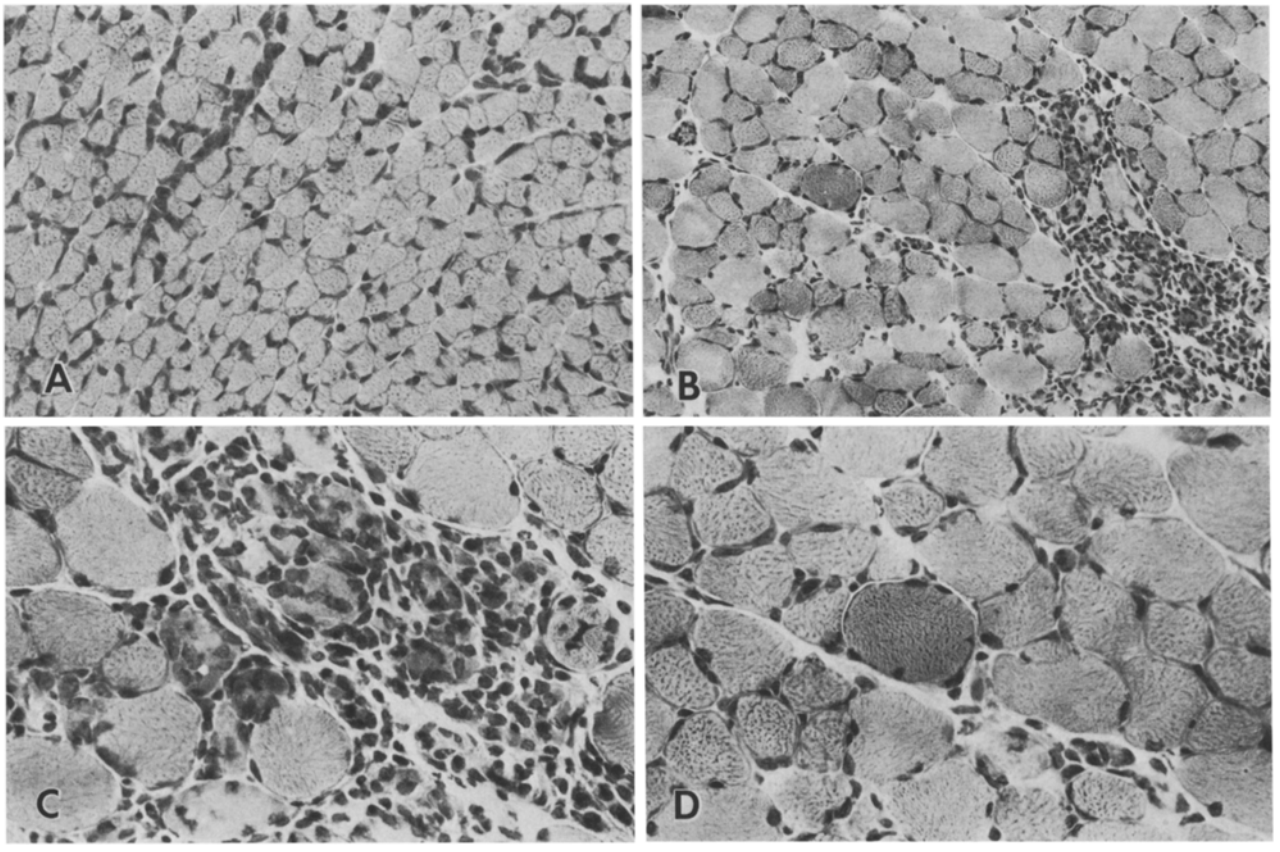

Fig. 3 A – D. Chronological alterations in tibialis anterior muscles from *mdx* mice at the ages of 5 days (**A**) and 20 days (**B – D**). Note that massive destruction of groups of muscle fibers occurs at 20 days (**B, C**) with occasional opaque (hypercontracted) fibers (**D**). **A – D** HE; **A, C, D** ×400; **B** ×200

At 120 and 180 days, almost all muscle fibers had centrally placed nuclei. Even at 180 days of age, there was no apparent perimysial or endomysial fibrosis. No adipose tissue replacement was identifiable. There was no quantitative or qualitative difference in the muscle pathology between EDL, soleus, rectus femoris, biceps brachii, pectoralis, and diaphragm muscles taken at 30 and 180 days (Fig. 4). The cardiac muscle was devoid of pathologic lesion. The incidence of centronucleated fibers at the various ages is illustrated in Fig. 5. The muscle pathology is summarized in Fig. 6.

Discussion

The most striking morphological feature in the skeletal muscles from *mdx* mouse is a massive muscle fiber destruction which begins abruptly at a critical time of 20 days of age followed by rapid regeneration. Since regenerating fibers possess centrally placed nuclei, the prevalence of centronucleated muscle fiber is thought to be an appropriate cumulative index of muscle regeneration (Karpati 1979). The centronucleated muscle fibers began to increase in number at 30 days of age and constituted almost all the muscle fibers around 120 days. Thus, we assume that at least one necrotic episode occurs in almost all fibers by 120 days after birth. Despite the presence of active fiber necrosis, the *mdx* mouse had no apparent progressive muscle weakness or atrophy. Since the regenerating fibers in groups are of uniform size and interstitial fibrosis is scant, the regenerative process may be complete and compensate for the muscle fiber degeneration.

The morphometric and histochemical analyses indicate that there is no significant difference in muscle fiber growth and fiber type differentiation between control and *mdx* mouse up to 20 days of age when massive muscle necrosis occurs. It is of great interest that such a massive muscle destruction occurs abruptly and simultaneously in various muscles without pre-existing abnormal fiber growth and differentiation such as have been identified in the dystrophic chicken (Nonaka and Sugita 1981) and in human muscular dystrophy (Mastaglia et al. 1970). The first question which arises is why such a massive muscle destruction is recognizable only at such a criti-

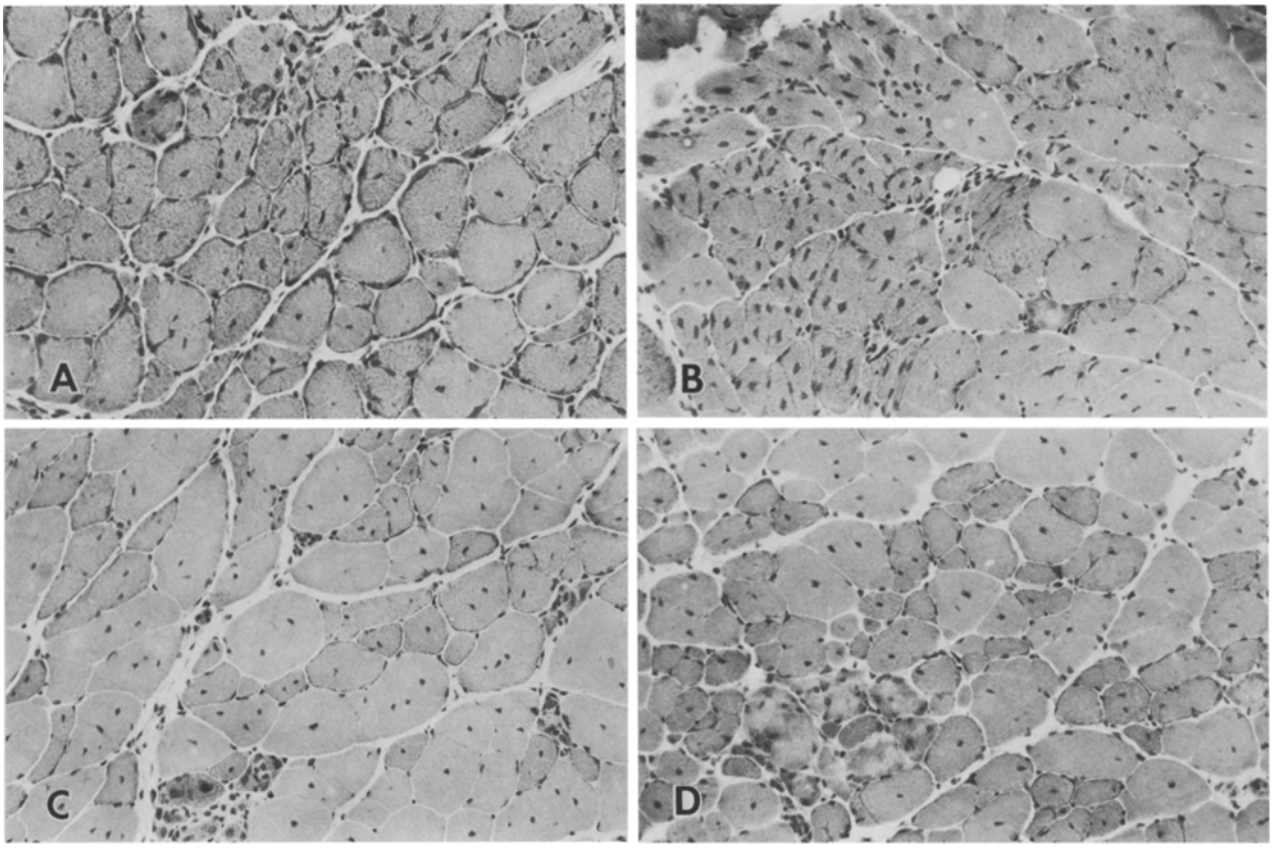

Fig. 4A–D. Cross sections of 180-day-old soleus (**A**), extensor digitorum longus (**B**), rectus femoris (**C**), and biceps brachii (**D**) muscles from *mdx* mice. Note the presence of necrotic and regenerating fibers in large groups with numerous centronucleated fibers in all muscles. **A–D** HE, ×200

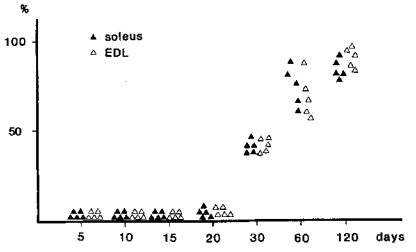

Fig. 5. The incidence of centronucleated fibers in soleus and EDL muscles at various ages

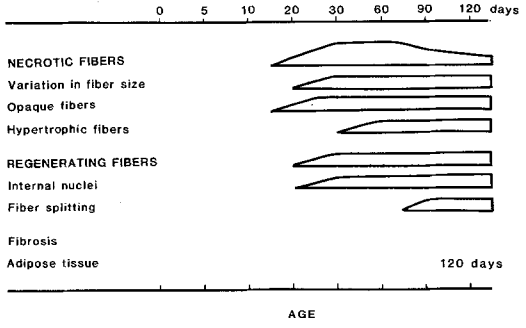

Fig. 6. The muscle pathology of *mdx* mouse in chronologic sequence

cal time, at 15–20 days of age. It has been evidenced that a certain degree of maturity in structural and chemical properties are required for the genetic expression of a dystrophic process, i.e., muscle fiber necrosis (Karpati et al. 1982). In other words, the immature fibers during development and early regenerating fibers after myonecrosis are assumed to be resistant to the intrinsic and extrinsic factors which damage the membrane system.

In Duchenne muscular dystrophy, most of the hypercontracted (opaque) fibers, which are probably a prelude to fiber necrosis, are well differentiated, and the undifferentiated type 2C fibers very rarely undergo fiber necrosis (Nonaka et al. 1981). Accordingly, it can be easily appreciated that the extent and incidence of fiber necrosis in the *mdx* mouse is reduced after 120 days of age because almost all muscle fibers have already experienced necrosis by 90–120 days of age, and most fibers are undergoing regeneration. When these regenerating fibers have matured, they again are prey to degeneration and undergo necrosis, but to a lesser extent as compared with those in the initial attack.

The skeletal muscle pathology in the dystrophic mouse (C57BL/6J-*dy*) inherited through an autosomal recessive trait (*dy* mouse) is distinguishable from that in the *mdx* mouse; the necrotic and regenerating process occurs in groups in the *mdx* mouse, but scatteringly in the *dy* mouse. An intensive fibrous tissue proliferation and adipose tissue replacement occur in the *dy* mouse as seen in human muscular dystrophy. Therefore, a comparative histological study of *dy* and *mdx* mouse may provide important information as to why muscle regeneration does not compensate for fiber necrosis in human muscular dystrophies, especially in Duchenne muscular dystrophy.

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