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ULTRASTRUCTURAL STUDIES ON MUSCULAR ATROPHY IN MAREK'S DISEASE II. MUSCULAR LESIONS IN SPONTANEOUS CASES

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The skeletal muscles of 14 spontaneous cases of MD were investigated by light and electron microscopy. The muscular lesions of MD were classified into 3 categories: Category I-myotrophic changes, Category II-myodegenerative changes, and Category III-neoplastic proliferative changes. The present investigation was focused on the Category I lesions.

Light microscopically, the atrophic fibers were sparsely distributed between the normal sized muscle fibers in mild cases. In the advanced stage, there were often clear-cut groups of atrophic fibers, and finally, groups of variable numbers of atrophic fibers were extensively seen.

Electron microscopically, irregularity of the fiber outline was commonly seen. The process of myofibrillary breakdown was conspicuous and seemed to start with disruption of the regular alignment of the myofilaments and streaming of Z-bands. The intermyofibrillar spaces were enlarged. The central nuclei with irregular shapes were often arranged in a row and increased in number. Targetoid fibers, honeycomb-like structures, and satellite cell activity were also observed.

These findings were similar to those seen in experimental denervation. Therefore, the authors attributed these atrophic changes to neurogenic origin.

Key words: *atrophic fibers, myofilaments, targetoid fibers, honeycomb-like structures*

INTRODUCTION

Marek's disease (MD) is a lymphoproliferative and neuropathic disorder induced by Marek's disease virus (MDV). Chickens with MD often show neurologic signs such as paresis of the legs and drooping of the wings. The peripheral nerves as well as

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the visceral organs are frequently involved in the lymphoproliferative, tumorous and non-tumorous responses.

FUJIMOTO and OKADA (1977) suggested that degenerative changes in the peripheral nerves in field and experimental cases of MD are primary demyelinative changes analogous to experimental allergic neuritis from light and electron microscopy. The inability to stand or leg weakness in chickens may be due to demyelination of the peripheral nerves. Despite the frequent occurrence of locomotor paresis, reports on muscular changes of paralysed limbs are limited to a few records.^{15,25,29)} WIGHT (1966) and OKADA (1970) classified muscular lesions of MD into 3 categories independently. Recently, we have investigated the skeletal muscular lesions in many cases of MD and found that most of them consisted of atrophic changes, which were considered to be of neurogenic origin. Therefore, in order to clarify the muscular lesions in MD, we conducted a comparative study on experimental denervation (report I) (8) and the skeletal muscular lesions of spontaneous cases of MD (report II) by light and electron microscopy.

MATERIALS AND METHODS

Muscles were collected from the *M. pectorales superficiales*, *M. gastrocnemius*, *M. semitendinosus*, *M. latissimus dorsi*, *M. serratus dorsalis*, and *M. gracilis* of 14 birds affected with spontaneous MD. Examined birds consisted of White Leghorn (Shaver) hens which were collected from the Hokkai Starchick Company or the East Poultry Farm, and the ages ranged from 116 to 160 days. The birds were killed by depletion of blood.

Light microscopy

Tissue samples from the muscles (described above), peripheral nerves (*N. ischiadicus*, *Pl. brachialis*, and *N. vagus*) and various parts of the visceral organs were fixed in 10% formalin and embedded in paraffin, following the usual dehydration procedure. Sections 4 μ m thick were stained with hematoxylin and eosin (H & E).

Electron microscopy

The muscle tissue samples were stretched and fixed in 2.5% cacodylate-buffered glutaraldehyde for 30 minutes. Then they were cut into small pieces (1mm) and fixed again. After that, they were postfixated in 1% phosphate-buffered osmium tetroxide and dehydrated in graded ethanol series and embedded in Epon 812. Blocks were cut by a glass knife with a PORTER-BLUM MT-2B ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate. Observations were made at 75 KV with an HU-12A HITACHI electron microscope.

RESULTS

Light microscopic findings

The muscular lesions of spontaneous cases of MD were classified into 3 categories

according to their characteristics: Category I-myotrophic changes, Category II-myodegenerative changes, and Category III-neoplastic proliferative changes of lymphoid cells. The present investigation was focused on the Category I lesions. According to the grade of the myotrophic changes, we classified the 14 cases into mild (4 cases), moderate (3 cases) and severe (7 cases) cases as illustrated in table I.

Mild cases: The atrophic fibers were located sparsely between the normal sized muscle fibers. In transverse sections, the atrophic fibers showed a decrease in size and were angulated (fig. 1). They stained markedly eosinophilic.

TABLE 1 *Microscopic changes of spontaneous cases of MD*

Case No.	Protocol No.	skeletal muscles			PN ¹⁾	visceral organs
		atrophy	degeneration	lymphoid cell proliferation		
1	E. 7828	+ ²⁾	—	+	R ⁴⁾	T _{II} ³⁾
2	E. 7838	+	—	+	T _{II} +R	T _{II}
3	E. 7869	+	±	+++	T _{II}	T _{II}
4	E. 7829	+	—	++	T _{II}	T _{II}
5	E. 8142	++	+	+++	R	T _{II}
6	E. 8143	++	—	+++	T _{II}	T _{II}
7	E. 8221	++	—	+	T _{II}	—
8	E. 8220	+++	—	+	T _{II} +R	T _{II}
9	E. 8226	+++	+	—	R	—
10	E. 8230	+++	—	—	R	—
11	E. 8204	+++	+	+++	T _{II}	T _{II}
12	E. 8187	+++	+	+++	—	·
13	E. 8202	+++	—	—	R	—
14	E. 8211	+++	+	+	T _{II} +R	—

1) PN: Peripheral nerves

2) Grade of lesions: —; no changes, +; mild, ++; moderate, +++; severe

3) T_{II}: Tumorous type lesions (Type II lesions)

4) R: Non-tumorous response type lesions

* Classification of MD lesions according to Fujimoto et al. (1971)³⁾.

Moderate cases: The shape of the fibers in transverse sections changed to polygonal shape. The nuclei were small, pyknotic and were often present in the center of the fiber, although even normal fibers had such structures in chickens. Small sized atrophic fibers were moderately distributed between fibers of normal size or fibers that were often swollen, either singly or in groups of variable numbers (fig. 2).

Severe cases: Most of the muscular fibers became atrophic, and various small sized fibers were present among the polygonal normal sized or swollen fibers. The shape of these small sized fibers was irregular and angulated. Nuclei were often present in the center of the fiber and apparently increased in number (fig. 3). The diameter of the fibers was extremely reduced as compared with that of the control. There were clear-cut groups of atrophic fibers (fig. 4). Finally, groups of variable numbers of atrophic fibers were seen without great variation from one field to another.

Electron microscopic findings

The most conspicuous changes of the atrophic fibers were the process of myofibrillary breakdown, such as disruption of the regular alignment of the myofilaments and streaming of Z-bands. Disappearance of myofilaments and disorganization and loss of Z-bands were evident (fig. 5). The contour of the sarcolemma was irregular and had numerous indentation due to devoid of contractile material in the subsarcolemmal sarcoplasm of the muscle fibers. The contractile myofilaments were also disappeared around the area of the myonucleus. Dilatation of the sarcoplasmic reticulum and myelin figures were also seen (fig. 5).

In the more advanced stage, the myofibrillary damages were more extensively observed. Streaming of the Z-bands often showed a zigzag appearance. T canals were often prominent, and autophagic vacuoles were also seen (fig. 6).

In some areas, myoatrophic fibers in different stages were also observed among the normal fibers (fig. 7). Atrophic fibers in the advanced stage showed apparently increased nuclei and mitochondria (fig. 7). In the area of the fasciculus of atrophic fibers, the fibers also had increased central nuclei which were arranged in a row. The contour of the sarcolemma was irregular and the diameter of the fibers was reduced. The nuclei were irregular in shape and they had large nucleoli and dispersed chromatin (fig. 8). The fibers contained focal lesions which were characterized by irregular myofilaments and whorl appearances with spotted disorganized Z-bands (figs. 8 & 9). They appeared as so-called "targetoid fibers", or "coiled fibers".

In the more advanced stage of muscular atrophy, the outline of the sarcolemma was irregular and indentation was so deep that it formed numerous folds of basal lamina (Figs. 10, 11, 12, & 13). Sometimes vacuolization of the sarcoplasmic reticulum was prominent (fig. 10). The central nuclei were obviously increased (fig. 11) and atrophic fibers were seen in groups. Satellite cells frequently seen on muscle

fibers were located between the sarcolemma and the basal lamina of a muscle fiber. Some of the satellite cells showed signs of increased activity and were separated from their parent muscle fibers (figs. 12 & 16).

In the atrophic muscles, there were degenerated motor endplates. The axon terminal showed disappearance of synaptic vesicles and was often replaced by Schwann cells. The spaces between the muscle fiber and the axon terminal were slightly enlarged and the electron opaque basal lamina appeared more widened (figs. 13 & 14). Reinnervation may be the result of regrowth of axons within Schwann cell tubes (figs. 13 & 14). In the atrophic muscles we often found honeycomb-like structures. They took the form of a more or less regular three-dimensional interconnecting array of tubules (figs. 15 & 16). They were often connected to vacuoles of the T system (fig. 16).

Muscular changes of categories II & III were also observed in the present spontaneous cases of MD (table I).

The myodegenerative changes (category II) corresponded to Zenker's hyaline degeneration and waxy or granular degeneration under light microscopy. The hyaline fibers showed marked hypercontraction of the myofibrils leading to the formation of contraction clumps or bands in one portion of the fiber (figs. 17 & 18). They also showed a complete absence of contractile element. The contents of the fiber underwent progressive disorganization and fragmentation, but the basal lamina was still preserved (fig. 18).

Category III consisted of a proliferation of various kinds of lymphoid tumor cells accompanied by some histiocytes, including macrophages (fig. 18). These cells also belong to T type lesions.³⁾

The muscle spindles showed almost no changes.

DISCUSSION

Previous reports on muscular lesions of MD refer to macroscopic tumorous nodules or microscopic proliferation of tumorous lymphoid cells.^{11,14,15,25,,28,29)} The lesions comprised invasion of muscle by neoplastic cells and did not represent true myopathies. WIGHT (1966) and OKADA (1970) classified the muscular lesions of MD into 3 categories or types independently. Category I (neurogenic atrophy) by WIGHT (1966) and type II (neurogenic and disuse atrophy) by OKADA (1970) both indicated atrophic changes of the muscle. Recently, we encountered unexpectedly many cases of atrophic changes of the muscles which were considered as neurogenic in MD.

Under light microscopy, the atrophic fibers showed a decrease in size and were angulated. They were located in variable frequency in different fields between the normal sized muscle fibers. In the advanced stage, the nuclei were often present in the central area of the fibers and were apparently increased in number. There were often clear-cut groups of atrophic fibers. Fibers of normal size or hypertrophic fibers

were disseminated between atrophic fibers. Finally, groups of variable numbers of atrophic fibers could be seen without great variation from one field to another. These changes were similar to those seen in the denervated muscles.^{7,8,10,27)} Under electron microscopy, one of the most common features were the irregularity of the fiber outline, which often formed numerous folds.^{7,10)} In the periphery of the muscles; an area devoid of contractile materials appeared, and this seemed to cause the indentation of the sarcolemma. The process of myofibrillary breakdown was conspicuous and seemed to start with the disruption of the regular alignment of the myofilaments and the streaming of Z-bands.^{10,12,16)} These changes represented the atrophic fibers. The myofibrils in the atrophic fibers were usually thinner than those in normal fibers and became misaligned and disrupted. The sarcoplasmic reticulum and T system became prominent.¹³⁾ There was frequently an accumulation of autophagic vacuoles.^{1,20)} The intermyofibrillar spaces were enlarged. The central nuclei were often arranged in a row and increased in number.²⁷⁾ The above findings were similar to those seen in the denervated muscle fibers.^{1,5,7,8,12,13,16,20,24,26,27)}

In the atrophic fibers, there were degenerated motor endplates. The axon terminal showed disappearance of synaptic vesicles^{9,17,18,19)} and it was often replaced by Schwann cells.⁹⁾ Then new neuromuscular junctions were found at the site of the original endplate.⁹⁾ Reinnervation may be the result of regrowth of axons within Schwann cell tubes.

Architectural changes in the myofibrillary system such as "target", "targetoid" or "coiled" fibers were observed in the present study as well as in the experimental denervation.^{22,23,30)} The term "target fibre" was introduced by ENGEL (1961).²⁾ However, these fibers are not specific for denervation since they may also be present in myopathic disorders.²³⁾

Satellite cells were frequently seen muscle fibers in the present cases. It is now generally considered that the satellite cells can be stimulated to undergo myogenic differentiation^{1,21)} though little is known of the factors which stimulate this process.¹⁾ HESS & ROSNER (1970) considered that the satellite cells arise from portions of denervated muscle fibers themselves, elongate and become myoblasts. In our cases, the satellite cells were clearly activated. These findings, therefore, suggested that the satellite cells participate in muscular regeneration.

In our cases, honeycomb-like structures were also seen. These structures were first described in denervation rat muscle, by PELLEGRINO & FRANZINI (1963). However, as they have been described in many conditions,¹¹⁾ their significance is yet unknown, but PRICE¹⁰⁾ has proposed that they are associated with the loss or absence of normal neural influence.

Categories II and III lesions were often found in our MD cases as shown in table 1. These changes were similar to the classification of WIGHT (1966) and OKADA (1970). The cause of the myodegeneration is still unknown. Some were considered

as due to secondary origin.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1 Case No. 3 *M. gracilis* Mild case. The small sized and angulated atrophic fibers are located sparsely between the normal ones. H. & E. stain $\times 150$
- Fig. 2 Case No. 10 *M. pectorales superficiales* Moderate case. Polygonal shaped atrophic fibers are moderately distributed between normal sized or often swollen fibers. H. & E. stain $\times 150$
- Fig. 3 Case No. 14 *M. pectorales superficiales* Severe case. Most of the muscle fibers become atrophic, and various small sized fibers are present. H. & E. stain $\times 150$
- Fig. 4 Case No. 12 *M. pectorales superficiales* Severe case. The diameter of the fibers is extremely reduced. Clear-cut groups of atrophic fibers are present. H. & E. stain $\times 150$

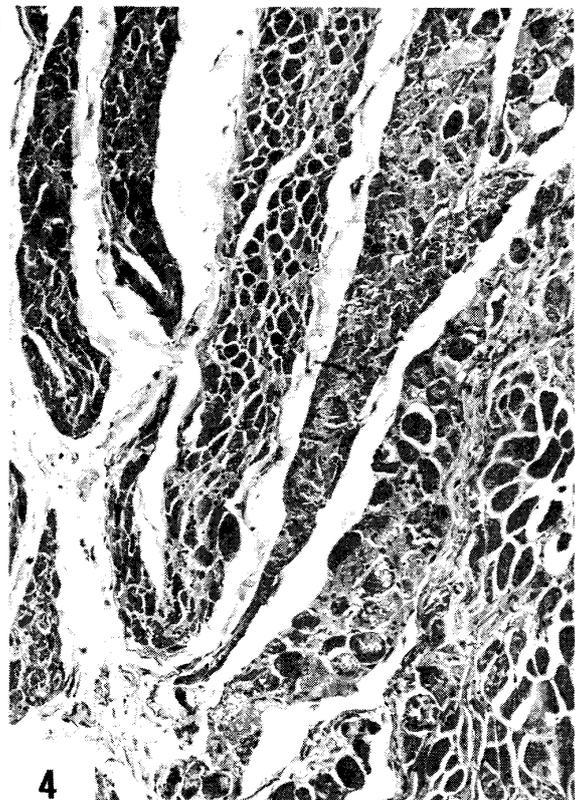
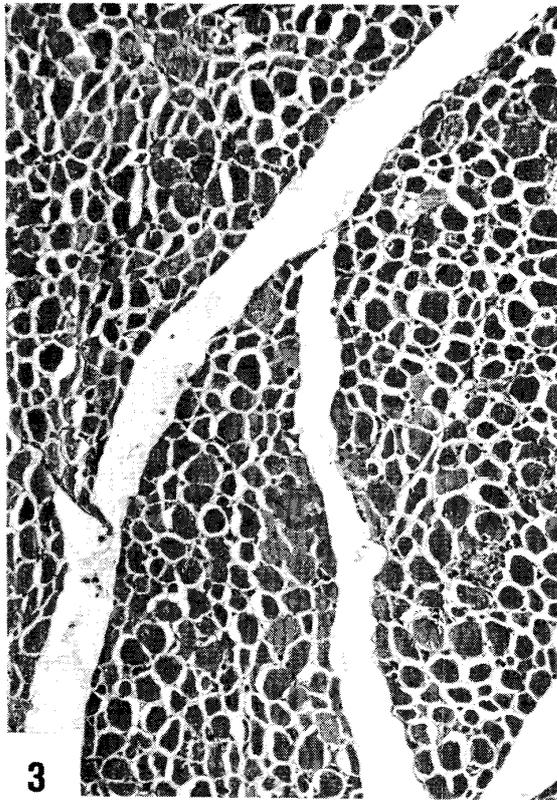
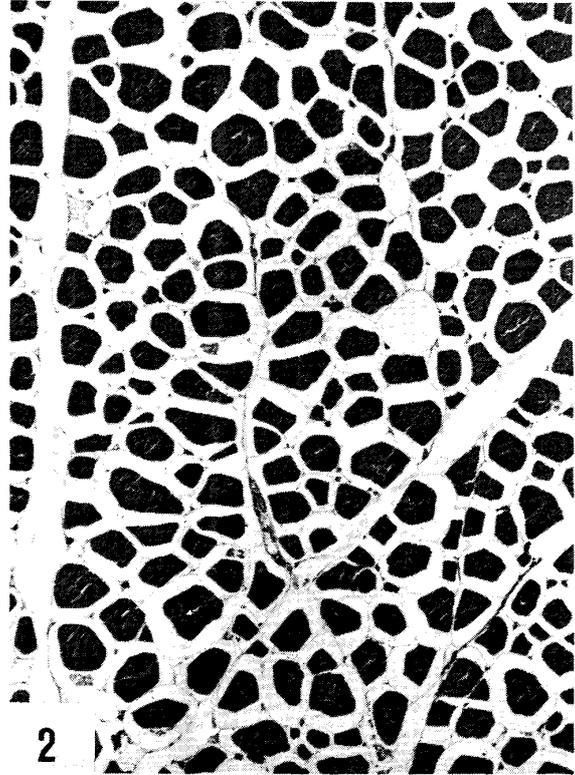
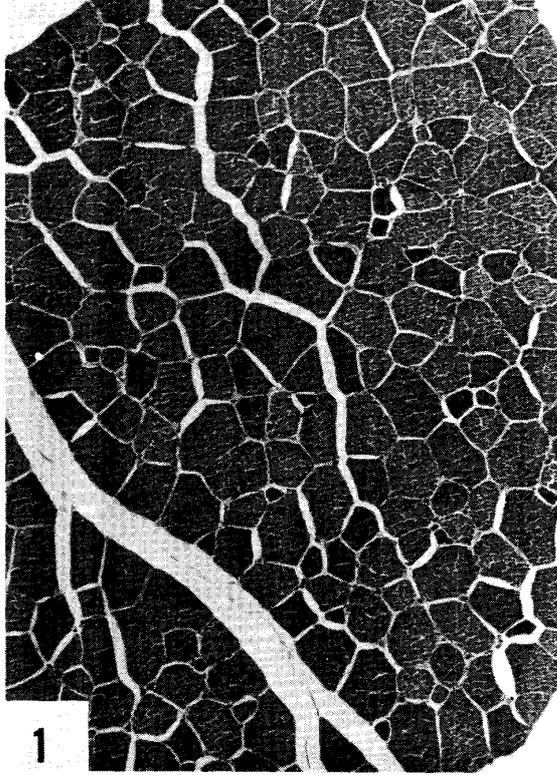


PLATE II

- Fig. 5 Case No. 13 *M. pectorales superficiales* The atrophic fibers. Disappearance of myofilaments, disorganization and loss of Z-bands are evident. $\times 13,000$
- Fig. 6 Case No. 14 *M. pectorales superficiales* The myofibrillary damages are extensively observed. Streaming of the Z-bands shows zigzag appearance. T. canals are prominent and autophagic vacuole are seen. $\times 13,000$

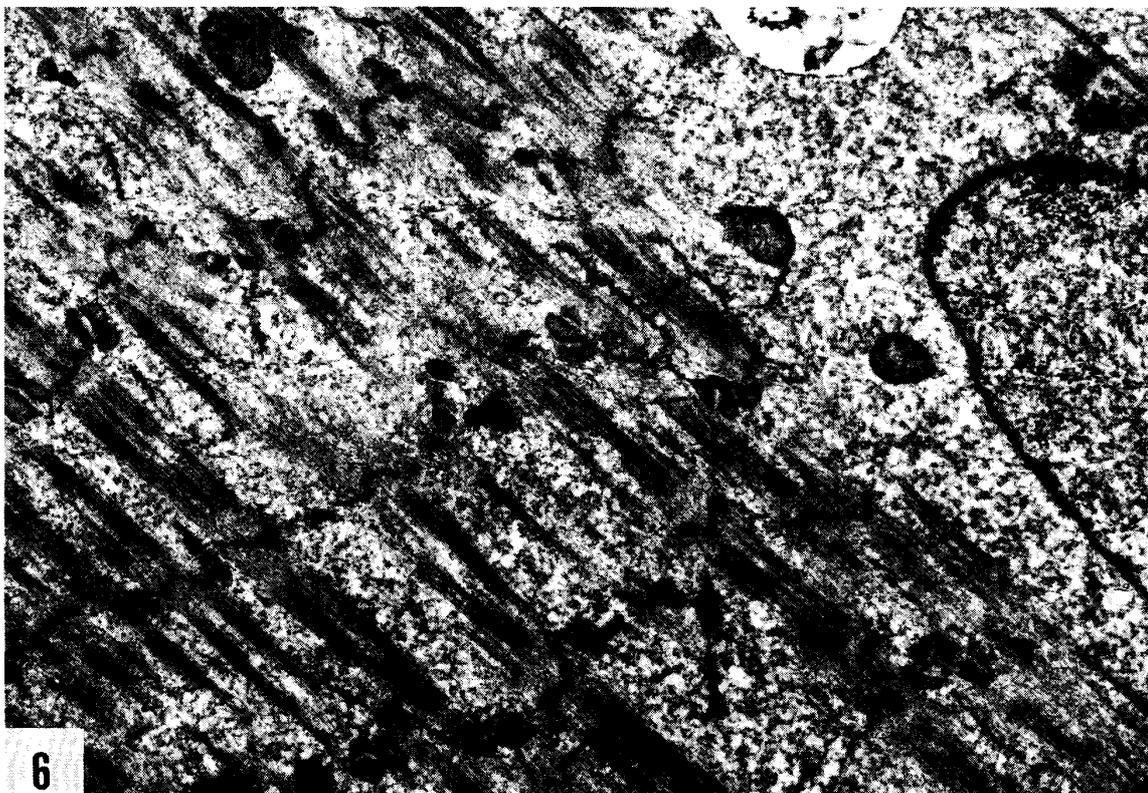
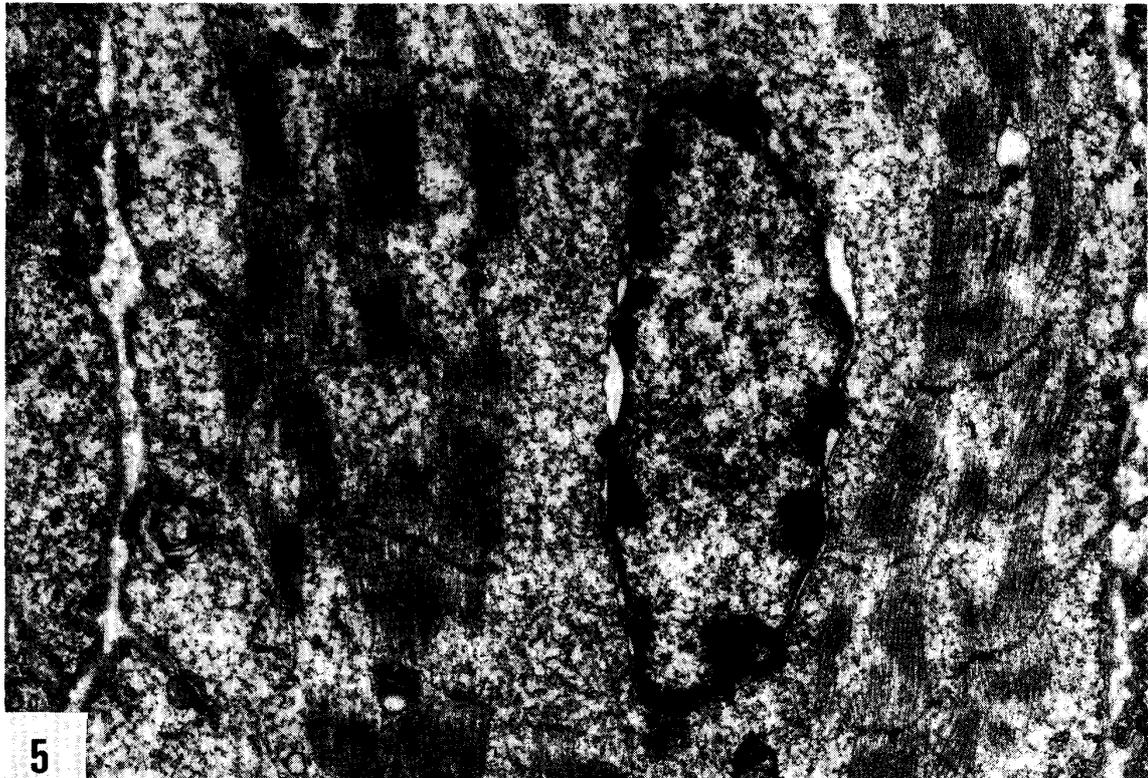


PLATE III

- Fig. 7 Case No.12 *M. pectorales superficiales* Myotrophic fibers in different stages are observed among normal fibers. \times 7,500
- Fig. 8 Case No. 12 *M. pectorales superficiales* The fasciculus of atrophic fibers. The fibers have increased central nuclei arranged in a row. \times 3,300

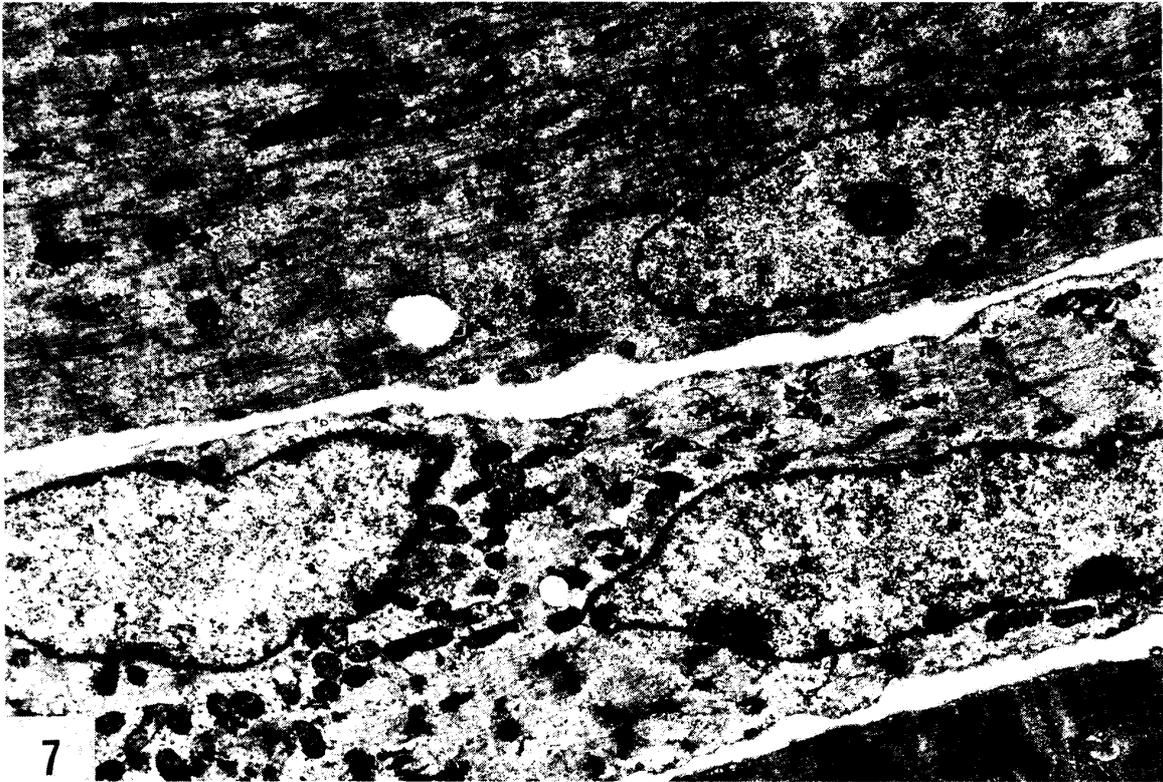


PLATE IV

- Fig. 9 Case No. 12 *M. pectorales superficiales* Targetoid fibers. Focal lesions are characterized by irregular myofilaments and whorl appearances with spotted disorganized Z-bands. $\times 8,400$
- Fig. 10 Case No. 12 *M. pectorales superficiales* The severely atrophied muscle fiber. The outline of the sarcolemma is irregular. Vacuolization of the sarcoplasmic reticulum is prominent.
 $\times 13,000$

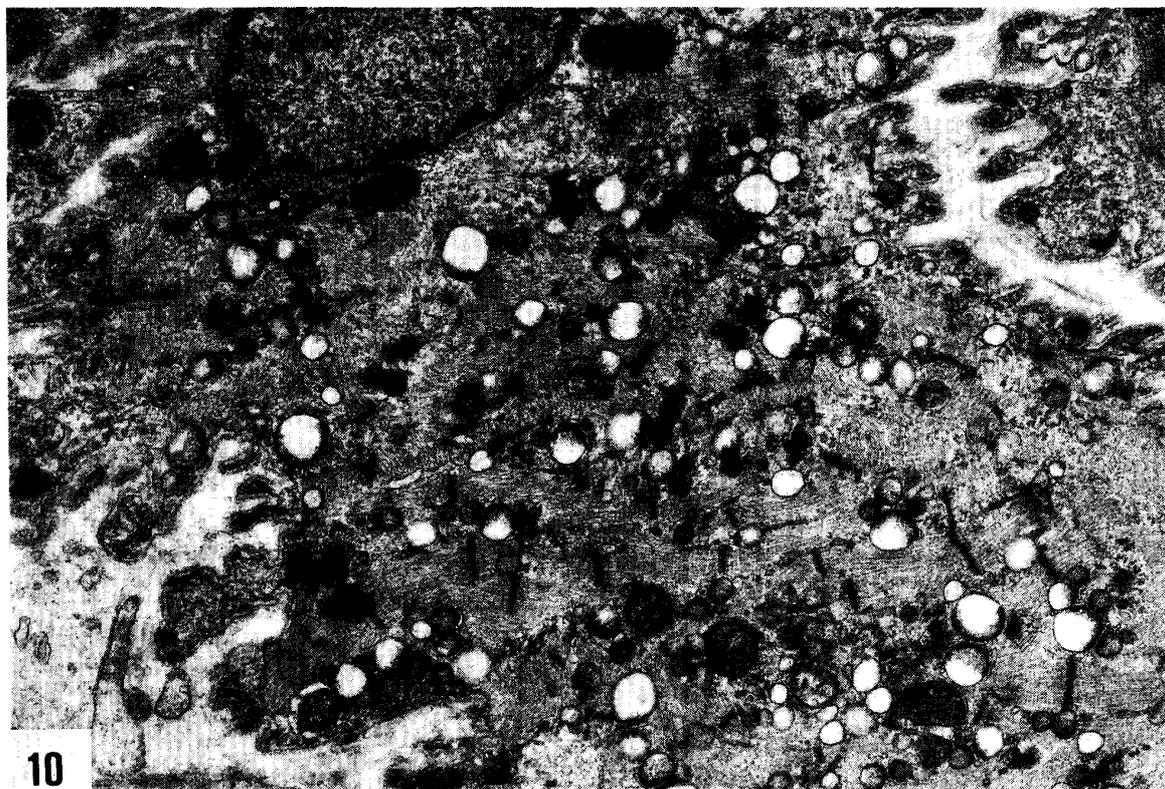


PLATE V

- Fig. 11 Case No. 12 *M. pectorales superficiales* The severely atrophied muscle fibers with obviously increased central nuclei are seen in groups. The outline of the sarcolemma was irregular and indentation is marked. $\times 4,200$
- Fig. 12 Case No. 12 *M. pectorales superficiales* The atrophied muscle fiber with severely indented sarcolemma has activated satellite cells. $\times 20,000$

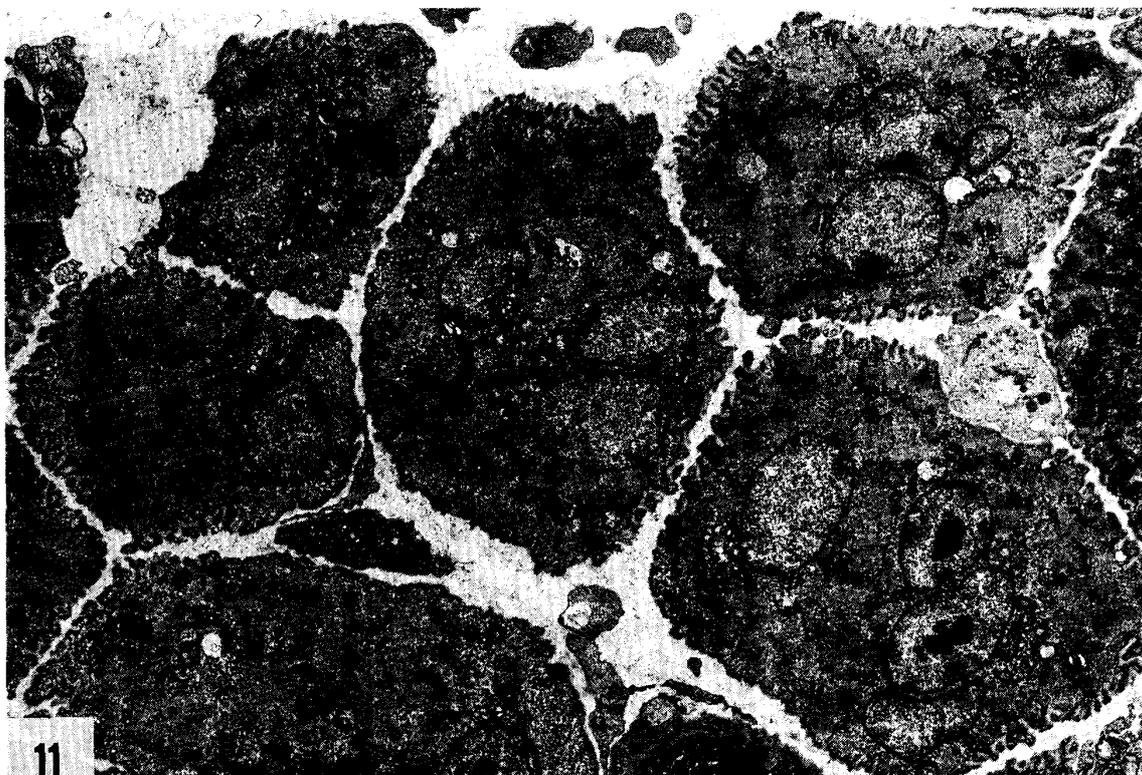


PLATE VI

- Fig. 13 Case No. 12 *M. pectorales superficiales* The atrophic muscle fiber with empty axon terminals. $\times 5,800$
- Fig. 14 Case No. 12 *M. pectorales superficiales* The axon terminal shows disappearance of synaptic vesicles. The cytoplasm of Schwann cell invades between the axon terminal and muscle fiber. $\times 25,000$

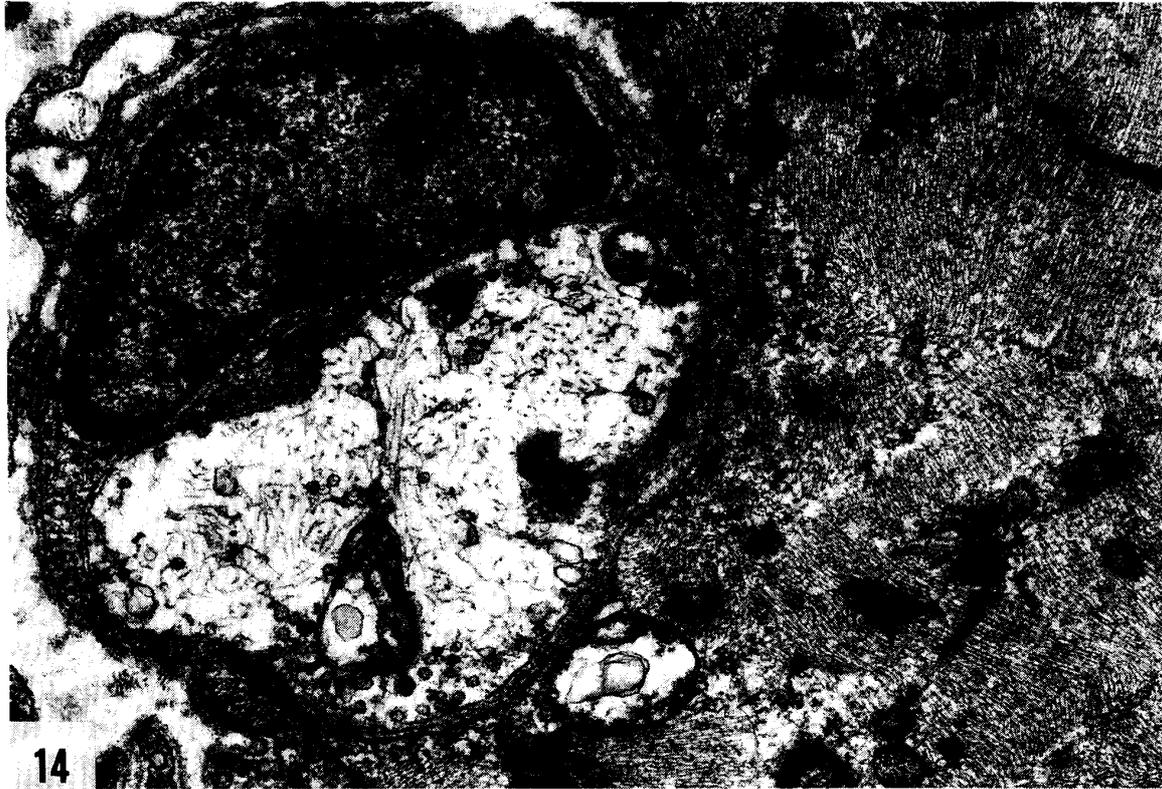
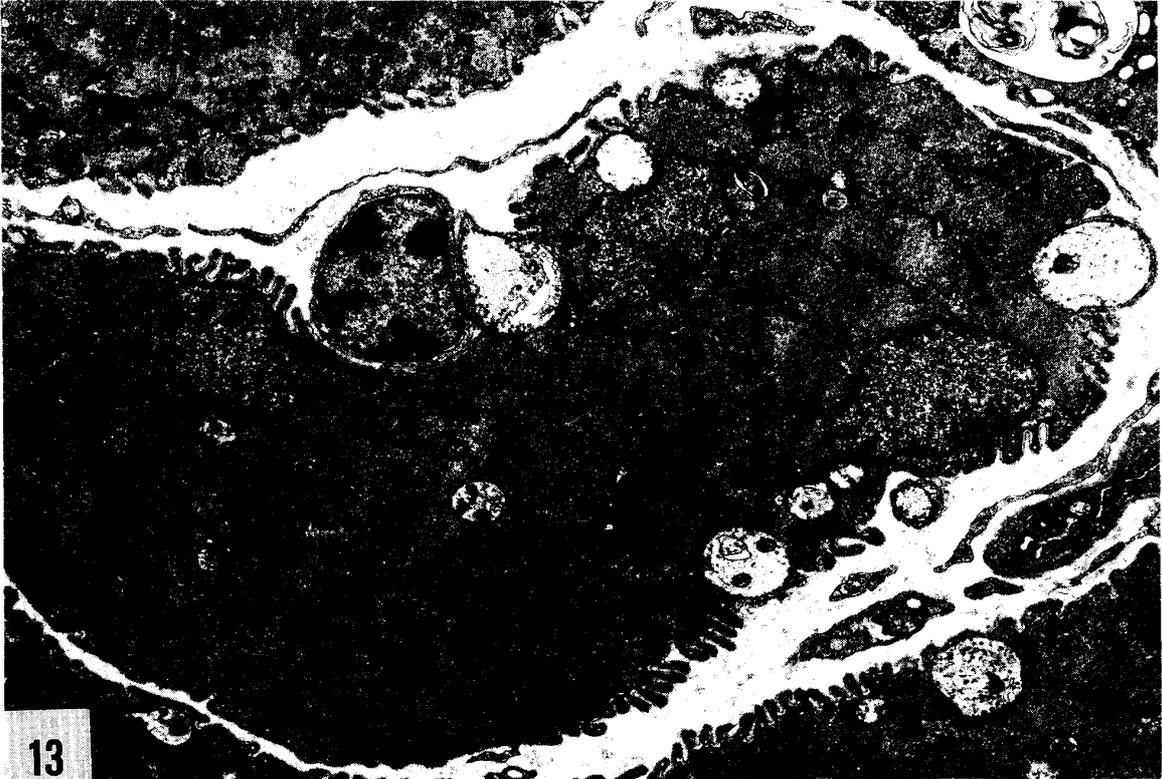


PLATE VI

Fig. 15 Case No. 3 *M. gastrocnemius* The atrophic fiber has many honeycomb-like structures. They are often connected to vacules of the T system. $\times 17,000$

Fig. 16 Case No. 5 *M. gracilis* The severe atrophied muscle fiber has prominent honeycomb-like structures. $\times 17,000$

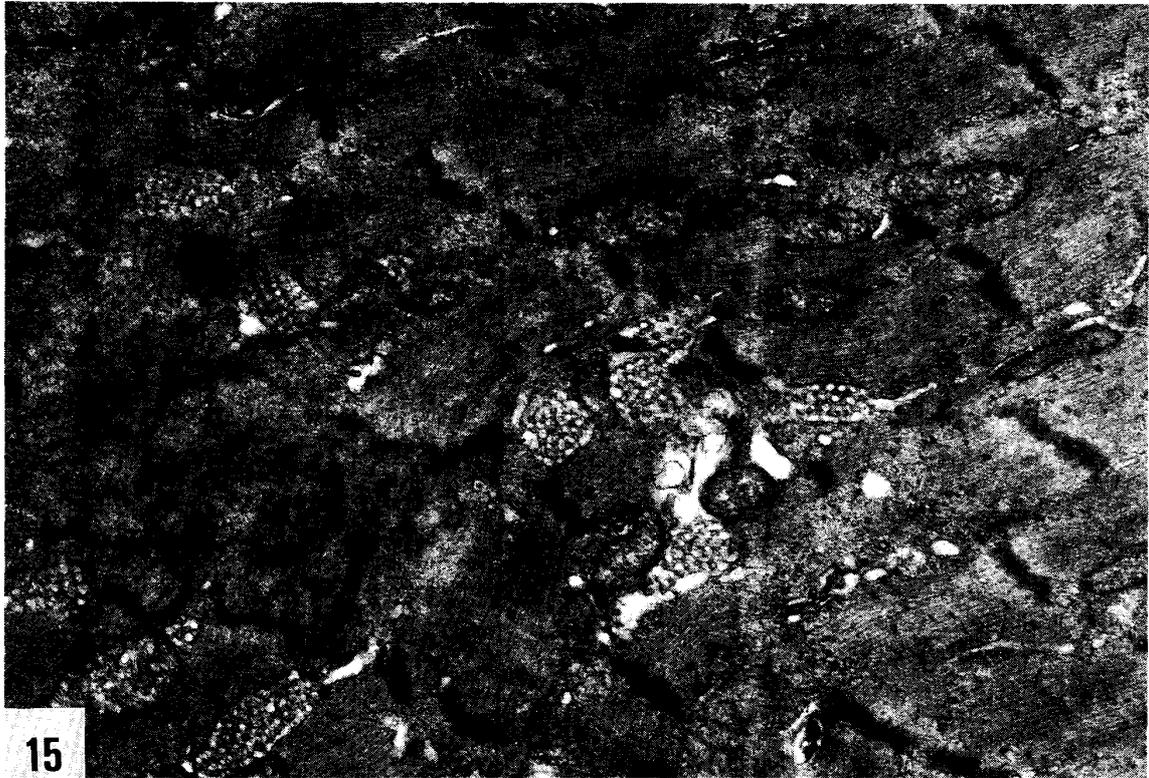


PLATE VIII

- Fig. 17 Case No. 11 *M. pectorales superficiales* Category II
lesion-myodegenerative change. Hypercontraction of the myofibrils leads to the formation of contraction clumps. $\times 3,300$
- Fig. 18 Case No. 12 *M. pectorales superficiales* Category III
lesion-neoplastic proliferative changes of lymphoid cells. Hypercontraction of the myofibrils are also seen. $\times 4,200$

