Mechanical Damage and Inflammation of Skeletal Muscles in the Disease Course of Muscular Dystrophic Mice

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Abstract

Muscular dystrophy is generally believed to be a disease characterized by degeneration of skeletal muscle fibers. Along this line of thinking, three types of "degeneration" theories for explaining the pathogenesis are now popular. Nevertheless, unfortunately, an effective treatment for this disease is still unknown, suggesting that the etiology might be misunderstood from the fundamental point of view. Accordingly, as a challenge to the degeneration theories, we previously proposed a muscle "defective maturation (growth)" theory and a further "bone-muscle growth imbalance" hypothesis based on our findings in hereditary muscular dystrophic *dy* mice (C57BL/6J *dy/dy*). The *dy* mice appear to suffer from a defect in a possible mechanism of bone-growth-induced muscle growth. Stretching-contraction-induced damage followed by repair, instead of degeneration-regeneration in the degeneration theories, would frequently occur locally along dystrophic muscle fibers on movement, causing overinflammation especially during growth periods: Some fibers damaged beyond repair would degenerate and drop away. Thus, our hypothesis implies a taboo (intensive exercise) and two possible symptomatic treatments (bone- or body-growth inhibitors and antiinflammatory drugs). This report shows schematically and experimentally that some of the pathological features characteristic of muscular dystrophy seem to be consistent with the bone-muscle imbalance hypothesis, but not with the degeneration theories.

Key Words: Bone, growth, inflammation, maturation, muscle, muscular dystrophy, myositis.

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Pathogenesis and Degeneration Theories

Schematic explanations for the pathogenesis of muscular dystrophy by popular degeneration theories (see reviews [9, 19, 33]) and the bone-muscle imbalance hypothesis [42-47, 56, 57] are presented in Fig. 1. According to the two degeneration theories (A and B in Fig. 1), pathological phenomena characteristic of muscular dystrophy, centronucleation and fiber-size variation (Fig. 2a), are thought to result from hypertrophy, degeneration and impaired regeneration of fibers; i.e., the regenerated fibers (or segments) are vaguely considered to remain immature, small-calibered and centronucleated. This means that the two phenomena must always occur simultaneously. However, as shown in Fig. 2b, pathological foci with marked fiber-size variation and few central nuclei are often observed. It is also noted that there are centronucleated fibers of normal or even large caliber (Fig. 2a). In this connection, Harman et al. [14] demonstrated previously that centronucleated fibers in *dy* muscles exhibited all degrees of staining and size variation.

The other degeneration theory (C in Fig. 1) was proposed to explain the pathogenesis of the other dystrophic *mdx* mice (C57BL/10 *mdx/mdx* or *mdx*/Y)[3, 40], stating that segmental degeneration of fibers causing dystrophic symptoms is prominent in muscles of *mdx* mice transiently around the growth period from 1 to 2 months and that almost complete regeneration occurs at the degenerated segments of fibers. In addition, in order to rationalize the

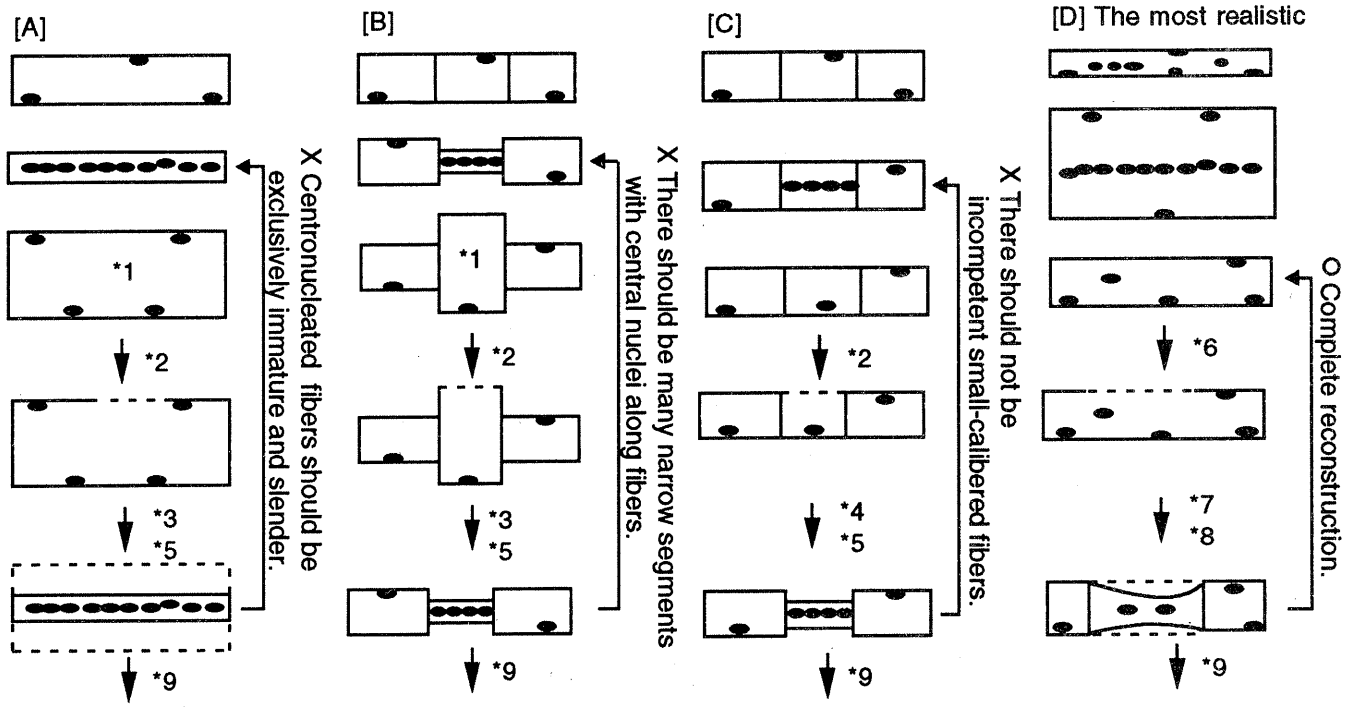

*Figure 1. Schematic explanations for the pathogenesis of dystrophic muscle fibers. [A] Muscle degeneration theory 1: Hypertrophy, degeneration, and impaired regeneration over the whole length of muscle fibers result in centronucleation and size variation of fibers. [B] Muscle degeneration theory 2: Segmental hypertrophy, degeneration, and impaired regeneration result in centronucleation and size variation of fibers. [C] Muscle degeneration theory 3 (*mdx *mice): Segmental degeneration and complete regeneration with central nuclei result in size variation of fibers. [D] Bone-muscle imbalance hypothesis: Defective maturation (growth) of fibers results in age-related aggravation of bone-muscle imbalance which is responsible for the secondary pathogenesis and progress of the disease. Fiber-size variation is the result of a variation in growth capability of fibers greatly shifted toward incompetence: Most fibers fail to grow but some of them grow almost normally.*

observation that adult *mdx* mice are not lean but apparently rather well-built, the regenerated fibers are conveniently presumed to be resistant to re-degeneration. If this were true, neither fiber-size variation (see Fig. 3c) nor muscle weakness [57] should remain in adult *mdx* mice.

Recently we showed that the frequency of centronucleated fibers with ordinary diameters increased suddenly after about 30 days of age when violent pathological changes (inflammation) became first observable in *mdx* muscles, indicating that the centronucleated fibers could not be the regenerating fibers, which at first should be extremely slender [57]. Williams et al. [65] also suggested that fibers with central nuclei are not necessarily regenerating fibers. One will notice in Fig. 3 (compare b with c) that with age, the number of central nuclei per cross-sectional area of fibers increases with normal and even large diameters, supporting our previous conclusion [57] that central nuclei instantaneously appear in fibers with ordinary calibers. If fibers with 2 central nuclei per cross-sectional area had experienced two degeneration-regeneration cycles, they should be much more slender compared with the other non-centronucleated fibers.

Finally, it must be pointed out that the two degeneration theories (A and B in Fig. 1) contain at least three etiological problems which appear to have no cause-effect relation with one another: 1. Why dystrophic fibers are paradoxically hypertrophied. 2. Why they are degenerated (atrophied). 3. Why the regeneration of degenerated dystrophic fibers is incomplete. Also, the other degeneration theory (C in Fig. 1) includes the contradictory problem of why the regenerated fibers can escape from re-degeneration in spite of the fact that natural *mdx* fibers degenerate. The degeneration theories seem to leave these fundamental points practically untouched.

Unreal Segmental Degeneration

Since the regeneration of fibers is thought to be unlikely to occur along the whole length of very long muscles (A in Fig. 1), recently, degeneration-(impaired) regeneration has generally been believed to take place at segments of

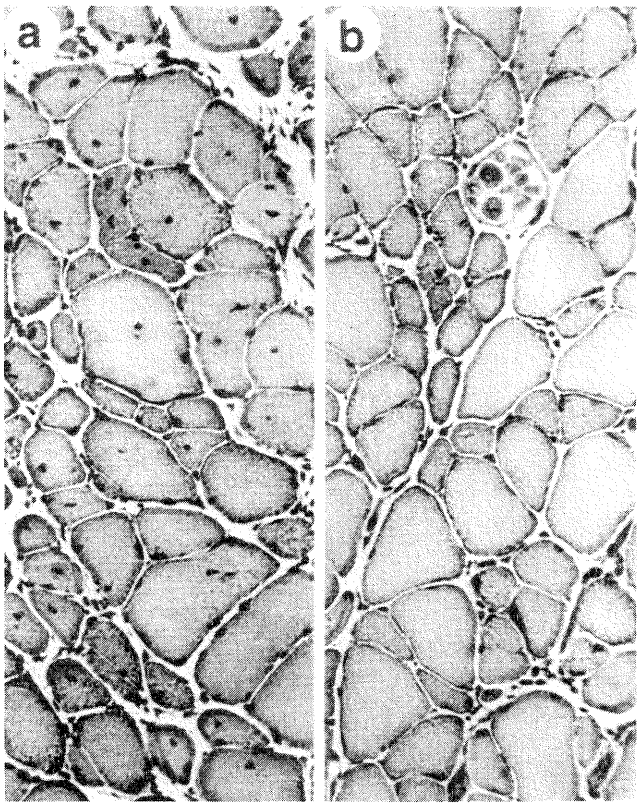

Figure 2. Increased size-variation of dy *muscle fibers with or without central nuclei. Two representative pathological foci (a, b) in a cross cryo-section of the antebrachial muscle of a 5-month-old* dy *mouse are shown. While size-variation is obvious both in a and b, centronucleation is prominent only in a but not in b, indicating that the two phenomena do not always occur simultaneously. Staining: Hematoxylin. Scale bar: 0.1 mm.*

fibers (B and C in Fig. 1). If this were true, rugged features (narrow parts with central nuclei) should be frequently observed along fibers (Fig. 4). Our findings thus far indicate that segmental degeneration-regeneration must be fictitious. In this regard, it is noteworthy that the entity for segments of skeletal muscle fibers is as yet obscure.

Bone-Muscle Imbalance Hypothesis

This hypothesis for explaining the pathogenesis in *dy* mice is composed of two phases: 1. The early pathosis is a defective maturation (growth) of muscle fibers [10, 37, 39, 61]. 2. Age-related aggravation of imbalance between growth-arrested muscles and growing bones plays a major role in the subsequent process [42-58, 61-63]. Actually, in contrast to devastated skeletal muscles in *dy* mice, striated muscles of tongue [12, 52], esophagus, ear [56] and nose [57], whether normal or dystrophic, which were not influenced by bone growth were almost free of pathological changes. Table 1 shows that the mean and SD/mean for muscle fiber diameter are normal in the nose but abnormal in the foreleg of *dy* mice. The SD/mean value indicates the degree of fiber-size variation. We have previously demonstrated that the abnormally increased variation in the diameter of skeletal muscle fibers in *dy* mice is attributable to their variation in growth capability greatly shifted toward incompetence: Some fibers grow normally but most of them fail to thrive [50, 51, 56].

Consistent with this hypothesis, various interesting facts have already been reported on man [5, 27, 35, 67-71] and animals [1, 2, 6, 8, 15, 20, 22, 23, 25, 26, 32, 59, 66] with myopathies by many authors (see our recent reviews [56, 57]). An increasing number of reports have emphasized the important role of mechanical injury [28, 64] in the disease process, relating to a possible physiological function [30, 36] of dystrophin [16, 38]. In some myopathies or senility, the disturbance of homeostasis, e.g., resulting in an imbalance between muscle and connective tissues, may be responsible for the pathogenesis. Changes in the hormonal milieu would alter the dystrophic course [24]. Here we must state that it remains to be revealed as to what degree the bone-muscle imbalance hypothesis can be applied to the pathogenesis of *mdx* mice.

Inflammation of Skeletal Muscles

According to the bone-muscle imbalance hypothesis, we have speculated that muscular dystrophy by nature may be accompanied with myositis caused by mechanical damage of skeletal muscles extraordinarily stretched transiently during growth periods. Actually, inflammation of muscles is observed in *dy* mice (Figs. 5 and 6) as well as in *mdx* mice (Figs. 3b and 7) [57] almost exclusively during the postnatal period around 1 to 2 months. During this period, mice grow rapidly in body weight (Fig. 8) [41]. Incidentally, Kasuga and Umazume [21] reported that the sar-

Table 1. Diameter of muscle fibers in noses and forelegs of dystrophic mice.

Mouse		Diameter* μm Nose	Foreleg
Normal	32 days	11.4 ± 0.16 (150)	30.7 ± 0.25 (202)
Dystrophic	32 days	11.3 ± 0.20 (122)	20.1 ± 0.46 (414)
Normal	6 months	12.7 ± 0.17 (137)	35.0 ± 0.22 (164)
Dystrophic	6 months	13.2 ± 0.20 (112)	24.5 ± 0.54 (245)

*Mean ± SD/mean (the number of analyzed fibers).

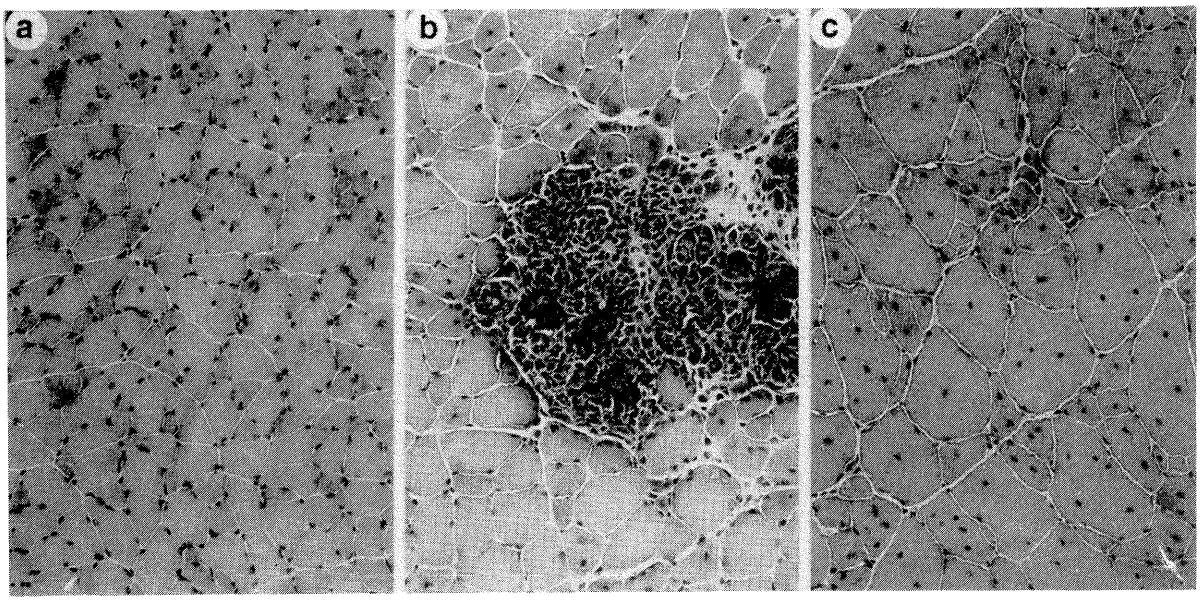

Figure 3. Inflammation of mdx *muscles. Foreleg triceps muscles from 25 (a), 40 (b) and 120-day-old (c)* mdx *mice were transversely cryo-sectioned and stained with hematoxylin. An inflammatory focus is seen in b, but not in a or c. Scale bar: 0.1 mm.*

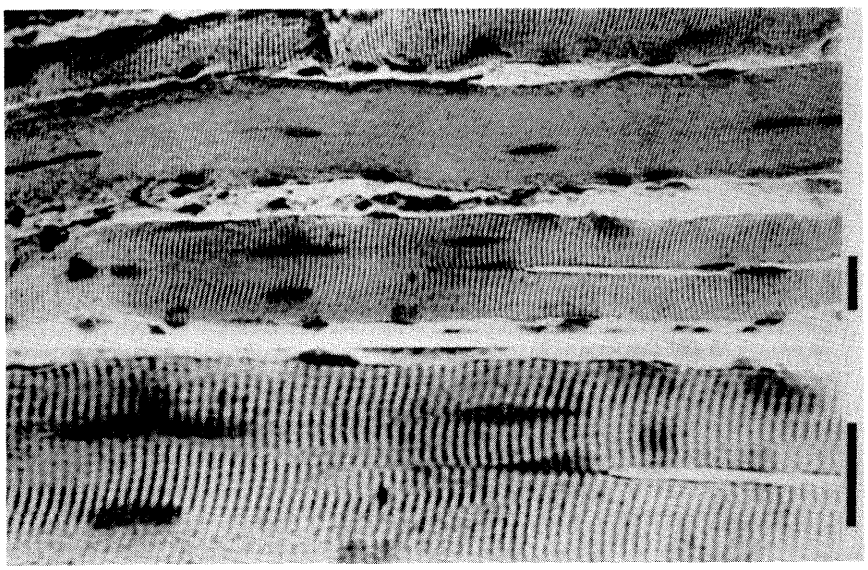

Figure 4. Central nuclei and fiber splitting in a longitudinal section of dy *muscle. Femoral muscles from a 4-month-old* dy *mouse were cryo-sectioned and stained for succinate dehydrogenase activity and with hematoxylin. Note that not rugged but smooth longitudinal features of fibers are obvious through parts with central nuclei. Scale bars: 20 μm.*

comere was stretched when muscle fibers of normal mice rapidly grew lengthwise.

It is known that there are some patients with muscular dystrophy who are suspected of having myositis from the biopsy of their muscles. These patients are diagnosed as having muscular dystrophy according to their clinical courses and family histories. Interestingly, some physical therapists say that muscles of patients with muscular dystrophy become feverish, i.e., inflammatory after undergoing strenuous sports activities (personal

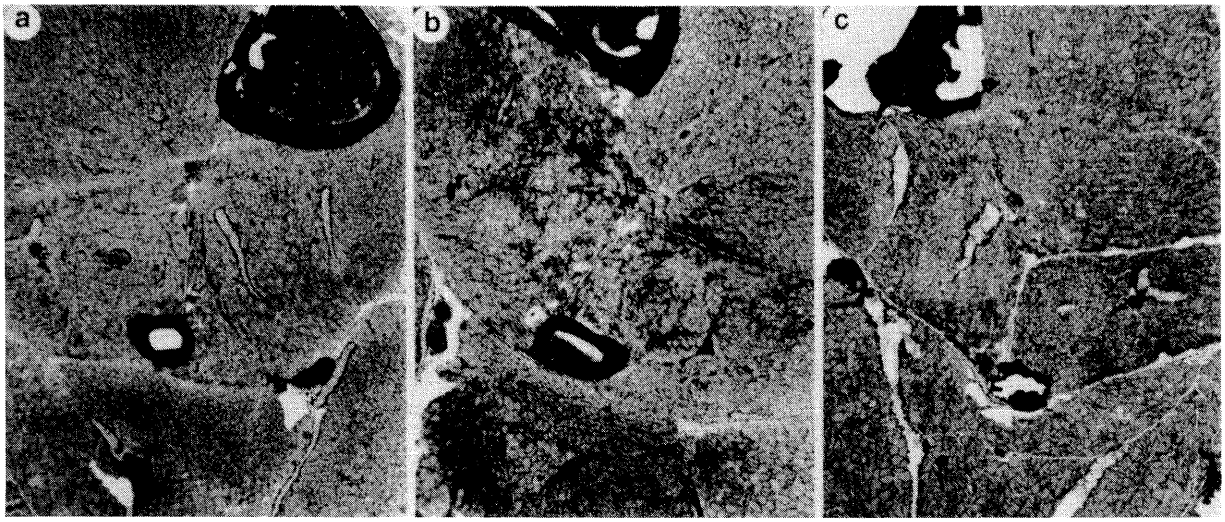

Figure 5. Inflammation of dy *hindleg muscles. Crura, bone and all tissue, of 21 (a), 27 (b) and 96-day-old (c)* dy *mice were transversely cryo-sectioned and stained with hematoxylin. Inflammation of muscles is seen in places of b, but hardly in a or c. Scale bar: 0.5 mm.*

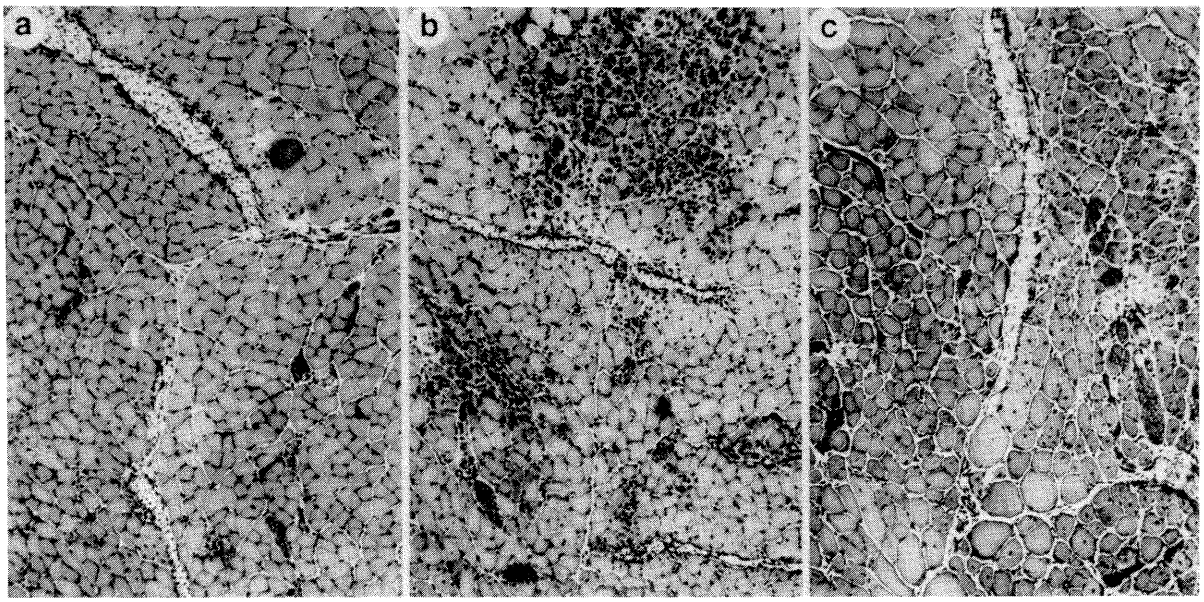

Figure 6. Inflammation of dy *foreleg muscles. Antebrachia, bone and all tissue, of 21 (a), 27 (b) and 96-day-old (c)* dy *mice were transversely cryo-sectioned and stained with hematoxylin. Inflammation of muscles is prominent in b, but not in a or c. Some investigators generally believe that the regenerated fibers characterized by having central nuclei would hardly re-degenerate, judging from the fact that the frequency of centronucleated fibers tends to increase with age or with the advance of the disease in dystrophic muscles. Contrary to this assumption, one can see a darkly-stained fiber, so-called an opaque fiber which is thought previously to have started degenerating but now to be rather an artifact, with a central nucleus around the upper 1/3 part of a. Scale bar: 0.2 mm.*

communication). Mukoyama et al. [34] reported two cases of polymyositis with muscle weakness and atrophy of the facioscapulohumeral type who showed moderate improvement of muscle weakness and lowering of serum CK level to the normal range with administration of prednisolone. There are several reports indicating that steroid therapy is effective in animal models [17] and cases of Duchenne muscular dystrophy [11, 13, 29].

Splitting and/or Fusion of Fibers

Fiber splitting (Fig. 4) is frequently observed in dystro-

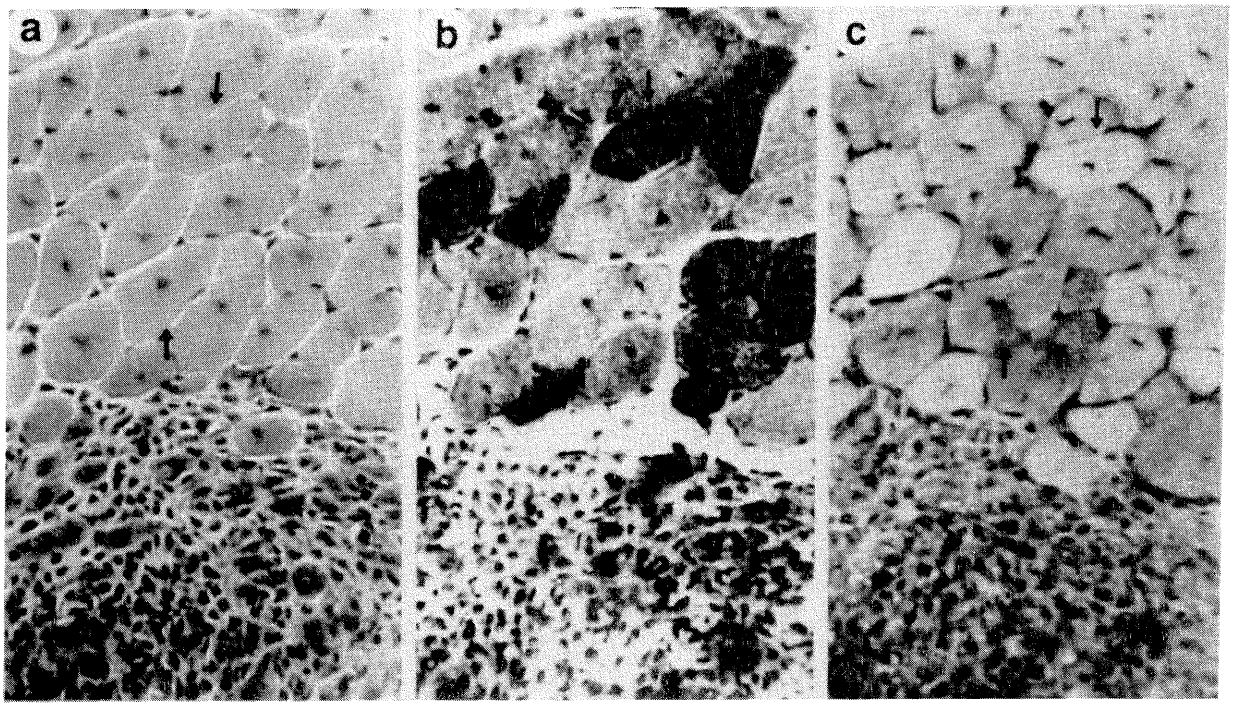

Figure 7. Centronucleated fibers are otherwise within the other fibers in size or stainability. Foreleg triceps muscles from a 40-day-old mdx *mouse were transversely cryo-sectioned, and three serial sections were stained with hematoxylin and eosin (a), for succinate dehydrogenase activity and with hematoxylin (b), and with periodic acid-Schiff and hematoxylin (c). One can see inflammation at the lower part of the photographs. Note that centronucleated fibers exhibit all degrees of staining intensity and size variation (see two examples marked with arrows). Scale bar: 50 µm.*

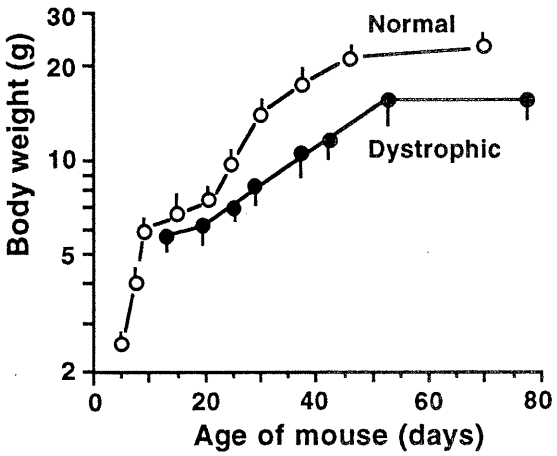

Figure 8. Growth curves of dystrophic and normal mice. Each mean with SD (bar) of body weight is the result of more than 3 animals. A growth defect in dy *mice is conspicuous.*

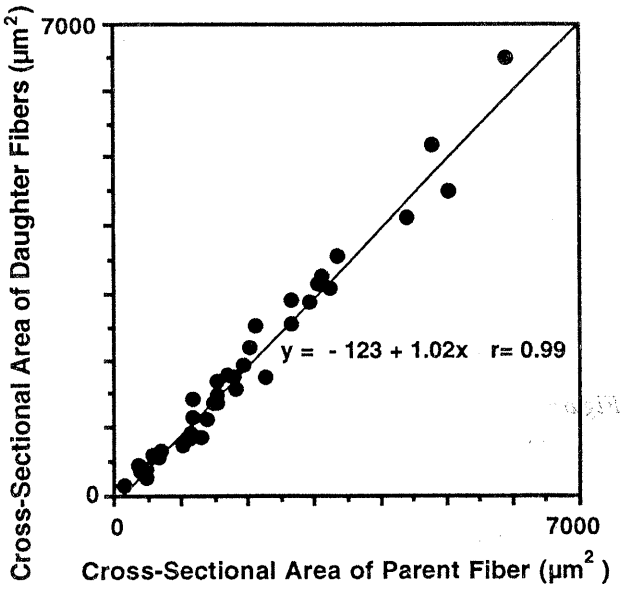

Figure 9. Relation between cross-sectional areas of parent and daughter fibers consequent upon splitting. Rectus femoris muscles of dy *mice were transversely cryo-sectioned and stained with hematoxylin and eosin. The cross-sectional area of fibers in serial sections was measured with a digitizer interfaced to a computer.*

phic muscles and, according to the degeneration theories, is generally thought to be a degenerative expression resulting in atrophy of fibers. However, contrary to this assumption, we found that on splitting, the cross-sectional area of fibers was conserved (Fig. 9). Interestingly, in the field of

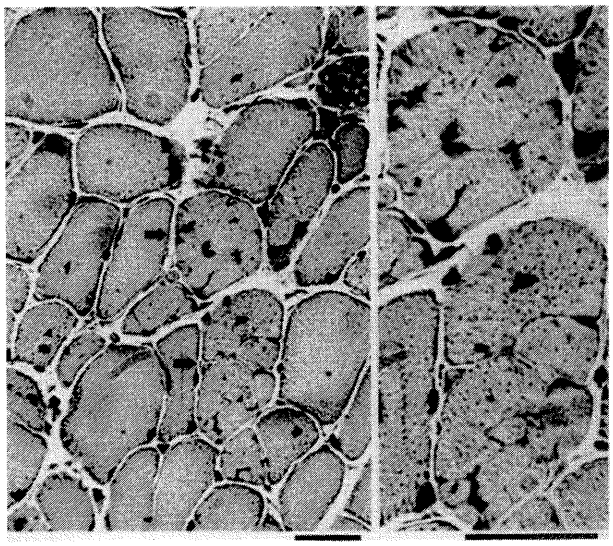

Figure 10. Fusion of a fascicle into a fiber? Crural muscles from a 3-month-old dy *mouse were transversely cryo-sectioned and stained with hematoxylin. A part (marked with arrows) in the left photograph was enlarged and presented in the right. Scale bars 30* μm.

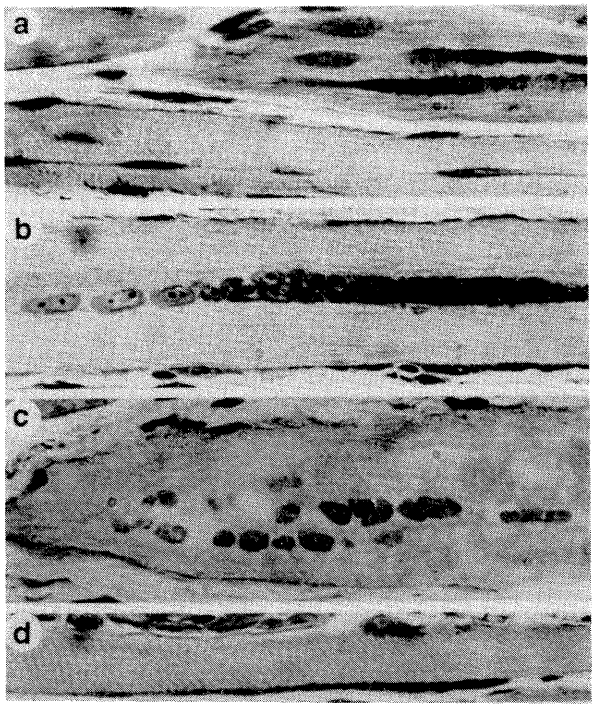

Figure 11. Various images of nuclei. Crural muscles from a 7-month-old dy *mouse were longitudinally cryo-sectioned and stained with hematoxylin. Single, chained, and clumped central nuclei are observed in a, b or c. Along the periphery of a fiber, clustered nuclei are noticed in d. Scale bar: 30* μm.

sports physiology there is an issue of whether or not fiber splitting increases the number of fibers in a muscle after exercise. On the other hand, others may assert that such possibly splitting fibers are fusing, since physically reverse phenomena, splitting and fusion, are indistinguishable from each other in static observations. According to this viewpoint, there may be a possibility that the progressive decrease in the number of fibers contained in dystrophic muscles is in part caused by fusion of a few or several fibers (or a fascicle) into one fiber: Such images can be seen in Fig. 10. It seems that fusion will be accompanied by centronucleation; i.e., peripheral nuclei in the fusing area will become central nuclei after fusion (Figs. 4 and 10). Detailed studies on the meaning of fiber splitting and/or fusion may shed light on the mechanism underlying the dynamic homeostasis of muscles.

Bone-Growth-Induced Muscle Growth

We have been focusing attention on the pathological and/or physiological meaning of central nuclei so as to obtain a key for elucidation of the pathogenetic mechanism of muscular dystrophy. Recently, we have noticed that central nuclei are not always round and are markedly varied in size and appearance as viewed in cross sections. This suggests a possibility to us that they may change their shapes in response to some functional demands. In order to gain insight into this curious subject, longitudinal cryo-sections of *dy* muscles were analyzed microscopically. Various types of central nuclei occurring singly, in a train or as a clump are seen in Fig. 11a & b. Furthermore, one can see a feature where many nuclei have suddenly emerged at the injured part of a fiber (Fig. 11c). Along with deformation, gathering and dispersion of nuclei seem to occur sometimes in muscles of physiological condition as well (Totsuka, unpublished results). Pycnotic nuclear clumps appear as if they were nests or graveyards of nuclei (Fig. 11 a & b). One may speculate that these nuclei in dystrophic fibers failed in fusion and/or differentiation. It is noteworthy that nuclear clumps are observed also at the periphery of the fibers (Fig. 11d). Changes in distribution (peripheral or central), shape (disc or spindle) or multiplicity (single, train or clump) of nuclei possibly may be caused by alterations in the state of fibers (e.g., extraordinary stretching), resulting in their functional diversity, and relating to the mechanism for maintaining the dynamic homeostasis of muscles and/or to a possible mechanism of bone-growth-induced muscle growth.

Relevance of dy *Mice to Human Muscular Dystrophy*

While *mdx* mice, which are accepted as being deficient in dystrophin, are therefore the ideal animal model for Duchenne muscular dystrophy, it remains to be revealed what type of human dystrophy the *dy* mice [31] are relevant to. Strangely, however, the progressive course and severity of muscle abnormality and disability in patients with Duchenne muscular dystrophy are well known to be much more similar to those in *dy* mice than in *mdx* mice. As a

characteristic feature, defects in growth are obvious in *dy* mice (Fig. 8) [41] and Duchenne dystrophic patients [4, 7], but not in *mdx* mice. Also, it is noteworthy that histological changes, e.g., central nuclei [18, 60], are similarly observed in muscles affected by various types of muscular dystrophy and even other diseases such as myotonia and myositis. In conclusion, our bone-muscle imbalance hypothesis may become a basis for considering secondary progressive courses of human muscle diseases.

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