Ultrastructural Changes in the Skeletal Muscle of Senile Rats with Significant Age-Dependent Motor Deficits

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Abstract

The anterior tibial (AT) muscle of 6 female Wistar rats aged 35-44 months, was examined by an electron microscopy. Previously, significant age-dependent functional and morphological deficits were found in these rats including [1] a strongly decreased muscle mass and force, [2] muscle stiffness, [3] spontaneous, tonic electromyographic activity, and [4] light-microscopic features of chronic denervation atrophy. In the present study diverse ultrastructural changes were found in muscle which correspond to chronic denervation atrophy. A number of already described abnormalities could be demonstrated in our material. However, sarcoplasmic reticulum (SR) tubular formations and extensive muscle fiber fragmentation have not been previously associated with senile changes in muscle. This can be explained by a very advanced age of our experimental animals and, in consequence, a more advanced denervation atrophy. Therefore it would appear that no single abnormality or set of morphological changes are characteristic of senile skeletal muscle.

Key words: skeletal muscle, ultrastructure, denervation atrophy, aging.

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It is well known that both the muscle strength andniuscle mass decline with age. The morphological changes that might explain these impairments have been studied by several investigators [2, 3, 4, 13, 17, 19, 38], Various morphological alterations described in old muscle were, in fact, consistent with those found in neurogenic atrophy. Indeed, a progressive loss of motor neurons and terminal axons seems to be the chief factor in the aging muscle [14, 22] Even where major diseases and environmental factors are avoided, there is nevertheless a steady decline of motor neurons with age.

The electron-microscopic data currently available for senile muscle remain limited [14, 35, 39]. The present study was, therefore, undertaken to determine ultrastructural characteristics of aging in rat skeletal muscle. Previously [27] significant age - dependent functional and morphological deficits were found in these rats including 1) strongly decreased muscle mass and force, 2) muscle stiffness, 3) spontaneous, tonic electromyographic activity, and 4) light microscopic features of chronic denervation atrophy.

Material and Methods

Experiments were carried out on 6 female Wistar rats aged 35-44 months. Biopsy specimen were taken from anterior tibial (AT) muscle under ether anesthesia. Dissected tissue was then fixed in 6% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, postfixed in 1% osmium tetrox-ide in the same buffer, dehydrated, and embeded in Spurr resine. Semithin sections were stained with toluidine blue for light microscopy. Ultrathin sections double stained with uranyl acetate and lead citrate were examined on JEM 1200 EX2 Electron Microscope.

Results

All examined skeletal muscle samples showed ultrastructural abnormalities. The pathological involvement of the muscle fibers, however, was not uniform. A wide spectrum of changes was observed on each examined grid. The abnormality most often encountered was the *muscle fiber atrophy* (Fig. 1,2,3,4). The degree of atrophy varied from slight to advanced and corresponded with t he intensity of myofibrillar degeneration and involvement of other muscle fiber elements. Both fiber types were affected, as

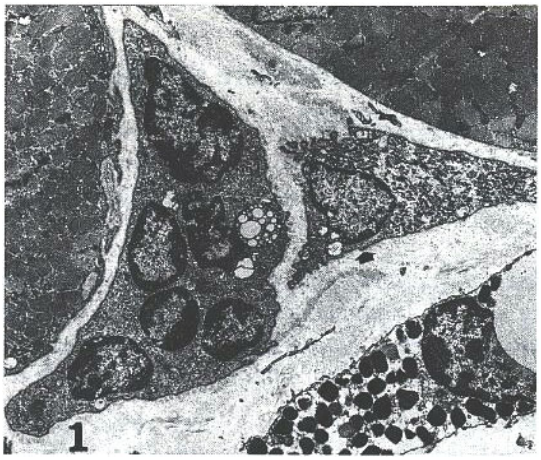

Figure 1. x 7350. Two severly atrophied muscle fibers showing advanced stage o fmyofibrillar degeneration. One fiber (arrow) contains many hyperchro-matic nuclei of irregular shape and lipofuscin granule. Only one, oval, pale nucleus is seen in the other fiber (arrowhead).

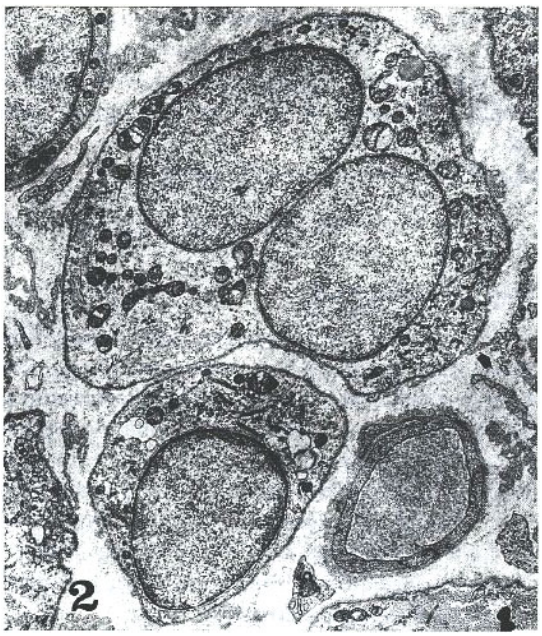

Figure 2. x 14 700. Split muscle fiber fragments almost devoid of myofibrils containing large, round, pale nuclei and few scattered mitochondria. Basement membrane processes and loops (arrows) are numerous.

shown by Z-line width. Most of the examined fibers, however, were too severely altered to be classified into particular fiber type.

Aggregation and deformity of muscle nuclei was observed in many atrophied fibers. Frequently the nuclear

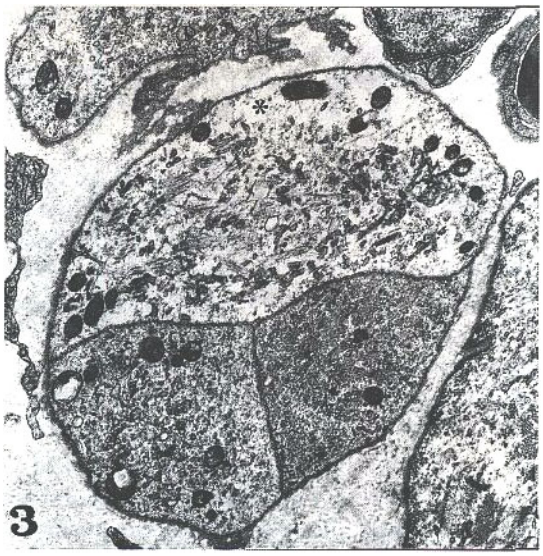

Figure 3.x 14 000. Numerous split fragments of muscle fibers (farrows) connected by long basement membrane processes (arrowheads). Atrophied muscle fragments contain almost no contractile material, few mitochondria, vacuoles and lamellar formation.

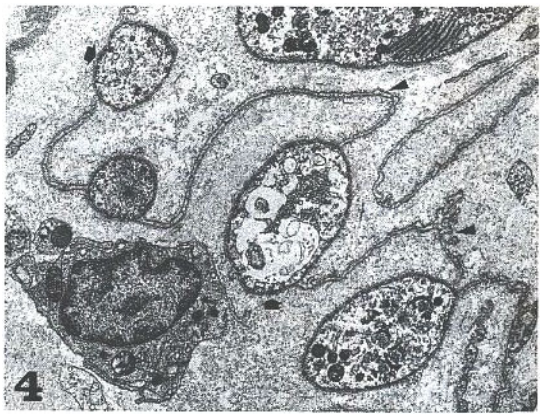

Figure 4. x 14 000. Split fragments of severely atrophied muscle fiber located under common basement membrane. A sharply demarcated area (asterisk) of myofibrillar disarray without recognizable sarcomeres and mitochondria.

membrane was deformed and deeply indented (Fig. 1). Such nuclei were often shrunken and hyperchromatic. Other nuclei, especially those located in small muscle fragments (Fig. 2) were round, pale and showed no features of degeneration. Internal migration of nuclei was also a frequent occurance.

Myofibrillar changes consisted of their disorganization and atrophy. The process was often not uniform, but segmental, focal and irregular. Such changes, however, appeared with varying intensity in almost all examined muscle fibers. Widened subsarcolemmal and intermyofibrillar spaces were also observed in the majority of fibers. Eventually atrophying fragments of fibers were almost devoid of myofibrils (Fig. 2, 3, 4). In such fibers only fragments of filaments, interspersed among masses of sarcoplasmic material were observed.

Disorganisation of sarcomere structure from slight to complete was found in all the fibers examined. In many instances the process was segmentai in pattern, but atrophied muscle fibers showed total or near complete disappearance of sarcomeres and loss of the Z-line occasionally. Core-targetoid formations with Z-line streaming and disintegration as well as-mitochondria! decrease and focal myofibrillar degeneration were unfrequent.

Mitochondria decreased in size and in number (Fig. 2,3, 4), their distribution in the majority of muscle fibers was altered, and they tended to aggregate into small, loose, subsarcolemmal clusters. In markedly atrophied muscle fibers areas completly devoid of mitochondria were observed.

Lipofuscin accumulation occurred in numerous both moderalely and severely atrophied fibers (Fig. 1), often aligned under the sarcolemma.

Myelin structures of diverse size and shape were frequently seen in markedly degenerated myofibers.

Lamellar formations presenting as parallel tubules with undulating borders were frequently observed (Fig. 3, 5). Each such lamellar structure consisted of a variable number of membrane - enclosed parallel cisternae. A dense material between membranes was arranged in symmetrical spiral or herringbone pattern. Lamellar complexes were especially prominent in the more atrophic muscle fibers. Frequently they were found close to the nucleus or near the plasma membrane (Fig. 1,6).

Fragmentation of muscle fibers was prominent. Split fragments (Fig. 2, 3, 4) were variable in size. Such fragments often occurred in clusters usually with the same internal structure, occasionally one of them showed more advanced degenerative changes. 1C is possible that several such fragments were situated under the common basement membrane (Fig. 4). In many cases split fragments were connected to the parent fiber, or were attached through a narrow cytoplasmic and/or basement membrane bridges (Fig. 3,4).

Basement membrane formed redundant projections and loops around highly atrophic fibers and their split fragments (Fig. 2,4). Also basement membrane bridges joining two distant muscle profiles were frequently encountered (Fig. 3,4).

Satellite cells were present in senile muscle although they were not numerous. They appeared similar to those in normal muscle of younger animals. Increased volume together with ribosomes and dense bodies resembling lipo-

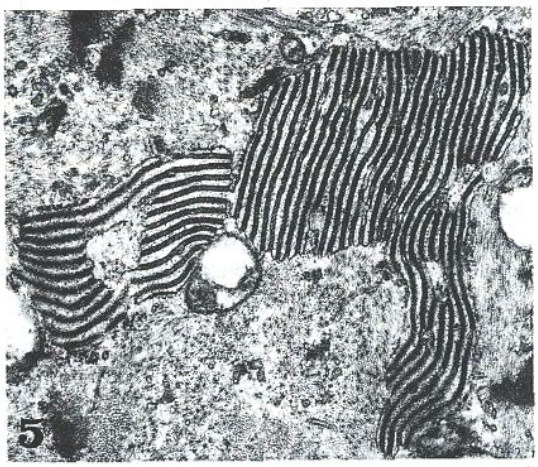

Figure 5. x 35 000. Lamellar inclusion of irregular profile in markedly atrophied muscle fiber. A dense material between membrane limited cisternae is arranged in symmetrical spiral pattern.

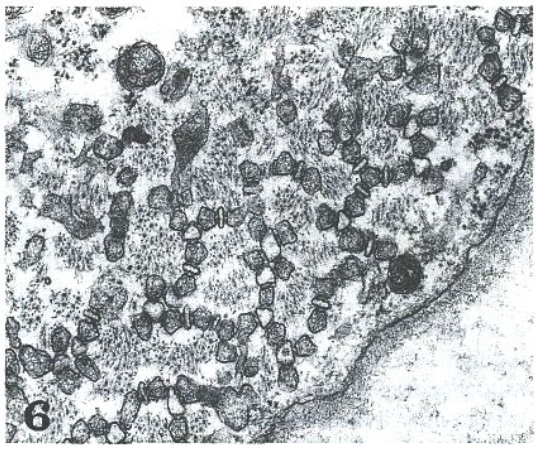

Figure 6. x 87 500. Dilated profiles of triadic junctions.

fuscine granules in cytoplasm were encountered in a few satellite cells.

Necrotic fibers invaded by rnacrophages were infrequent in examined muscles.

Discussion

The diverse ultrastructural changes, seen in senile rat muscle correspond to chronic denervation muscle atrophy. AH observed abnormalities were consistant with those described in the literature on long-standing denervation both in experimental animals [8, 10, 12, 32] and humans [9, 11, 18,26,37], AH those ultrastructural abnormalities, except for tubular formations, have already been described in aged animals [14] and humans [35,39]. Tubular formations were observed in experimentally chronically dener-vated muscle and defined as a "helical complexes" [24], "lamellar inclusions" [18] and "sarcoplasmic reticulum tubular formations" [8]. These inclusions were believed by

Engel and Stonnington [8] to be formed from junctional sarcoplasmic reticulum tubules. Miledi and Slater [24] stressed, that such formations "may serve to identify denervated muscle fibers in some pathological conditions". We are not aware of any reports of similar tubular formation in conditions other than experimental denervation. Their presence in unlesioned senile rat muscle is reported here for the first time.

It is somewhat surprising that in earlier studies on senile rat skeletal muscle such abnormalities were not encountered. The reason may be the older age of our animals and consecutively, the more advanced denervation atrophy as compared to other previously reported studies [35,39].

It is evident from the current electron-microscopic study that the abnormalities occur in senile muscle in both types of muscle fibers types. In many affected fibers the advanced degree of atrophy made ultrastructural evaluation of fiber type impossible [28, 30]. Tomonaga [39] in his report admitted that measurement of Z-band width yielded no uniform results. Nevertheless, he tentatively concluded that fibers showing changes of Z-band streaming were probably alt red fibers, while most of the fibers undergoing myofibrillar degeneration were white or intermediate. Because of advanced degenerative changes seen in our material we were unable to conclude whether there was any preferential atrophy of particular muscle fiber type.

Lipofuscin accumulation was frequently seen in numerous fibers of the tibialis anterior muscle. It is generally accepted that lipofuschi accumulation is prominent in long-lived postmitotic cells of man and animals and is considered to be a useful marker of aging in skeletal muscle [35].

The origin of lipofuscin granules in muscle remains uncertain - they might be lysosomal residual bodies originating in autophagic vacuoles [7] or might originate from mitochondrial lipids. In denervation, lipofuscin accumulation in muscle has been observed even in young patient after long-standing denervation [37] and in young chronically denervated animals [8].

A remarkable feature of senile rat muscle in our material was an extensive fiber fragmentation. Muscle fiber splitting has already been reported in senile rodent muscle by Caccia [4] but no details of this process were given. Muscle fiber splitting is a frequent alteration both in primary myogenic diseases as well as in chronic denervation. It is postulated [9] that nondenervated fibers carrying a heavy workload are particularly susceptible to hypertrophy and consecutive fragmentation. As the denervation progresses all split muscle fibers undergo atrophy. In our material split fragments, variable in size, were often closely apposed under a common basement membrane or connected through narrow cytoplasmic or basement membrane bridges. A similar picture was reported by Schrodt and Walker [32] and by Miledi and Slater [24] in chronically denervated skeletal rat muscle. The latter authors suggested that fragmentation of muscle fibers in denervated muscle might somehow equate to the reverse process of fusion in embryonic life. They speculated that the muscle fiber might break into segments related to the single myoblast cell original nuclei. Indeed, in our material, the majority of nuclei in the split fragments bear some resemblance to muscle nuclei seen in fetal life. The nuclei are prominent round, pale and in no instance resemble the irregular hyperchromatic nuclei observed in denervated muscle.

Redundancy and folding of basement membrane was a common finding in our material. Fujisawa [13] observed redundant basement membrane around atrophic fibers in senile rat muscle. Miledi and Slater [24] reported ectolemmal bridges and loops in experimental conditions after prolonged denervation in the rat diaphragm and gastrocnemus muscle. Papillary projections of the muscle fiber surface and redundant loops of basement membrane around atrophic fibers in denervated rat muscle were also described by Engel and Stonnington [8]. It is accepted in the literature that this phenomenon is of no diagnostic significance and is observed in muscle fiber atrophy regardless of a nature of the disease [5, 9, 23, 24, 26]. Redundant basement membrane processes and bridges could simply represent the wrinkling of the original basement membrane due to shrinking of the atrophying muscle fiber. It is also possible that new basement membrane is produced. Recent observation on increased fibronectin mRNA content in aging fibroblasts as well as elevated amount of detectable fibronectin protein during cellular aging [20] support this hypothesis.

hi our specimens of the senile muscles some satellite cells, although not too numerous, were seen. It is known from numerous studies that satellite cells are present in muscles of senile rats [16], mice [34] and humans [31,39]. It has been shown however that the number of satellite cells decreases in mice and rats of advanced age [1,15,36]. The appearance of satellite cells in the biopsies of muscles from elderly patients were characterised by Tomonaga [39] and considered as degenerated. Satellite cells observed in our material showed no abnormalities. Lipofuscin granules present in the cytoplasm of satellite cells have already been described in the literature in normal muscle of several species, animals [33] as well as humans [6]. Some of satellite cells in our study showed signs of increased activity which correspond to the changes described in denervated muscle [34]. This finding supports the suggestion, that senile muscle retains its ability to regenerate although the extent of regeneration is lower then in young animals [29]. However we did not identify regenerating muscle fibers in our material.

Previous light microscopic examination of the muscles from the same group of rats showed [27], prominent neurogenic changes as well as some myopathic abnormalities. In electron microscopic evaluation the "true myopathic" changes such as necrosis were minimal whereas the rest of findings classified as myopathic in light microscopy (i.e. hyalinization, internal location of nuclei and muscle fiber splitting) may also be found in the neurogenic muscle

atrophy. The constellation of pathological alterations seen in our material supports the latter conclusion. Another difference between our light [27] and electron microscopic evaluation of the same rat senile muscle is that no core/targetoid changes were observed by light microscopy. The core/targetoid fibers identified by EM were, in fact, rather few in the examined muscle and, moreover, sometimes the changes were minima!. The low incidence of such fibers may be indicative of an abortive course of the reinnerva-tion process in senile muscle.

Thus it could appear that there is no single characteristic abnormality or set of morphological changes which characterizes functionally altered senile muscle. Our findings confirm the conclusion that the pathological changes seen in senile muscle might result from the involvement of motoneurons, terminal axons and motor endplates [13,16, 17,31].

The mechanism of skeletal muscle denervation in senescence seems to be very complex and involve different levels of the motor system such as upper and lower motor neurons, nervous pathways, spinal roots, peripheral nerves and neuromuscular junctions. Factors such as disuse may also be important in senile muscle atrophy. However, morphological changes observed by us in senile muscle are probably not the result of inactivation per se, since Ludat-scher et al. [21] found that edurance-trained old mice had even more pronounced structural changes in muscle than their sedentary age-matched controls.

Previous reports on ultrastructure of senile rat muscle [4, 14] do not describe many of the structural changes reported here. The presence of lamellar complexes, intensive muscle fiber splitting and prominent basement membrane alterations in our material may be explained as a more advanced stage of muscle senescence since older animals were examined in this study.

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