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An Ultrastructure Investigation of Intrafusal Muscle Fibers in Myotonic Dystrophy

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Summary. Ultrastructure features of muscle spindles from two patients with myotonic dystrophy are described. Intrafusal muscle fibers exhibit extensive splitting with nuclear bag fibers affected more so than nuclear chain fibers. No sensory endings are present on nuclear chain fibers nor on one nuclear bag fiber throughout the equatorial and myotube regions. Small motor end plates are evident on various segments of split intrafusal fibers in the polar region and some of these extend into the myotube region. Satellite cells are numerous on both nuclear bag and nuclear chain fibers. These frequently occupy the cleft space between segments of split intrafusal fibers. The myotonic dystrophy muscle spindle ultrastructure features seem to closely resemble the appearance of developing mammalian muscle spindles as illustrated with opossum fetal tissue.

Key words: Muscle spindles – Myotonia – Intrafusal fibers – Satellite cells.

Introduction

The light microscopic and histochemical features of intrafusal muscle fibers and the ultrastructure changes in extrafusal muscle fibers in myotonic dystrophy have been well delineated since Daniel and Strich (1964) first reported on the light microscopic features of muscle spindles in myotonic dystrophy (Aleu and Afifi, 1964; Cazzato and Walton, 1968; Heene, 1973; Klinkerfuss, 1967; Mussini et al., 1970; Samaha et al., 1967; Schotland, 1970; Schroder and Adams, 1968; Swash, 1972). From these investigations the one feature unique to myotonic dystrophy is that many muscle spindles consist of numerous small intrafusal muscle fibers with abnormal innervation patterns. These small muscle fibers apparently develop by a process of longitudinal splitting from parent fibers.

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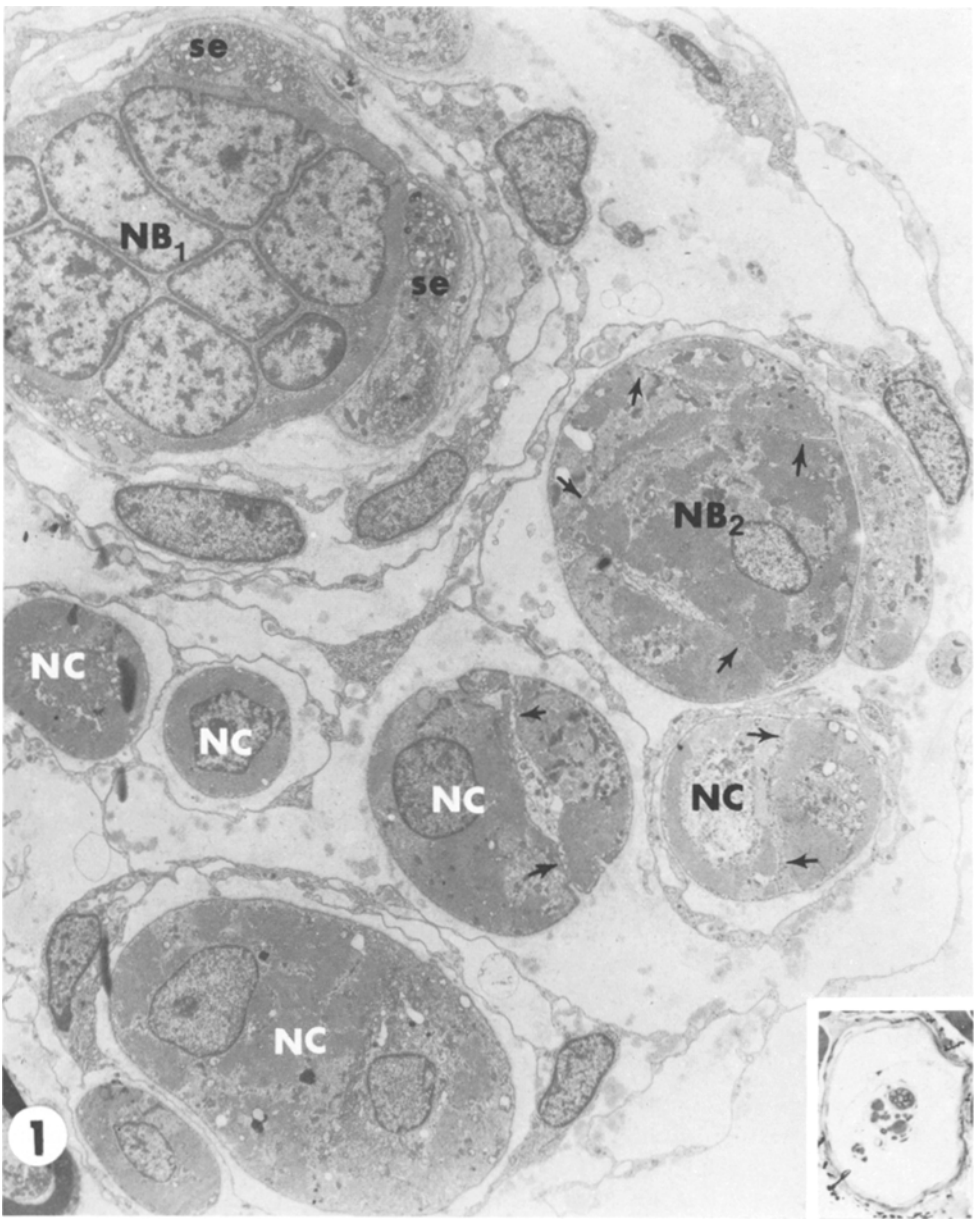


Fig. 1. Equatorial region of myotonic muscle spindle. One normal appearing nuclear bag fiber (NB_1) encased by sensory endings (*se*) is evident. A second large intrafusal fiber assumed to be a nuclear bag fiber when followed in serial sections consists of several segments (NB_2). Some nuclear chain fibers (*NC*) appear normal except for lack of sensory endings, others consist of two segments. Arrows mark separation sites into various segments. ×3468. Inset – One micron light microscopy section of entire spindle taken immediately after grids for Figure 1. ×250

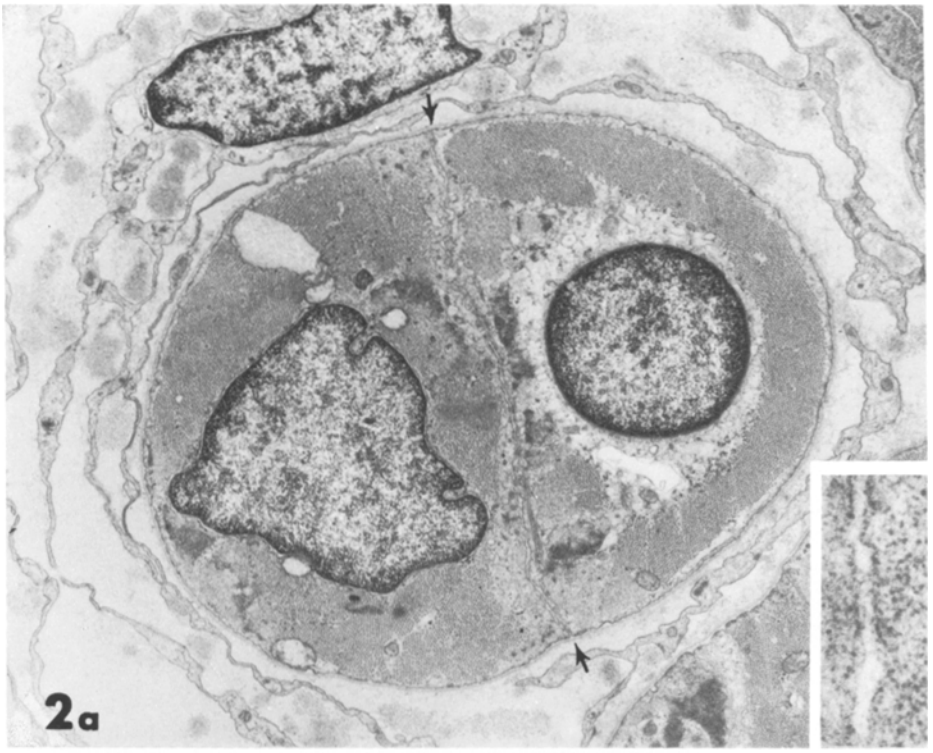
In order to examine the pathogenesis of this splitting, Swash and Fox (1975) described two muscle spindles serial sectioned throughout the polar and equatorial regions from a 52 year old myotonic dystrophy patient. At selected levels, thin sections were taken for electron microscopy to further delineate the features seen in the two-micron serial sections, and a third muscle spindle from the same patient was studied histochemically. Their results demonstrated that the splitting process is extremely complex in the midpolar region but that each split fragment remains separated from the parent fiber for only a short portion of its length and then fuses back with adjacent fibers. At the tips of the polar region and in the equatorial region such splitting does not occur or is greatly reduced. Ultrastructurally, each cluster of split fragments was surrounded by a common basement membrane with individual fragments separated by a clear space 130–300 Angstroms wide. Some fragments demonstrated degenerative changes, rodlets, vacuoles, lipid deposits, myelin figures, and sarcoplasmic masses, whereas others appeared normal. Sensory nerve endings appeared normal but motor endings were small, difficult to identify, and limited to trail type endings. Swash and Fox proposed that the abnormality of intrafusal fiber splitting may be due to mechanical stresses perhaps associated with the myotonia and that the small motor endings present may represent a proliferation of fusimotor-nerves in an attempt to innervate the fragmented abnormal muscle fibers.

In an attempt to further clarify the pathogenesis of intrafusal fiber splitting we have serial sectioned for electron microscopy portions of four muscle spindles from two patients with myotonic dystrophy and compared these with developing muscle spindles in mammalian fetal tissue. It is the purpose of this paper to present our findings which either add clarity or are in addition to those of Swash and Fox.

Materials and Methods

The investigation was carried out on tibialis anterior muscle biopsies from a 24 year old male and his 48 year old father, both with typical adult myotonic dystrophy. The biopsies were taken in muscle clamps and immediately immersed into 0.5 M cacodylate buffered 3% glutaraldehyde, pH 7.4, for 15–30 min. The specimens were then released from the clamps, trimmed into small blocks, and reimmersed in fresh glutaraldehyde for two hours. They were then washed in two ten minute cacodylate buffer baths, post-fixed in veronal acetate buffered 2% OsO4 for one and one-half hours, dehydrated in a graded series of alcohols, cleared with propylene oxide and flat embedded in Epon 812. Following heat polymerization (60° C, 48 h) one micron transverse sections were cut from each block and examined by light microscopy to locate muscle spindles. If spindles were identified, they were isolated in the plastic blocks under a dissecting microscope and serial

Fig. 2. a Nuclear chain fiber in equatorial region appears to be splitting into two fibers. No sensory endings are evident and both segments are encased by same basement membrane (arrows). ×6020. Inset – High magnification of adjacent membranes in area of separation. ×101,440. **b** Developing muscle spindle in 20 day old opossum fetus. Various intrafusal fibers appear to be developing as segments from one fiber within a common basement membrane. Two developing myoblasts (b_1, b_2) closely resemble satellite cell appearances but contain myofilaments in contrast to satellite cells. Arrows indicate borders of plasma membranes. ×4277



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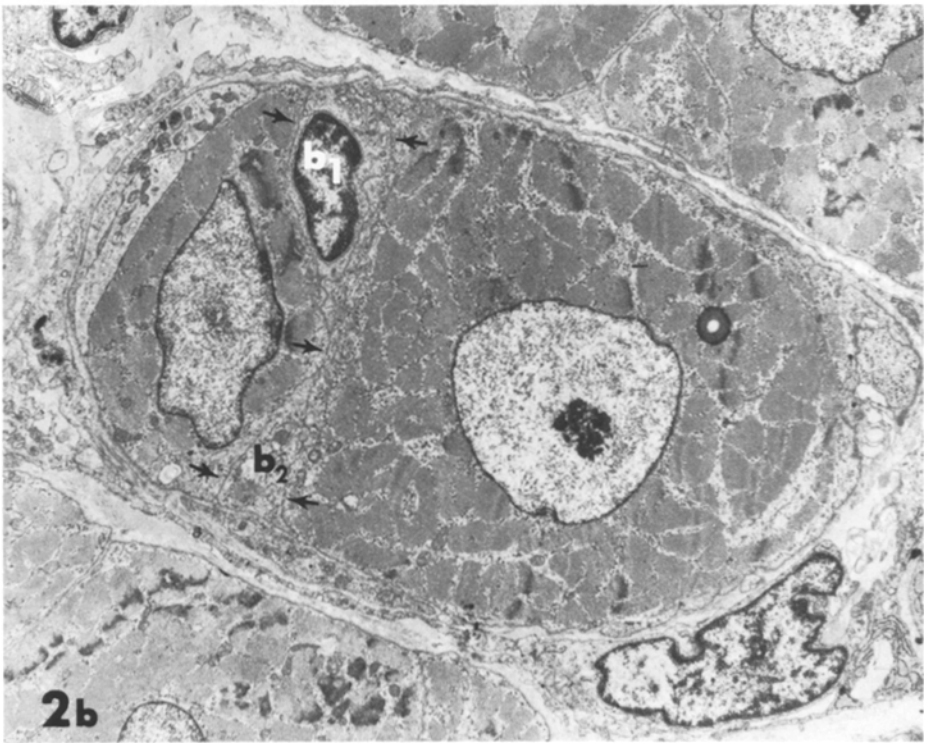

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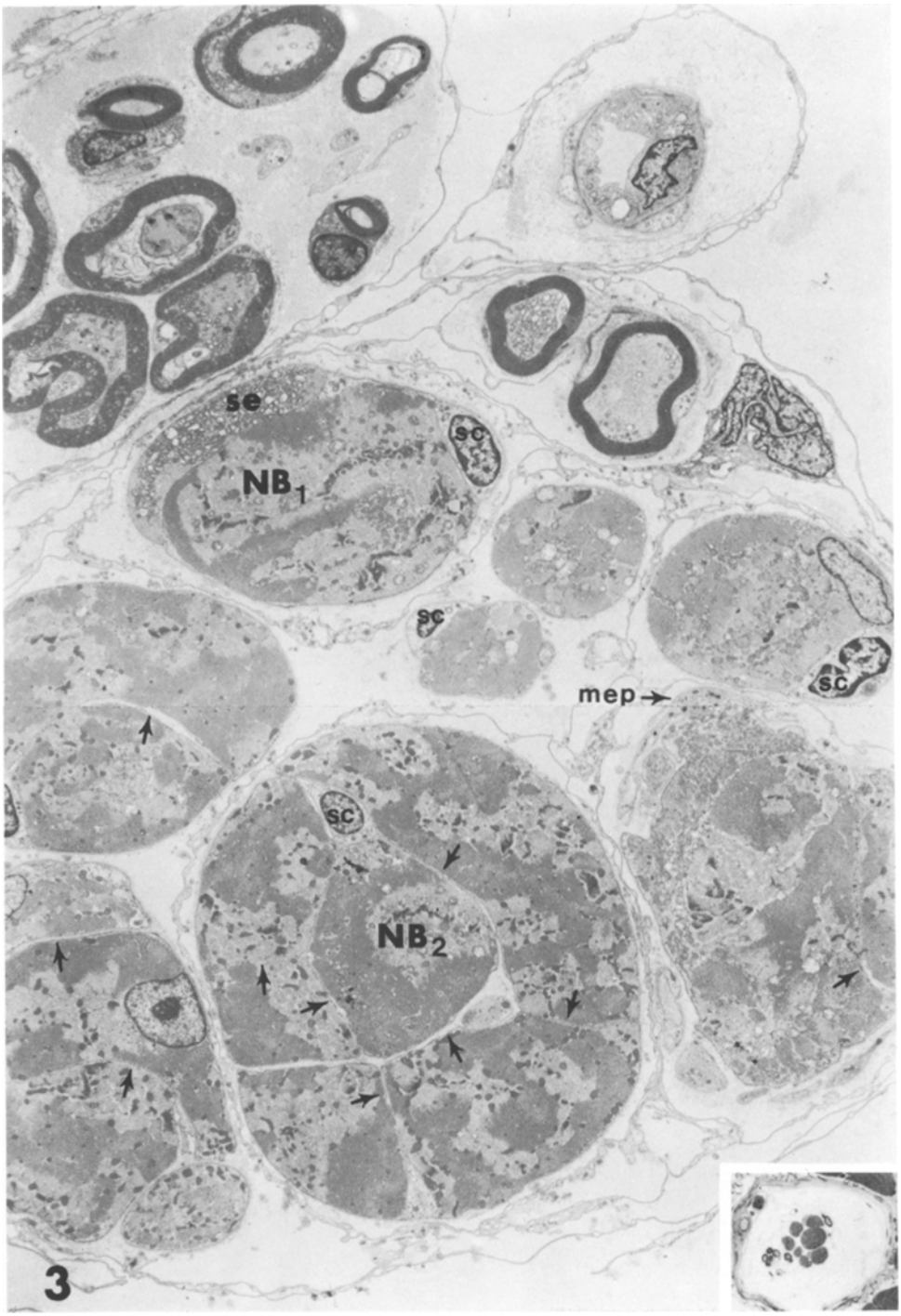


Fig. 3. Myotube region of same spindle in Figure 1. Nuclear bag fiber (NB_1) seen in Figure 1 is still encased by a sensory ending (*se*). Adjacent large fiber (NB_2) consists of multisegments and is devoid of sensory innervation. Smaller fibers consist of several segments and one fiber supports a small motor end plate (*mep*). Numerous satellite cells (*sc*) are evident on both nuclear chain and nuclear bag fibers. Arrows indicate divisions into various segments. ×2482. Inset – One micron light microscopy section taken immediately after grids for Figures 3 and 6. ×250

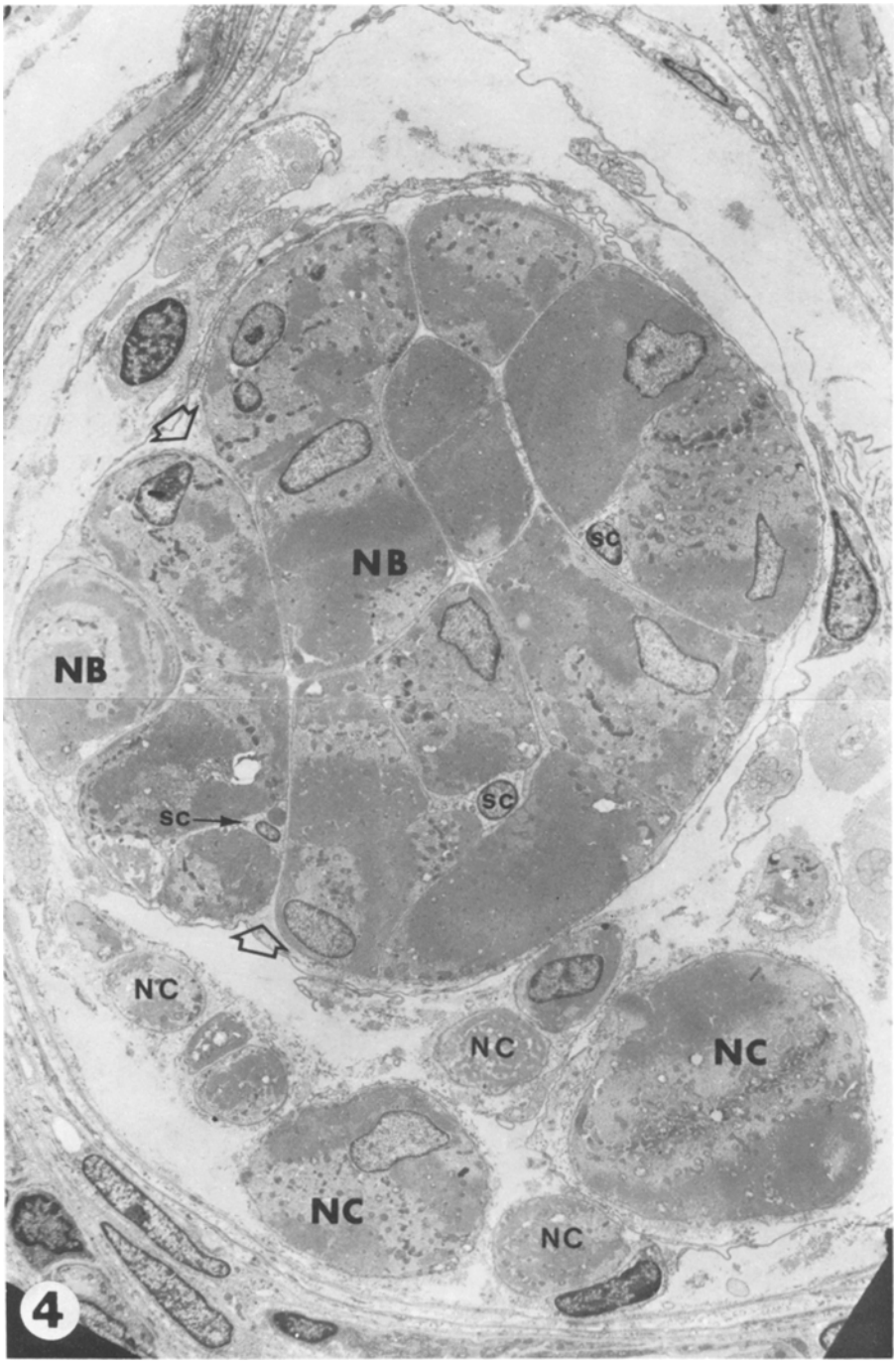


Fig. 4. Polar region of same spindle in Figures 1 and 3. Both large nuclear bag fibers (*NB*) now consist of multisegments and closely approximate each other. Basement membrane material separates the division between the two main fiber components (arrows), whereas various segments within one fiber are encased by a common basement membrane. Each segment contains its own nucleus. Nuclear chain fibers (*NC*) are small and difficult to discern main fibers from segments of a fiber. Three satellite cells (*sc*) are evident. $\times 2465$

thin sections for electron microscopy were cut throughout the remaining length of each spindle. The thin sections were mounted on 200 mesh copper grids, stained with uranyl acetate and lead citrate and examined in an RCA EMU-3H electron microscope at 50 KV.

Results

Two muscle spindles from each patient were isolated in the plastic blocks. From these we observed the splitting of intrafusal fibers; however, splitting did not extend the full length of the fibers and there were marked differences between nuclear bag and nuclear chain fibers. The best results were obtained from the 24 year old, hence selected sections through one of the spindles from this patient are presented.

Intrafusal Muscle Fibers. In the equatorial region, one normal appearing nuclear bag fiber encased by primary sensory endings was evident. A second large intrafusal fiber assumed to be a nuclear bag fiber due to its size was without nuclear bag features and consisted of numerous segments all within a common basement membrane (Fig. 1). No sensory endings were evident on this fiber. Some adjacent nuclear chain fibers appeared normal except for lack of sensory endings. Others appeared to be split into two segments each of which contained its own nuclei when followed in series (Figs. 1 and 2a). High magnification revealed no clue as to how or why the fibers undergo splitting (Fig. 2a inset).

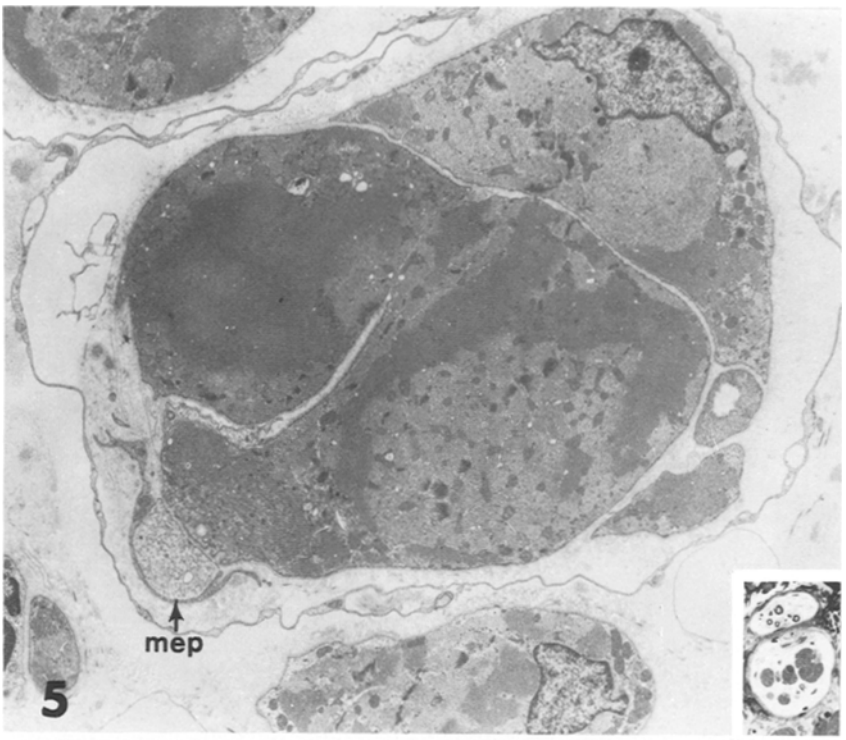
In the myotube region the nuclear bag fiber illustrated in Figure one was still encased by sensory endings and had not split. The other large intrafusal fiber consisted of multisegments without any sensory innervation evident throughout the equatorial or myotube regions. Nuclear chain fibers were small, without sensory innervation, and varied from an intact fiber to a split fiber consisting of two to four or five segments (Fig. 3).

In the polar region both large intrafusal fibers consisted of many segments. Further, the two large fibers came very close together when followed in series, although basement membrane material was present between the two main fiber components throughout most of their length. Nuclear chain fibers appeared

Fig. 5. Polar intrafusal fiber consisting of several segments with small motor end plate (*mep*) on one of the segments. ×4530. Inset—One micron light microscopy section taken immediately after grids for Figure 5. ×250

Fig. 6. Sections taken immediately after grids for Figure 3 illustrating same motor end plate (*mep*) seen in myotube region in Figure 3. Note satellite cell (*sc*) and narrow cleft between segments (arrows). ×4530. Inset—One micron light microscopy section taken immediately after grids for Figures 3 and 6

Fig. 7. a Developing intrafusal fiber (*IF*)—satellite cell (*sc*) complex in 50 day old opossum fetus. Satellite cell and muscle fiber are approximately same diameter and appear identical to those in myotonic muscle spindle as seen in Figure 7b. Arrows indicate borders of plasma membranes. ×12,400. **b** Intrafusal fiber (*IF*)—satellite cell (*sc*) complex in polar region of myotonic muscle spindle. Satellite cell and muscle fiber are approximately same diameter and appear identical to those in developing muscle spindles as seen in Figure 7a. Arrows indicate borders of plasma membranes. ×25,200



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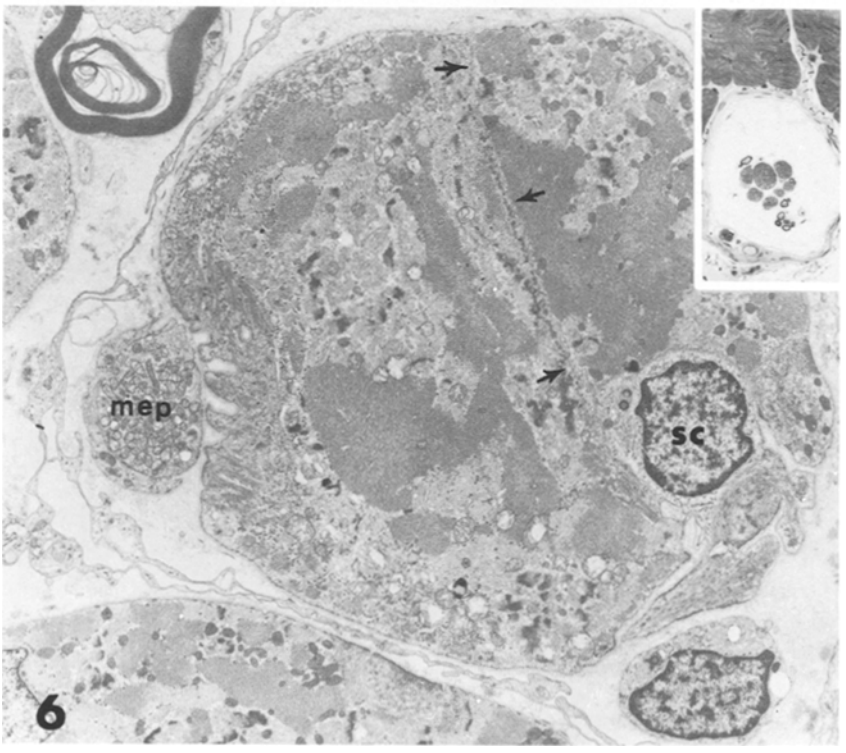

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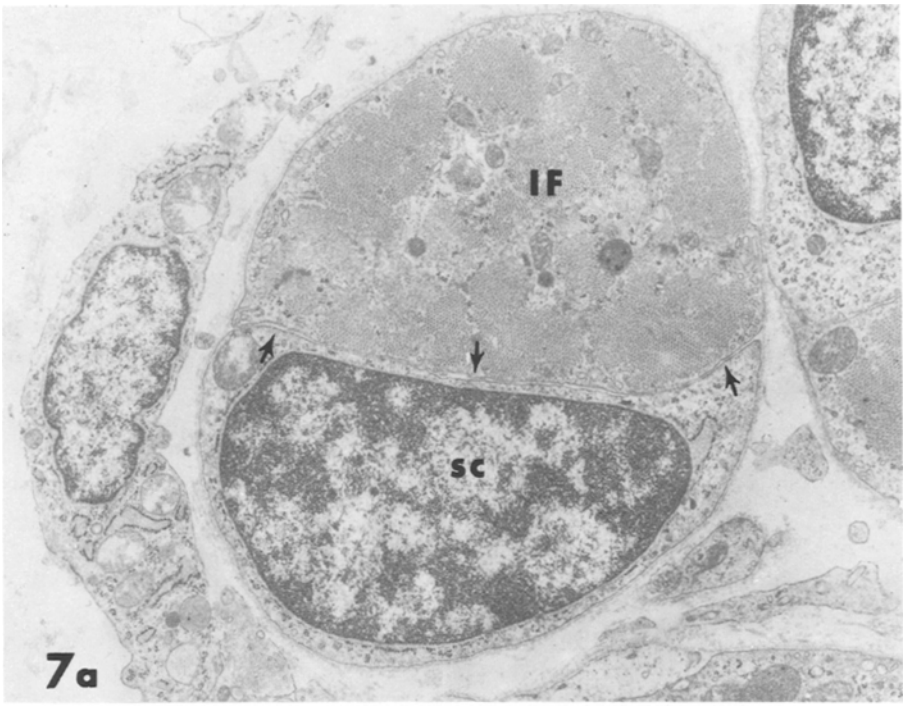

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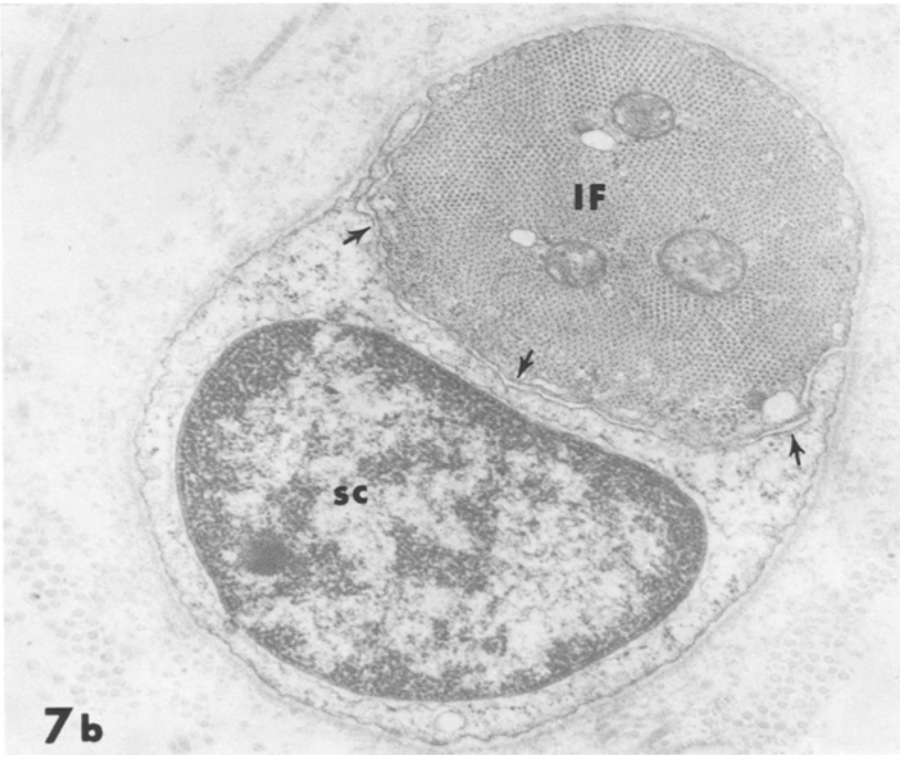

7b

intact or at most consisted of only two or three segments for a short period of their length. Nuclear bag fibers therefore appeared much more affected by the splitting process than nuclear chain fibers.

Innervation. Sensory innervation appeared normal on the one nuclear bag fiber (Fig. 1). No other intrafusal fiber exhibited sensory endings throughout the series of cross sections. Therefore, no sensory endings were seen on any nuclear chain fiber nor on the large fiber which appeared segmented in the myotube and equatorial regions as well as in the polar region.

Motor endings were very small and consisted of shallow junctional folds but no mound of sarcoplasm beneath the sarcolemma (Figs. 5 and 6). Some of these extended into the myotube area on segmented fibers adjacent to the nuclear bag fiber engulfed by sensory coils (Figs. 3 and 6). Two end-plates on different segments of one fiber in the same cross-section were seen in one instance in the polar region.

Satellite Cells. Numerous satellite cells were present on both nuclear bag and nuclear chain fibers in the myotube and polar regions. Some of these were in typical satellite positions and in the polar region appeared nearly as large as their host intrafusal fiber (Figs. 3, 4, 6, 7b). In other instances, small mononuclear cells without myofilaments occupied the cleft space between segments of split intrafusal (Figs. 3 and 4). These cells appeared similar to satellite cell morphology except for their unusual position and number.

Related studies. Developing mammalian muscle spindles from opossum fetal tissue were also isolated and compared with the myotonic dystrophy muscle spindles. During early development the fetal spindles closely resemble the appearance of a segmented intrafusal fiber. They consist of one or two developing intrafusal fibers and a few poorly differentiated cells all within a common basement membrane (Fig. 2b). The poorly differentiated cells are in positions similar to satellite cells but myofilaments can frequently be identified in their cytoplasm. Hence, we classify these as intrafusal myoblasts instead of satellite cells. At a later stage of development the individual fibers of the muscle spindle separate with each possessing its own basement membrane. In the polar region many of the intrafusal muscle fibers then host satellite cells which may be nearly as large as the host fiber (Fig. 7a). These closely resemble the appearance of the polar intrafusal fiber-satellite cell relationship seen in Figure 7b from the myotonic dystrophy biopsy.

Discussion

Since many muscle spindles in myotonic dystrophy demonstrate extensive splitting of intrafusal muscle fibers it is important to determine the nature of this process. Swash (1972) suggested this splitting may be secondary to changes in the pattern of fusimotor innervation. When Swash and Fox (1975) recently obtained the first ultrastructure micrographs of myotonic muscle spindles they changed their hypothesis to conclude that the abnormalities in fusimotor innervation were more likely secondary to the complex process of intrafusal fiber

splitting, i.e., gamma motor neurons undergo sprouting to supply the individual segments of split intrafusal fibers with fusimotor innervation. From our results we support this latter hypothesis, however, it does not explain the etiology of intrafusal fiber splitting.

It has been suggested (Swash and Fox, 1975) splitting may be due to abnormal stress placed upon the intrafusal fibers in association with the myotonia or with the myotonia after discharges. Support for this hypothesis comes from Hall-Craggs (1972) demonstration of extrafusal fiber splitting in heavily overloaded muscles. Such overloading could conceivably occur in intrafusal fibers through abnormal fusimotor innervation patterns, but this is in opposition to the hypothesis that the abnormalities in fusimotor innervation are secondary to fragmentation of intrafusal fibers. Alternatively, abnormal stress on intrafusal fibers might occur through the links with extrafusal fibers if the extrafusal fiber contraction-relaxation patterns were altered as in myotonia. If so, one might expect nuclear bag fibers to be affected more so than nuclear chain fibers since they are longer and terminate outside the spindle capsule in close apposition to extrafusal fibers. Our findings suggest this could be a factor since the majority of the intrafusal fiber segments in our cases were derived from nuclear bag fibers. Nuclear chain fibers seldom appeared split into more than three segments and generally over a short distance whereas nuclear bag fibers demonstrated up to sixteen segments in one cross section. The difficulty in accepting such a hypothesis however is that prolonged contraction-relaxation patterns should unload the spindle rather than place increased stress upon it, unless such prolonged extrafusal contraction also results in increased fusimotor activity in an attempt to maintain a functional spindle setting relative to the contracted extrafusal fiber length.

An alternative consideration to the stress hypothesis as inducing intrafusal fiber splitting is suggested by the ultrastructure similarities between developing muscle spindles and myotonic dystrophy spindles. Our preliminary ultrastructure results indicate mammalian spindles are first recognizable as single intrafusal fibers. As development continues several intrafusal fibers, myoblasts, and satellite cells all become evident within a common basement membrane. These later separate as sensory neurons encircle their perimeter. It is noteworthy that the myotonic intrafusal fibers exhibiting splitting in the equatorial and myotube regions in our cases were not encased by sensory nerves. The nucelar bag fiber which did support sensory terminals split only in the polar region beyond the limits of sensory innervation. Perhaps this indicates an essential sensory neural influence to switch from developmental processes to a mature functional intrafusal fiber. In absence of such influence an individual fiber may continue to develop similar to fetal tissue resulting in many small fibers with numerous satellite cells and abnormal fusimotor innervation patterns. The motor end plates we found appeared abnormal in that they possessed shallow junctional folds but no mound of sarcoplasm beneath the sarcolemma. Thus they possessed characteristics of both plate and trail type end plates (Barker et al., 1970) and extended into the sensory region of the muscle spindle.

The weakness in considering the absence of sensory innervation as a possible factor in intrafusal fiber splitting is of course the fact that normal muscle

spindles do not possess sensory innervation in the polar region and their polar regions do not appear to be splitting. At this time we can only speculate that this may indicate the absence of sensory innervation is a secondary response to splitting, and that the splitting process affects individual intrafusal fibers at different rates, or, that primary and secondary sensory neurons are affected differently by myotonic dystrophy which may in turn be related to intrafusal fiber splitting. In the present study we could not classify the few sensory endings we did see as to primary or secondary or determine if they were all of one type. Additional ultrastructure studies comparing congenital myotonia with myotonic dystrophy muscle spindles might prove helpful in clarifying the role of sensory innervation and maturation or splitting of intrafusal fibers.

Swash and Fox further speculated that splitting may induce the formation of satellite cell structures and that at least some of the small fibers are new muscle fibers derived from satellite cells rather than segments of a split fiber. They suggested those fragments containing a nucleus might be regarded as satellite structures, but, if such fragments contain myofilaments they do not meet the accepted definition of satellite cells (Muir, 1970) and it is impossible to determine their derivation. Further, in our experience nearly every segment of a split intrafusal fiber contains its own nucleus if followed in serial sections. Consequently, we could not positively identify any satellite cells in their micrographs. However, we do agree with the speculation that some of the small mononuclear fibers seen in previous studies may be satellite cells, as we found them quite numerous on both nuclear bag and nuclear chain fibers throughout the myotube and polar regions. In the polar region some of these cells appeared in normal satellite cell position on the periphery of an intact fiber. Others associated with the multisegmented nuclear bag fibers often occupied the cleft space between two segments and appeared as though a cytoplasmic process of the cell was wedging its way between the fragments. Whether these cells are factors in initiating the splitting process or are secondary responses to splitting remains unknown but they are clearly numerous enough to account for some of the many small mononuclear cells reported in previous investigations. Further, in normal mammalian muscle spindles, Banker and Girvin (1971) indicated such cells are only on nuclear bag fibers. In myotonic dystrophy they not only appear to be more numerous but are as common on nuclear chain fibers as on nuclear bag fibers.

In summary, the results of our investigation have demonstrated the following:

1. Nuclear bag fibers are affected more than nuclear chain fibers by the splitting process.

2. Motor end plates are difficult to classify as plate or trail endings as they possess some features of both types.

3. Motor end plates appear to innervate individual segments of the split fibers and extend into the sensory region of the muscle spindle on the segmented fibers.

4. Satellite cells are very numerous on the segmented fibers, often occupying the cleft space between segments.

5. The intrafusal fibers exhibiting extensive splitting in the equatorial and myotube regions were devoid of sensory innervation in our cases.

From these findings and those of Swash and Fox we support the conclusion that abnormal fusimotor innervation is probably secondary to intrafusal fiber splitting. Whether the stress hypothesis as a cause of intrafusal fiber splitting is valid has not been determined. However, we feel the fact that nuclear bag fiber appear to be affected more than nuclear chain fibers may indicate an important intrafusal-extrafusal fiber relationship in the splitting process. Finally, as an alternative approach, we suggest that the development of primary and secondary sensory innervation in determining mature intrafusal fibers is in need of further investigation since many of our fibers appeared devoid of sensory endings. Thus, instead of mature fibers splitting myotonic dystrophy may involve immaturation of intrafusal fibers.

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