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Target-Targetoid Phenomenon of the Human Muscle Fibers

A Histological, Histochemical and Ultrastructural Study

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Summary. Target and targetoid fibers in a muscle biopsy from a patient with paralysis of the deltoid and supraspinatus muscles were studied by light and electron microscopy. The probable cause of the neuropathy was tumor compression.

Target and targetoid change was exclusively confined to hypertrophic or normal-sized fibers. Morphometric evaluation of the target and targetoid fibers showed no significant difference between them. With the electron microscope, up to 4 structural zones were seen in the typical target fiber but many were devoid of either zone 2 (halo) or zone 3, or both.

It was conceivable that focal irregularity and streaming of Z-bands were the primary alterations in the process of target-targetoid fiber formation, and that this phenomenon was induced both by partial residual innervation as well as re-innervation.

Key words: Target-targetoid phenomenon – Histochemistry – Electron microscopy – Z-band alteration – Incomplete innervation.

Introduction

The target fiber was first described by Engel (1961) in muscle biopsies showing the classical features of denervation. Target fibers can generally be regarded as a specific findings in denervation atrophy of the neuronal type (Engel, 1961; Kovarsky et al., 1973). However, they may also be demonstrated in other pathological conditions, such as congenital myopathy (Schotland, 1967) and familial periodic paralysis (Schafig et al., 1969).

A target fiber has usually three distinct concentric zones. The central zone lacks striation and contains longitudinally oriented wave-like threads. Occasion-

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ally an intermediate zone is missing, and the fiber with central and outer zones is said to be targetoid. Schmitt and Volk (1975) described these histological findings in a case of denervation atrophy and suggested a continuous transition between the different lesions. More recently Schmitt (1976) has regarded the development of the target as a secondary degeneration following interruption of the nutritional supply, after morphometric investigation. Despite the considerabe amount of morphological, histochemical and ultrastructural information on target fibers, their morphogenesis and pathogenesis are still unknown.

The purpose of this paper is to describe histological, morphometric and electron microscopic studies of target-targetoid fibers in order to gain further insight into the morphogenesis of these structures.

Material and Methods

A biopsy of the right deltoid muscle was taken, using an isometric clamp, from a 33-year-old male. This man had weakness of the deltoid and supraspinatus muscles, presumably due to tumor compression of the cervical nerve plexus.

The biopsy was fixed in 10% formalin. Section were stained with hematoxylin-eosin, phosphotungstic acid hematoxylin (PTAH), periodic acid Schiff, Azan-Mallory and Gomori tirchrome.

Another fragment was immediately frozen in cooled isopentane at −70° C. Sections were stained with modified Gomori trichrome and with methods for demonstration of succinic dehydrogenase (SDH), NADH-diaphorase, phosphorylase, myofibrillar ATP-ase and cholinesterase.

A third fragment was fixed in 2.5% glutaraldehyde solution, postfixed in osmic acid, dehydrated and embedded in Epon 812. Ultrathin sections were treated with uranyl acetate and lead citrate.

Results

Muscle fibers, in cross section, ranged from 10 to 110 μ in diameter. Irregularly scattered groups of 3 to 10 or more small fibers were found interspersed between the target fibers. The majority of these small fibers showed angulation, a characteristic feature of denervation atrophy.

The intramuscular fat and blood vessels were normal, and intramuscular nerves and muscle spindles were not seen. In transverse sections three zone target fibers (Fig. 1a) and two zone targetoid fibers were seen. Some fibers contained targets in the periphery. These were called peripheral target fibers (Fig. 1b). Two zone fibers without an intermediate zone were regarded as targetoid fibers (Fig. 1c).

The central zone stained dark red in Gomori trichrome and contained variable numbers of coarse wavy threads and granular material, which seemed to coalesce to form a large core (Fig. 1g). In longitudinal sections, the core did not extend over the whole length of the muscle fiber; sometimes it was very small and restricted to a few sarcomeres. In serial sections, typical target profiles seemed to change into targetoid one. Finally the fiber again returned to target profile, or became a normal-appearing muscle fiber.

In a population of 1167 randomly chosen muscle fibers from a cross section, 1.0% of the fibers were found to be target and 4.6% targetoid; 4.0% peripheral target fibers were seen. The distribution of the these fibers over the whole

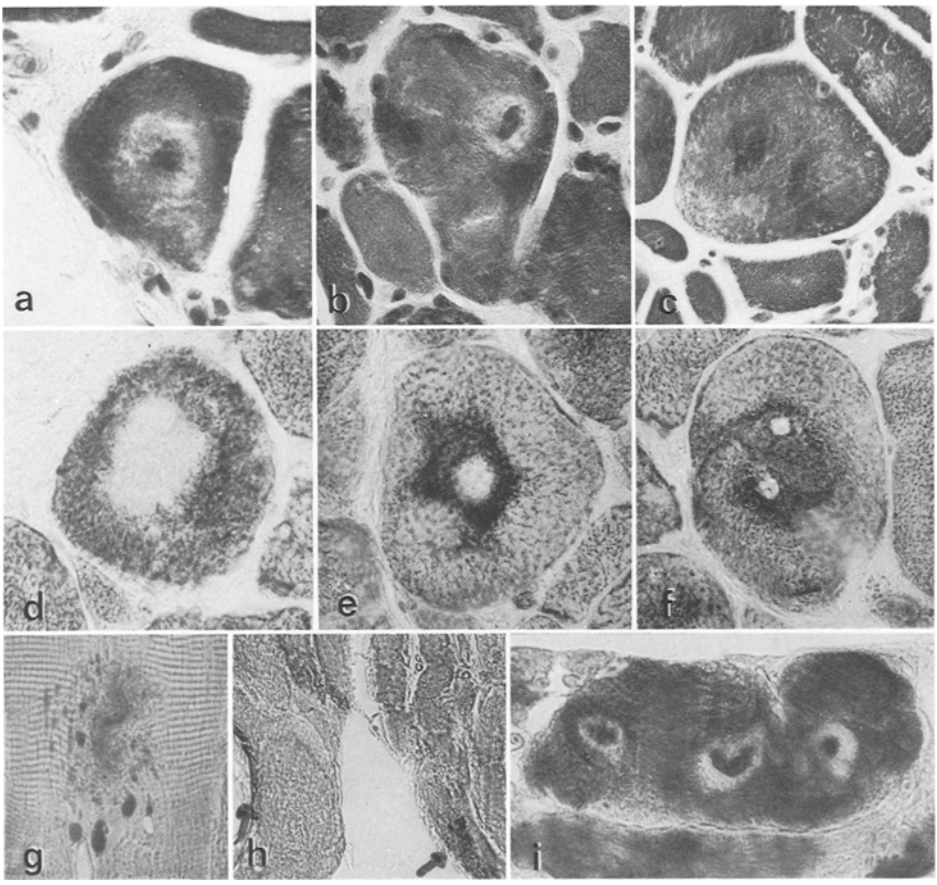

Fig. 1. **a** Target fiber; **b** peripheral target fiber; **c** targetoid fiber; **d** targetoid fiber; **e** target fiber; **f** two targets in a single fiber. **g** Many wave-like dense bands and granules in the central area in a muscle fiber; **h** cholinesterase activity is seen in the atrophic and hypertrophic fibers; **i** longitudinal section of targetoid fiber. **a–c** Gomori trichrome; **d–f** modified Gomori trichrome. **g** PTAH, **h** cholinesterase, **i** PTAH

cross section was not constant; some areas had a predominance of one type. The mean diameter of the target fibers was 63 μ; the mean of the targetoid was 74 μ; the mean of the peripheral target fibers was 55 μ; the mean of the fibers without target-targetoids was 27 μ. The target and targetoid change appeared to be present almost in the normal-sized or hypertrophic fibers, and only rarely in the atrophic fibers (Table 1).

Histochemically the central zone contained markedly reduced or absent enzyme activity and the next zone, when present, gave evidence of intense activity. The outer zone showed an occasionally moth-eaten appearance, or irregular reticular networks. Some fibers had two or more targets. Most target and targetoid fibers seemed to be type 1 fibers, but differentiation into type I and type II was not always clear.

Table 1. Results of the measurements of target, targetoid, peripheral target and other fibers

	Numer	Mean diameter	Range
Gomori trichrome			
targetoid	56	74 μ	45–115 μ
target	12	63 μ	35– 85 μ
peripheral	47	55 μ	20– 85 μ
other fibers	1052	27 μ	10– 75 μ
SDH			
targetoid	68	72 μ	40–120 μ
target	56	73 μ	45–105 μ
other fibers	1310	31 μ	10– 92 μ

The enzyme profiles of target and targetoid fibers were not comparable to those stained with Gomori trichrome; the histochemical central zone involved both the central core and the intermediate zone seen in conventional histological preparations. The intermediate zone with high enzyme activity, was not identified by histology (Fig. 1d–f). Thus the morphometric measurements were also performed on histochemical slides, giving a value similar to those found on the histological preparations. Cholinesterase activity was demonstrated in the end-plates of a few fibers with targets as well as those of the fibers without targets (Fig. 1h).

Electron microscopically zone I (central core in histology) varied in structure; the zone consisted chiefly of electron dense material with a fibrillary structure, comparable to Z-band material in its electron density (Fig. 2). Cellular organelles were scanty and only glycogen granules were seen. However, some cores had large numbers of wave-like dense bands separated by bundles of myofilaments (Fig. 3 and 4a). At a higher magnification, the band contained large numbers of actin-like wavy filaments which were usually parallel to the long axis and lay 60 to 150 Å apart, measuring about 60 Å in diameter. The dense bands always lacked the tetragonal arrays characteristics of normal Z-lines or nemaline rods (Fig. 4c). The myofilaments between the dense bands were often irregularly oriented but might be normal in structure and size, and the usual relationship between thick and thin filaments was often preserved. Some myosin filaments appeared to insert into the dense bands (Fig. 4b). The other cores consisted of large collections of tiny dense bands and streaming of Z-lines interspersed by normal or slightly disoriented myofilaments. Many mitochondria and dilated sarcotubular profiles were present within or around the core.

Zone 2 (halo in histology) consisted of severely disorganized myofilaments including Z-band fragments and sarcoplasmic reticulum, which were often grouped together, the latter usually having a swollen appearance. This zone contained glycogen granules. The mitochondria were variable in number. The targetoid fiber was devoid of zone 2. Some atypical target fibers had the zone on one side and were devoid of it elsewhere. The border between zone 1 and zone 2, if present, was usually ill-defined.

Zone 3 (high enzyme activity zone) varied in different fibers. The normal sarcomere pattern was often preserved. The Z-lines were often wave-like in

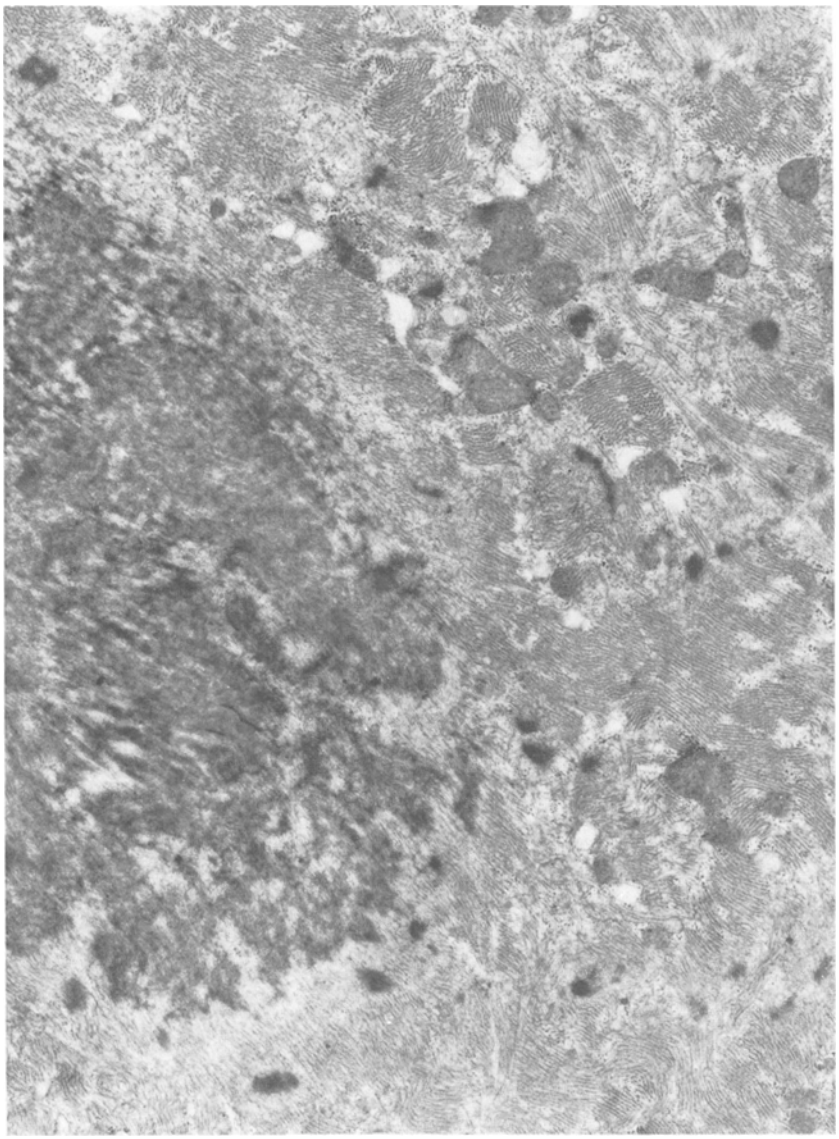

Fig. 2. The central dense bands consist exclusively electron dense material with fibrillary structure. Myofilaments adjacent to core area are relatively well oriented in the upper field. Myofilaments are disoriented in the lower field. ×16,000

appearance, disrupted, and increasingly disoriented near the central zone. Mitochondria were usually increased in number and small mitochondrial aggregations were observed. Their structure was not remarkable. The sarcotubular systems varied greatly in number and shape in the different portions.

The outer zone showed the least abnormality. The I and A bands were usually normal. The Z-lines occasionally displayed a zig-zag appearance. Sarco-

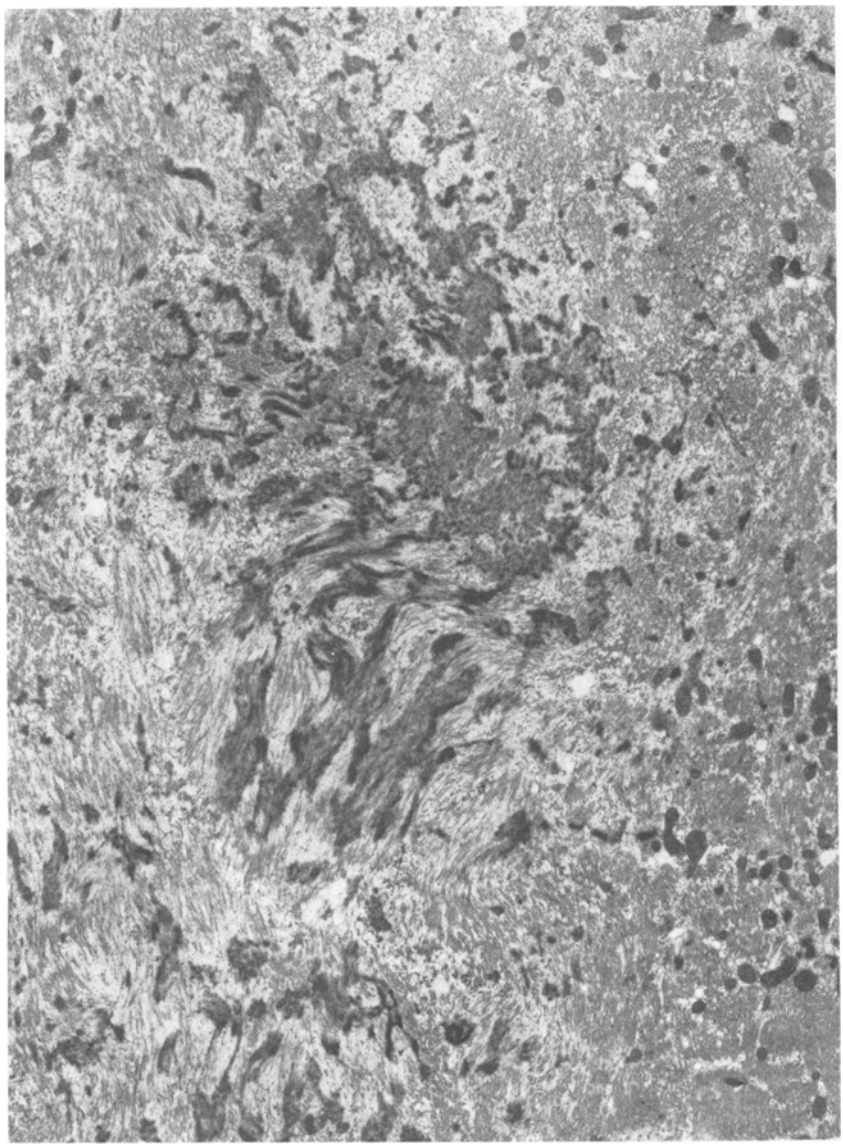

Fig. 3. Zone 1 consists of large collections of wavy dense streaks, interspersed by thin filaments and normal-appearing myofilaments with hexagonal arrays. In the right field the core is directly continuous with zone 3, and zone 2 is absent but in the left zone 2 present. × 10,000

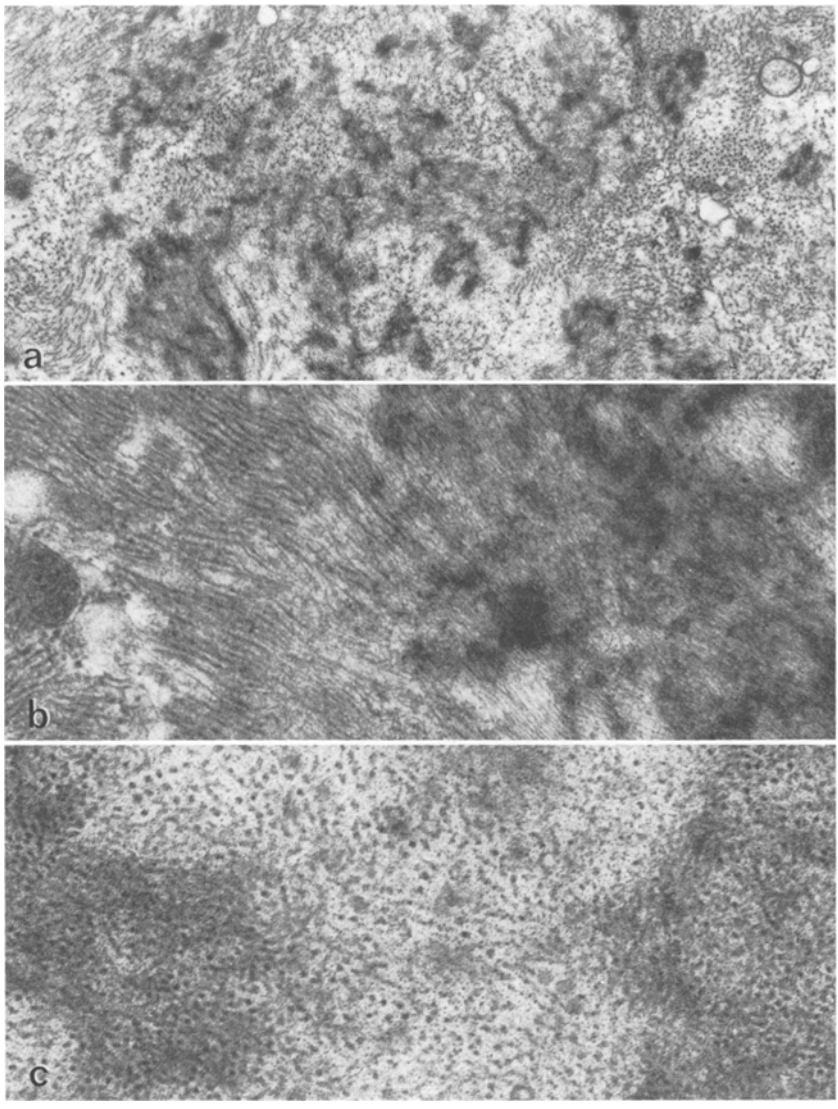

Fig. 4. a Myosin and actin filaments are seen between dense streaks. The usual relationship between thick and thin filaments may be preserved. **b** Myosin filaments appear to insert into the dense streaks. **c** A part of dense band does not have tetragonal arrays characteristic of normal Z-lines or nemaline rods. The diameters of the fibrils are about 60 Å, 150 Å apart. **a** ×30,000; **b** 29,000; **c** ×48,000

tubular systems were often dilated. Vacuoles with multilamellar membranes and/or amorphous material were present. Duplication and papillary projection of the sarcolemma were occasionally seen but were not considered significant.

In most fibers without target-targetoid change, sporadic irregularity of the Z-lines, irregular arrangements of myofilaments and decreased numbers of mitochondria were seen. Only a few cytoplasmic and sarcoplasmic bodies were present. In a few fibers, autophagic vacuoles and lipid bodies were also seen. Motor end-plates were not seen in any of the blocks examined.

Discussion

Light microscopically target fibers are said to have three distinct concentric zones in the central portion of the affected muscle fiber; targetoid fibers lack the intermediate clear zone. Histochemical and histological profiles of the target-targetoid fibers are not comparable and the morphometric measurements were therefore performed on both histological and histochemical slides. The results from each type of preparation reveal a significant difference between the values for target-targetoid fibers and the other fibers; the target-targetoid zones were confined to normal-sized or hypertrophic fibers, and rarely present within atrophic fibers. There is no significant difference between the values for the target, targetoid and peripheral target fibers. Ultrastructural sections showed great variation, and zones 2 and 3 may be absent. Zone I is sometimes only partially bordered by zone 2 (Fig. 3). These findings suggest that at least in our material there is no fundamental difference between target and targetoid muscle fibers. The transitional fiber alteration (Schmitt and Volk, 1975; Schmitt, 1976) was not seen in this case.

The histology and ultrastructure of the target and targetoid fibers in this case were somewhat different from those of Kavarsky et al. (1973), Tomonaga and Sluga (1969) and Schmitt (1976). The alterations here appear to be much more variable, especially in the central part. There is no distinct intermediate zone. Some central cores have much granular substance in addition to wave-like dense bands (Fig. 1g). Some of the wave-like dense streaks seem to be composed of a focal irregularity of the Z-lines closely resembling the earliest Z-disk alteration in Z-band streaming. These coalesce to form a larger and classical target structure in the advanced stage, indicating the evolution of core formation.

Thin filaments within the dense streaks are measured at about 60 Å in diameter and are probably actin filaments. Myosin filaments can not be identitied, which is not consistent with Coster's observation (1976).

It is generally accepted that zone I is probably derived from Z-line material (Tomonaga and Sluga, 1969). The dense streaks are considered to be a nonspecific alteration since they have been observed in multicore disease (Heffner et al., 1976; Mukoyama et al., 1973), central core disease (Santa and Engel, 1973), in the cytoplasmic body (Macdonald and Engel, 1969) and in muscle biopsies from healthy young adults (Metzler et al., 1976). They are different in structure from nemaline rod or Z-line itself since they lack the tetragonal arrays of filaments. Association of target-core fibers and rods has been reported

by Afifi (1965). The histochemical and ultrastructural characteristics of target and central core are similar and they may have a similar pathogenesis (Karpati et al., 1971; Santa and Engel, 1973).

The target fiber is regarded as a characteristic manifestation of the denervated myofiber subsequent re-innervation (Engel, 1961; Kovarsky et al., 1973) or of a long-standing incomplete denervation (Tomonaga and Sluga, 1969). However, experimental denervation of the peroneal muscle induces muscle atrophy alone with disappearance of histochemical fiber classification but no tendency to induce target or core lesions (Kinoshita, 1973). Some authors (Reuck et al., 1974; Dubowitz, 1970) consider that the re-innervation process plays an important role in the formation of the lesions.

Target fibers have also been described in muscle biopsy specimens from congenital myopathy (Schotland, 1967), familial periodic paralysis (Schafig et al., 1969) and experimental models (Dubowitz, 1970). The possibility of denervation in these situations has not been eliminated. There are profound changes in the discharge pattern of motor nerves supplying tenotomized muscle (McMinn et al., 1967) and denervation prevents formation of cores following tenotomy (Engel, 1966). In both our and Coster's case, cholinesterase activity is demonstrated in several end-plates of hypertrophic, atrophic and target-targetoid fibers, indicating that these fibers are innervated. The target or core structure first develops between 10 to 20 days in experimentally triethyltin-treated rats and by 23 days the lesions are more numerous and better developed. Nerve fiber degeneration, however, is not seen until 20 days and there is no evidence of axonal sprouting or of re-innervation. In addition, the intensity and distribution of cholinesterase activity in the subneural apparatus of the motor end-plates is normal (Graham et al., 1976).

The nerve ending of the neuromuscular junction containes some core vesicles from which a certain trophic substance may be discharged by nerve stimulation into the sole plate. This process might have an important trophic effect on muscle fibers (Yonezawa et al., 1973).

There is evidence for true denervation and re-innervation in this case at least. It is possible that incomplete innervation is a primary source of target fiber formation in muscle in developing denervation and re-innervation.

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