A light and electron microscopic study in serial sections of dystrophic extraocular muscle fibers

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Whole epon-embedded dystrophic extraocular muscles were serially sectioned at 20 microns. These sections were cleared for light microscopy and surveyed for abnormal fibers. Various morphologic disruptions associated with gross changes in diameter over hundreds of microns were observed. Electron microscopic samples reveal a continuum of different kinds of structural changes related to such variations in fiber diameter. These data indicate that a large assortment of ultrastructural changes can occur simultaneously in the same cell.

Key words: extraocular muscle, muscular dystrophy, serial sectioning, morphologic continuum, fiber alteration.

Many myopathies display similar non-specific ultrastructural changes of the various intracellular organelles.1-3 Generally, it is not possible to determine a morphologic starting point of the degenerative process, nor can one readily ascertain a continuity between the various changes observed; indeed, the different abnormalities do not appear to be simultaneously present in the same fiber.4-6

In the present preliminary study, several dystrophic extraocular muscle fibers were followed for hundreds of microns in serial sections of epon-embedded tissue, by light and electron microscopy. Some muscle cells were found to display a continuum of numerous morphologic changes, with foci of maximal degeneration closely contiguous to areas of marked swelling.

Materials and methods

Five dystrophic mice of the Bar Harbor Strain 129, age 10 weeks, were used. The animals were perfused with 1 per cent paraformaldehyde–1 per cent glutaraldehyde through the heart. The extraocular muscles were dissected free and immersed in cold 4 per cent glutaraldehyde for 10 hours, postfixed in 1 per cent osmium tetroxide for 2 hours, and flat-embedded in Epon 812. The epon-embedded muscles were serially sectioned transversely at 20 microns by steel knife on a sliding microtome. Groups of 10 sections were cleared for light microscopy by curing a layer of epon onto the sections which were sandwiched between a polystyrene slide and polished polyethylene. Upon curing, the polyethylene peels off leaving the sections adhering to the polystyrene slide. Such sections can now be viewed by phase
Figs. 1 through 6. Twenty micron serial sections of epon-embedded mouse superior rectus extraocular muscle under phase contrast. Proceeding from Figs. 1 through 6, one cell (arrow) is decreasing in size, while another dystrophic cell (arrowhead) is increasing. x780.
Results

In the dystrophic animals studied, the medial rectus, superior rectus, levator, and retractor bulbi muscles all showed moderate to marked abnormalities. Only the medial and superior recti, however, contained fibers that exhibited the abnormal pattern discussed in this paper.

By phase contrast, swollen cells containing globular masses were readily detectable. As previously reported, these globular masses are mitochondrial aggregates (Fig. 1, single arrow). When followed in serial 20 micron sections (Figs. 1 through 6) such cells are seen to rapidly decrease in diameter and become indistinguishable from neighboring cells within 100 to 150 microns. This series of sections also contains another such cell (arrowhead) which is decreasing in size in the reverse order of the figures. In most cases these cells virtually disappear and leave no trace in the subsequent serial sections. Occasionally, sufficient residual debris remains so that fibers may be followed further. Approximately, 10 per cent of the cells were fol-
Fig. 16. Excessive lipid (L) bodies and autophagic vacuoles (AV). ×15,000.

Fig. 17. Evidence of membrane breakdown (arrowhead), autophagic vacuoles (AV), and swollen sarcoplasmic reticulum (SR). ×15,000.

Fig. 18. Hypertrophic fiber containing swollen and proliferated sarcoplasmic reticulum (SR). Dilated and clustered mitochondria (M) are also evident. ×15,000.

Fig. 19. An advanced hypertrophic stage with highly swollen and clustered mitochondria (M). ×15,000.

followed in this manner. A representative fiber is seen in Figs. 7 through 15 (1 micron sections recut from the original 20 micron sections). A swollen cell (Fig. 7) rapidly decreases in diameter within 80 microns (Figs. 8 and 9), reduces to a barely detectable residue within an additional 80 microns (Fig. 10), and is again seen to increase in size to a swollen state within 120 microns (Figs. 11 to 13). This fiber then returns to a normal appearing diameter within 120 microns (Figs. 14 and
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Fig. 20. Accumulations of swollen mitochondria (M) in various stages of degeneration and absence of a limiting membrane are evident in this atrophic stage. ×6,000.

Fig. 21. Near the point of maximum degeneration, this fiber contains a lobulated nucleus (N) and degenerative mitochondria (M). ×6,000.

Fig. 22. The point of maximum atrophy is characterized by highly swollen mitochondria (M) enclosed in a limiting membrane. ×4,500.

which it then retains. The continuity of this cell was ascertained by electron microscopy through that portion of its length where it could not be clearly identified by light microscopy, such as in Figs. 9 and 10.

Sampling the fine structure of this fiber and others taken over this distance indicates a large assortment of ultrastructural changes that parallel the gross diameter variations and the gradual resumption of apparent normalcy.

The earliest major ultrastructural changes appear to be the presence of autophagic vacuoles and apparent excess of lipids (Fig. 16). Just before cellular hy-
pertrophy begins, one can observe an increased dilation of the sarcoplasmic reticulum and some membrane breakdown (Fig. 17). When the swelling of the cell has reached a stage where its diameter has increased by almost 50 per cent and it has assumed a somewhat rounded appearance, the sarcoplasmic reticulum is highly swollen and proliferated and the mitochondria have begun to swell, cluster, and lose their osmiophilic properties (Fig. 18).

When the cell has reached its maximal hypertrophied state, about twice its normal diameter, mitochondrial clustering is advanced (Fig. 19). As the fiber decreases to almost half its original diameter, it contains little more than an accumulation of highly swollen mitochondria in advanced stages of degeneration. The cell membrane is virtually absent (Fig. 20). Near the focus of maximal degeneration, a tenuous plasma membrane surrounds the cellular remains in some regions (Fig. 21). At the point of greatest decomposition, several swollen mitochondria, some of which have assumed gigantic dimensions, are enclosed within a limiting membrane (Fig. 22).

Discussion

The type of degeneration observed in this study is characterized by marked hypertrophy over a distance of 100 to 150 microns to virtually total cellular decomposition. In the opposite direction, swollen cells assume a normal appearing diameter within 100 to 200 microns, but retain some signs of disruption for an additional 200 to 300 microns. Apparently, this pattern occurs more or less symmetrically in both directions away from a focus (or foci) of maximal destruction. Conceivably, such degeneration is spreading along the fiber; if so, the associated continuum of ultrastructural changes along the fibers' length would indicate the time course of the degenerative process for any given point on the fiber as the spreading cellular disruption sweeps by that point. Present findings indicate that a muscle cells' plasma membrane is disrupted at many points along the fiber length. It is possible that the initiating insult to the cell is to the plasma membrane leading to its breakdown and to secondary and nonspecific changes in subcellular organization. One could further speculate that the primary injury to the plasma membrane occurs at the level of a myoneural junction(s). Previous papers have suggested an involvement of the myoneural junction in the etiology of muscular dystrophy in mouse.

The results in this paper indicate that a large assortment of structural changes can occur simultaneously in the same cell, within several hundred microns. Observations based on sampling over a relatively short distance would provide an impression of very different appearances in the respective fibers, even if all were undergoing the same degenerative process.

It would seem that additional information as to what may be taking place in an abnormal muscle could be obtained from observations on single fibers taken over the better part of a millimeter. Serial 15 to 25 micron sections of epon blocks facilitates following fibers of interest; it also provides a rapid and convenient survey of large volumes of tissue whereby such cells of interest may be detected initially.

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