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Comparison of *dy* and dy^{2J}, Two Alleles Expressing Forms of Muscular Dystrophy in the Mouse (39283)

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In 1970 we described a progressive hereditary myopathy of mice caused by a second allele, dy^{2J}, at the *dy*-locus (1). The new mutation occurred in the inbred strain WK/ReJ originally developed for studies of an anemia determined by the *W*-locus. There are three major pleiotropic effects of *W*; homozygotes suffer from a severe macrocytic anemia, they are sterile due to a shortage of primary germ cells, and their pigment is restricted to the retina; 90% die within the first week of life. In contrast, *W*/+ heterozygotes are viable and not anemic. Within the WK/ReJ colony, two dystrophic phenotypes occurred, dy^{2J}/dy^{2J} +/+ and dy^{2J}/dy^{2J} *W*/+. The latter could easily be recognized by a white belly spot. During ultrastructural studies of muscle from the two types of dystrophic mice, we became aware of two kinds of lesions (2). In addition to changes characteristic of the original *dy* mutation, qualitative changes occurred in the mitochondria of the muscle fibers in dy^{2J}/dy^{2J} *W*/+ heterozygotes. The mitochondria were swollen and the cristae formed a lattice pattern. These mitochondria with "sticky cristae" were not found in dy^{2J}/dy^{2J} +/+ mice. Thus, dy^{2J} and *W* combined effects on the quality of muscle changes. These and other studies suggest, therefore, that pleiotropic gene effects and interactions between genes may modify or add to the various forms and expressions of muscular dystrophy. Clinically, WK/ReJ dy^{2J}/dy^{2J} mice are less severely affected than 129/ReJ or 129B6F$_1$ *dy*/*dy* mice, and dy^{2J}/dy^{2J} mutants breed and have a near normal life. To unequivocally decide on differences between *dy* and dy^{2J}, the two mutations and resulting syndromes must be compared on an identical genetical background. Both genes have now been established in the C57BL/6J strain.

Methods. One- and three-month-old male C57BL/6J mice of the following genotypes were compared: *dy*/*dy*, *dy*/+, dy^{2J}/dy^{2J}, dy^{2J}/+, and +/+. The respective mutations were also compared to earlier histological reports made on these same genes as they appeared on their strain of origin, 129/ReJ-*dy*, WK/ReJ-dy^{2J}, as well as 129B6F$_1$-*dy* (3–5).

Gastrocnemius and adductor femoris muscles together with sciatic nerve were excised and fixed in Fekete's acetic alcohol formalin and were processed by standard paraffin procedures. Paraffin-embedded tissues were serially sectioned at 7 μm and alternately were stained with hematoxylin and eosin, luxol fast blue, and alcian blue-PAS (3).

Results and discussion. Following the introduction of the dy^{2J} gene onto the C57BL/6J background, the clinical signs became more severe and progressed more rapidly than in the WK/ReJ strain; yet the age of onset of symptoms did not change. Also, whereas homozygous pairs of WK/ReJ-dy^{2J}/dy^{2J} mice could occasionally breed, the ability of C57BL/6J-dy^{2J}/dy^{2J} males to breed successfully dropped drastically even with nutritional supplements added to their diet. Since pairs of C57BL/6J-dy^{2J}/dy^{2J} do not breed at all, the stock is maintained by crossing male homozygotes with heterozygote females (dy^{2J}/+). Despite the increased severity of symptoms in C57BL/6J-dy^{2J}/dy^{2J} mice, their lifespan remained essentially normal (Southard, J. L., personal communication).

Histologically, dystrophic fibers show nuclear rowing, central location of nuclei, and loss of striation. Also, there are variations in fiber size, necrosis, and fiber breakdown with some regenerative attempts. Glycogen accumulates in fibers during early necrosis. The same qualitative changes are observed in both *dy* and dy^{2J} homozygotes; none occurs in heterozygotes. The earliest lesions

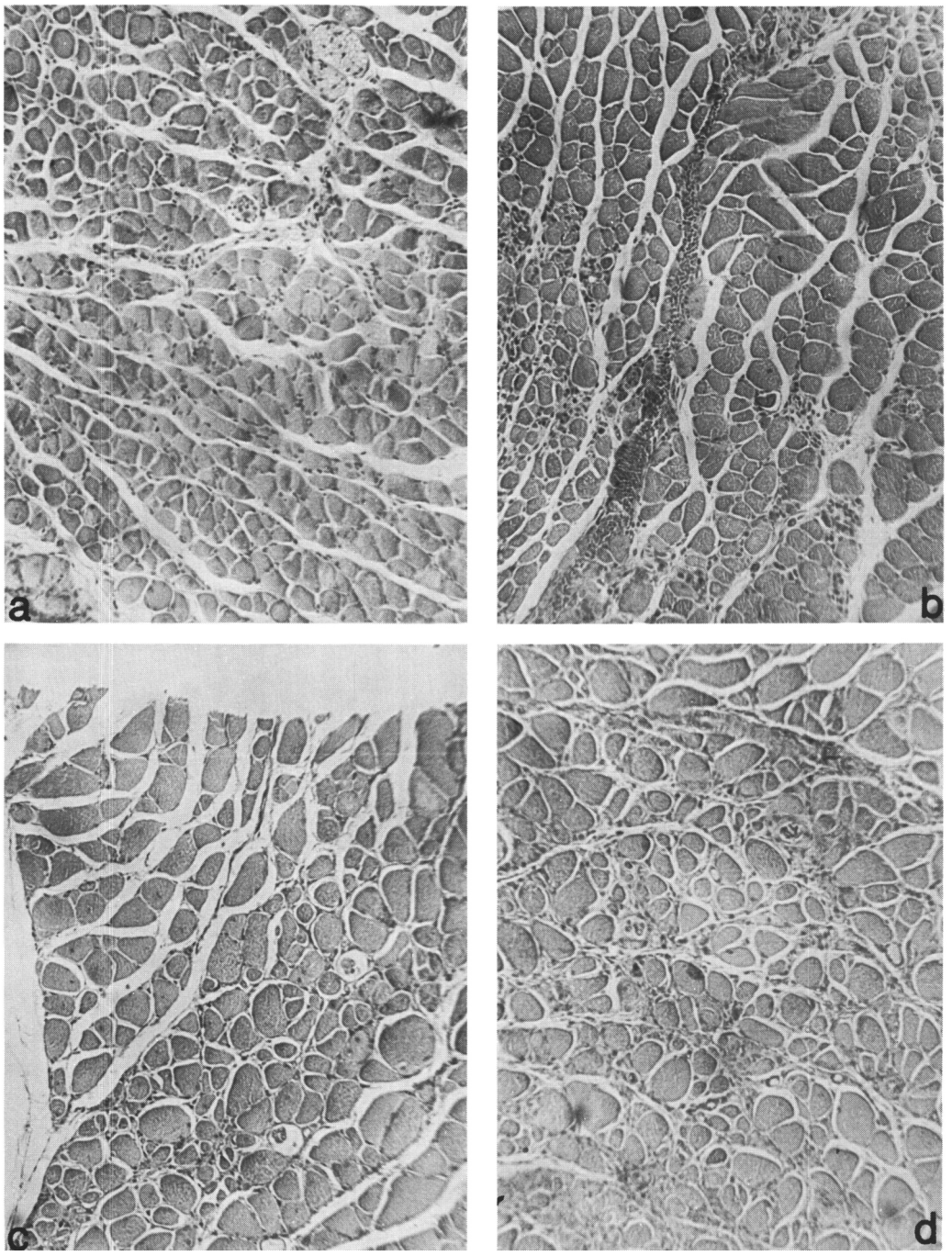

FIG. 1. Cross section through gastrocnemius muscle. At 1 month of age there is excessive nucleation signifying regenerative attempts. This occurs only focally in (a) C57BL/6J-dy^{2J}/dy^{2J} but is generalized in (b) C57BL/6J-*dy*/*dy*. By 3 months of age the number of lesions has increased with more fibers affected and more frequent appearance of centrally located nuclei than at 1 month. The lesions have become more generalized in (c) C57BL/6J-dy^{2J}/dy^{2J} and appear more like the allelic form in (d) C57BL/6J-*dy*/*dy*. Hematoxylin and eosin × 135.

are more focal in dy^{2J}/dy^{2J} and more diffuse in the *dy/dy* mutant, yet they are equally extensive at 3 months of age (Fig. 1a–d). On an identical genetic background the two alleles, *dy* and dy^{2J}, are essentially the same indicating that the remainder of the genotype exerts a major influence on the expression of muscular dystrophy. We have previously reported a specific example of interactions between genes modifying or adding to the expressions of muscular dystrophy, namely dominant spotting (*W*) and steel (*Sl*) (2). Clearly, the muscular dystrophy caused by the dy^{2J} on a C57BL/6J background is a more severe disease than on the WK/ReJ background, whereas that caused by *dy* on a C57BL/6J background is similar to that in the original 129/ReJ strain (5).

Summary. The muscular dystrophies caused by *dy* and dy^{2J} on a C57BL/6J genetic background are similar in quality. At 1 month, slight differences occur in distribution of the muscle lesions, diffuse and focal, respectively, but at 3 months little, if any, differences exist. The *dy* dystrophy appears the same histologically on either the C57BL/6J or original 129/ReJ and 129B6F$_1$ backgrounds.

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