Muscle Histology in Carriers of Duchenne Muscular Dystrophy

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There have been many studies with the light microscope of histological changes in dystrophic muscle. Recent reviews include those of Greenfield, Shy, Alvord, and Berg (1957), Adams, Denny-Brown, and Pearson (1962), and Pearson (1963). Pearson (1963) has pointed out that the majority of investigations have been of moderately advanced cases and that it is still not known in which portion of the muscle fibre the initial dystrophic process is manifest: sarcoplasm, sarcolemmal sheath, contractile fibres, mitochondria, or nuclei. In order to investigate this problem, Pearson has studied preclinical cases of muscular dystrophy where it would be expected that early changes would not be obscured by secondary changes that occur later in the disease. Though studies of preclinical cases have demonstrated that definite pathological changes in the muscle are present even before the onset of clinical weakness, they have not been very helpful in determining the initial manifestation of the dystrophic process, for even in preclinical cases histological changes are fairly well advanced. In a 4-month-old infant with preclinical muscular dystrophy, Pearson (1962) found widespread hyalinization of muscle fibres and marked variation in fibre size.

As an alternative to investigating preclinical cases, it was considered that information concerning early structural changes in dystrophic muscle might be obtained by studying muscle biopsies from carriers of muscular dystrophy: the rationale being that in carriers of muscular dystrophy, according to the Lyon hypothesis concerning gene action in the X chromosome (Lyon, 1961, 1962), all gradations in histological appearance from perfect normality to gross abnormality might be expected. The problem has been investigated by recording subjective impressions of apparent histological abnormalities and by making measurements of fibre size and the number of central and peripheral (sarcolemmal) nuclei.

Another reason for studying muscle specimens from carriers was to determine whether there was any correlation between histological appearances and serum levels of creatine kinase, since this enzyme has been found to be significantly raised in a proportion of carriers of Duchenne muscular dystrophy (Demos, Dreyfus, Schapira, and Schapira, 1962; Hughes, 1963; Pearce, Pennington, and Walton, 1964; Pearson, Fowler, and Wright, 1963; Richterich, Rosin, Aebi, and Rossi, 1963; Sugita and Tyler, 1963).

Method

A carrier of X-linked Duchenne muscular dystrophy has been defined as a woman with at least two affected sons or one affected son but a family history of other male relatives having been similarly affected, i.e. a brother or maternal uncle.

All the carriers were carefully examined for evidence of any muscle weakness, and creatine kinase determinations were made on fresh specimens of serum by the method of Tanzer and Gilvarg (1959). The results are expressed in International units (International Union of Biochemistry, Report of the Commission on Enzymes, 1961), one unit being that amount of enzyme which will catalyse the transformation of 1 μM creatine/minute/1,000 ml. serum. In this laboratory the upper limit for normal healthy women is 1.5 units (Emery and Pascasio, 1965).

The microscopic structure of gastrocnemius muscle was studied in three healthy women with no history of any neuromuscular disease and nine carriers of Duchenne muscular dystrophy. The ages of the carriers ranged from 29 to 55 (mean 37.5) and the three controls were
30, 41, and 48 years old. All specimens were taken from the belly of the muscle and were obtained from healthy controls during the operation of varicose vein ligation. Biopsy specimens were obtained from the carriers under local anaesthesia, care being taken not to inject any of the anaesthetic into the part of the muscle to be excised. A block of tissue about 2 cm. long and 1 cm. wide, with the muscle fibres running parallel to the long axis, was excised. In order to minimize the longitudinal shrinkage of muscle fibres that occurs during fixation (Greenfield et al., 1957, p. 97; Adams et al., 1962, p. 710), the block of tissue was placed on a thin piece of card and gently pressed. After about 5 minutes, when the tissue had become well adhered to the card, it was immersed in fixative.

All the muscle specimens in this investigation were fixed in Formalin-Zenker's solution (5 parts of 40% Formalin to 95 parts of Zenker's fixative) for 15 to 18 hours. Transverse and longitudinal sections were cut at 6 μ and stained with haematoxylin and eosin. All the slides were studied without any knowledge as to the identity of the subject.

Results

Two carriers (Nos. 1 and 2 in the Table) who were found to have muscle weakness have been reported previously, and their status as carriers has been discussed (Emery, 1963). Enlarged calves were found not only in these two carriers, with muscle weakness but also in a third carrier (No. 12 in the Table) with no clinical evidence of any muscle weakness. It may be that the enlarged calves in this woman represent normal hypertrophy. However, her serum creatine kinase was 19.8 units which, in this series, is high for carriers. Calf enlargement may therefore be a microsymptom of muscle disease in carriers (Dubowitz, 1963; Walton, 1964).

With regard to the histological findings, all the controls and two of the carriers (No. 9 and No. 11) appeared to be perfectly normal. Sections from carriers No. 10 and No. 13 were classified as possibly abnormal because many of the fibres appeared to be swollen and, because of rounding-off, to have lost the normal polygonal outline. Swelling of muscle fibres was the only apparent abnormality in these two carriers. In carrier No. 24, not only did many of the fibres seem to be swollen, but several were stained deep red (eosinophilic) and cross-striations and the outline of myofibrils were less clearly defined ('hyaline fibres'). Hyaline fibres were also seen in the sections of muscle from the controls, but here they were very few and always located on the periphery of sections, as though due to fixation or staining artefact.

However, in carrier No. 24 and the remainder of the carriers in whom 'hyaline fibres' were considered.

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TABLE

SUMMARY OF THE HISTOLOGICAL FINDINGS IN 9 CARRIERS OF DUCHENNE MUSCULAR DYSTROPHY

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Grade</th>
<th>Swollen Fibres</th>
<th>Hyaline Change</th>
<th>Fibre Size Variation</th>
<th>Increase in Nuclei</th>
<th>Necrosis</th>
<th>Regeneration</th>
<th>C.T. and Fat Infiltration</th>
<th>Fibre Size (μ)</th>
<th>Serum Creatine Kinase</th>
</tr>
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<tbody>
<tr>
<td>9</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50.45</td>
<td>1.5</td>
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<tr>
<td>11</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>13</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>52.20</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>63.84</td>
<td>2.1</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>49.70</td>
<td>3.9</td>
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<tr>
<td>12</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>71.37</td>
<td>9.8</td>
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<td>+</td>
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<td>-</td>
<td>-</td>
<td>81.90</td>
<td>3.2</td>
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<tr>
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<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72.59</td>
<td>3.2</td>
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<tr>
<td>2</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>78.03</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Degrees of abnormality have been arbitrarily classified from 0 (normal) to 6 (most abnormal).

C.T. is connective tissue.
to be an abnormal finding, these fibres were much more frequent and were situated within fasciculi. In some of the deeply staining eosinophilic fibres, the sarcoplasm appeared to have been converted to floccular masses. In carrier No. 12 there appeared to be increased variation in fibre size (Fig. 1). In carrier No. 20 swelling of muscle fibres, hyalinization and increased variation in fibre size were present as well as an apparent increase in the number of sarcolemmal nuclei. A few fibres were necrotic. Necrotic fibres differed from normal fibres in that they stained grey-blue and not pink, there were no clearly defined myofibrils, and associated nuclei were shrunken and pyknotic. This appearance of the nuclei is characteristic of fibres undergoing necrosis (Pearce and Walton, 1962). Some necrotic fibres were vacuolated and others were being phagocytosed by histiocytes (Fig. 1 and 2). In carriers No. 1 and No. 2 some necrotic fibres had been completely phagocytosed, leaving only the endomysial tube within which regeneration of muscle fibres presumably occurs (Kitiyakara and Angevine, 1960). In carrier No. 20 no regenerating fibres were found and very few were present in sections from carriers No. 1 and No. 2. The fibres believed to be regenerating were rounded, smaller than adjacent fibres, basophilic, and associated with vesicular nuclei with prominent nucleoli (Fig. 1). These appearances have been accepted by others as evidence of fibre regeneration (Pearce and Walton, 1962; Walker and Drager, 1962; Gilbert and Hawk, 1963). The final stage was proliferation of connective tissue and fatty infiltration, but this was
seen only in carrier No. 2 and was not extensive. The histological findings in the nine carriers have been summarized in the Table.

Measurements of fibre size and number of nuclei are given in the Table and represented graphically in Fig. 3. The mean fibre diameter for the three controls was 45·3 μ (S.D. 9·5), 49·8 μ (S.D. 8·0), and 52·5 μ (S.D. 10·6). These results compare well with those obtained by Schwalbe and Mayenda (1890) of 47·5 μ and by Feinstein, Lindegård, Nyman, and Wohlfart (1955) of 54·1 μ for the gastrocnemius muscle. Other investigators have obtained values greater (62·5 μ, Halban, 1894) and less than (31·6 μ, Sissons, 1963) placed nuclei per 100 fibres. From the results of this study (Fig. 3) it seems that, in the gastrocnemius muscle, fibres exceeding 80 μ in diameter or more than 10 nuclei per fibre are abnormal. Central nuclei were found in as many as 6% of fibres in the three controls.

With the exception of carrier No. 24, where sampling of muscle fibres may not have been completely random, the increase in fibre size in
carriers closely paralleled the degree of histological abnormality determined independently. The results of the measurements of fibre size confirmed the impression that swelling of muscle fibres is apparently the earliest change, and that increase in sarcolemmal and central nuclei is found only when structural changes are moderately advanced (Fig. 3).

Since there can be no certainty that the data are normally distributed, Kendall's non-parametric rank method (Siegel, 1956, p. 213) was used in calculating correlation coefficients. The correlation coefficient between apparent structural changes and serum creatine kinase levels was +0.69 and highly significant (p = 0.006). The correlation coefficient between fibre size and serum creatine kinase levels was +0.44, but not significant.

As might have been expected, muscle biopsies appeared most abnormal in the 2 carriers with muscle weakness.

Discussion

The results of the present investigation suggest that swelling of muscle fibres is the earliest structural change in dystrophic muscle. This has also been suggested as the earliest change by Pearce and Walton (1962) from the results of their extensive studies of biopsies from affected individuals. They were unable to determine whether this enlargement represented a genuine hypertrophy of fibres; attempts to demonstrate that these enlarged fibres contained an increased number of myofibrils were unsuccessful for technical reasons. Pearson (1963) has criticized the views of those investigators who have considered hyalination of muscle fibres as merely fixation or staining artefact. From his studies of preclinical cases he has concluded that hyalinization probably represents one of the earliest forms of fibre alteration. The results of the present investigation support Pearson's opinion. There were several reasons for considering such fibres abnormal in this study. First, when similar fibres were seen in normal muscle they were invariably situated at the periphery of sections and always few in number, whereas in carriers they were situated within fasciculi and were much more numerous. Secondly, their presence was associated with other histological abnormalities that were significantly correlated with the level of serum creatine kinase.

According to the Lyon hypothesis concerning gene action in the X chromosome (Lyon, 1961, 1962), it might have been expected that in carriers of X-linked Duchenne muscular dystrophy each muscle fasciculus would be a mosaic of necrotic and normal fibres, the proportion of necrotic fibres reflecting the proportion of cells in which the active X chromosome was the one bearing the muscular dystrophy gene. In each of two female carriers, Pearson et al. (1963) observed only two populations of muscle fibres, one normal and the other dystrophic, and concluded that this was evidence of X chromosome mosaicism. In the present investigation two populations of cells were not found. Instead structural changes appear to have progressed to different extents in different carriers and all gradations from apparent normality to quite marked abnormality were observed. These findings seem more in accord with modern views regarding the development of the multinucleate muscle fibre: in vitro experiments (Stockdale and Holtzer, 1961) and electron-microscope studies (Bergman, 1962; Hay, 1963) suggest that multinucleate muscle fibres arise by fusion of uninnucleate myoblasts. A possible explanation for the histological findings could therefore be as follows: If the myotomes are mosaics of nuclei, in some of which the active X chromosome is the one bearing the muscular dystrophy gene whereas in others the active X chromosome is the one bearing the normal gene, then fusion of uninnucleate myoblasts derived from the myotomes would result in multinucleate muscle fibres which would also possess two different types of nuclei in differing proportions. The proportion of nuclei of the two different types in any muscle fibre would depend on the proportion of the two types in the myotome and by chance in the myoblasts which fuse together to form a fibre. Perhaps the proportion of nuclei in which the active X chromosome is the one bearing the muscular dystrophy gene within any fibre determines whether it will be normal, swollen, hyalinized, or even necrotic (Fig. 4).

Difficulties in attempting to demonstrate X chromosome mosaicism in such conditions as choroideraemia and ocular albinism (Beutler and Baluda, 1964) might also be the result of morphogenetic changes occurring during development in tissues that originally exhibited mosaicism.

Dubowitz (1963) recently reported his histological findings in muscle biopsy specimens from four carriers of Duchenne muscular dystrophy, but pointed out that one of the difficulties he had in interpreting minimal pathological changes was to know the limits of normality. Another important factor to be considered in such studies is the somewhat subjective interpretation of muscle histology. By taking the precautions of using controls and studying specimens without any
fibres. Histological studies of muscle biopsy specimens would seem to be a useful adjunct to the estimation of serum creatine kinase in detecting carriers of Duchenne muscular dystrophy.

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REFERENCES


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