63

A Study of Muscular Atrophy
after Tenotomy and Denervation
—— Single Fiber Electromyography and Tissue ——

Masayasu MIZUNO

Department of Rehabilitation Medicine, Kawasaki Medical School, Kurashiki 701-01, Japan

ABSTRACT. To study atrophic muscles after tenotomy and denervation, we used as subjects leghorn superficial pectoral muscle (type II fiber muscle), and examined changes in Single Fiber Electromyography (SFEMG) and tissue for four weeks.

The amplitude of SFEMG gradually decreased, the duration became prolonged, and waves like those of type I fibers were found with an amplitude of less than 2.4 mV and a duration of over 2 msec. The amplitude was smaller in denervated muscle than in tenotomized muscle. The duration of the tenotomized muscle was more uneven than that of denervated muscle.

Histological findings (ATPase stain) showed that the muscle fibers gradually atrophied and that the type of muscle fibers did not change. Although SFEMG showed that tenotomy and denervation caused type II fibers to change in to type I fibers, histological findings showed no change.

Key words : single fiber electromyography — ATPase stain — tenotomy — denervation

Recently with developments and problems in rehabilitation, the disuse syndrome has been brought to light.[1] It should be noticed that disuse muscle atrophy is a great functional disorder to physical exercise. Therefore, we performed tenotomy on leghorn superficial pectoral muscle and denervated it, to test the following hypothesis electromyographically and histologically :

(1) that SFEMG would show a change in disuse atrophied muscle, and that it would reveal a difference between the tenotomized muscle and denervated muscle.
(2) that the disuse atrophied muscle would show a change in muscle fiber type.

In this experiment, SFEMG was used to measure the action potential of single fibers. The needle electrodes for SFEMG are 25 μm in diameter, whereas conventional ones are 100–200 μm in diameter. Therefore, the action potential of single fibers of about 50 μm in diameter can be isolated and identified (Fig. 1).

水野雅康

The needle electrode for conventional EMG

200μm

The needle electrode for single fiber EMG

25μm
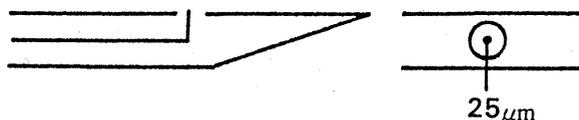
Fig. 1. The needle electrodes.   The needle electrodes for SFEMG are only 25 μm in diameter, whereas conventional needle electrodes are 100~200 μm in diameter. Therefore, the action potential of a single fiber of about 50 μm in diameter can be isolated and identified.

SUBJECTS AND METHODS

As subjects, 8 white leghorns' superficial pectoral muscles were used. Their ages were from 8 to 14 months, and their weight was 1.74±0.06 kg. They were bred in the cages.

The eight white leghorns were divided into two equal groups as follows:
(1) *Tenotomized group*

To obtain control group measurements, the animals were subjected to general anesthesia with Na–pentobarbital 20 mg/kg injected into the humeral veins.   Their pectoral nerves controling the superficial pectoral muscles were exposed in the vicinity of the scapulohumeral joints; supramaximal stimulation by a rectangular wave of 0.1 msec duration; and the amplitude and duration of SFEMG were measured at three or four points in each muscle, 20 waves in total.   Only the action potential of single fibers was measured by inching the electrode because two potentials are led out when the electrode is found between two muscle fibers.   The position at which the recording electrode was to be inserted was adjusted so that the amplitude would be obtained on the oscilloscope at its maximum, and the amplitude between the positive peak and the negative peak was measured.   The duration was measured in the base–line between the two points where the action potential began and stopped at the point where it had begun.   The earth was set on the leghorn superficial pectoral muscle.

Then the superficial pectoral muscle was tenotomized at the humerus, after which SFEMG was examined after one week, two weeks, and four weeks respectively, using the same method as that of the control.

(2) *Denervated group*

To measure SFEMG by stimulating superficial pectoral muscle directly, two TOP Pole needles (insulated electrode needle–23G) were inserted into the muscle 5 mm a part for the infusion of 0.3 ml of physiological saline.   After

that, the muscle was stimulated by electricity under the same conditions as in the tenotomized group, and SFEMG was examined along the course of muscle fiber at a point 20 mm away from the stimulated part. The amplitude and duration of the SFEMG were measured at three or four points in each muscle, 20 waves in total. The earth was set between the stimulated part and the recording needle electrode (Fig. 2).

Then a pectoral nerve was cut in the vicinity of the scapulohumeral joint before its proximal and its distal stumps were ligated with 5-0 nylon thread to prevent the nerve from regenerating and reinnervating. 15 waves from each muscle were measured by SFEMG using the same method as that of the control after one week, two weeks, and four weeks respectively. The number of waves was limited to 15 to prevent damage to the muscles by the exciting and recording electrodes.

The room temperature of the laboratory for both groups was kept at 22 ~24°C. The leghorns were kept there for half an hour before experiment in order to accustom them to the room temperature. Nihon Kohden Neuropack 4 was used for electromyography, and Nihon Kohden Single Fiber Electrode was used as the needle electrode. Almost simultaneously with SFEMG, a muscle biopsy specimen was obtained from the superficial pectoral muscle, and a 10 μm cryostat section was obtained by freezing with dry ice acetone, and then, through ATPase stain (pH 4.3·10.3), muscle fiber types were classified and diameters of muscle fibers were measured. A Nikon Eyepiece Micrometer was used to measure the diameters of 100 fibers per muscle. I also compared the histological findings with SFEMG. I employed t-test to decide the difference was statistically significant at $p < 0.01$.

RESULTS

The average weight of the tenotomized leghorns was 1.74 ± 0.06 kg and that of the denervated animals was 1.73 ± 0.07 kg, and thus the difference being not significant between the two groups.
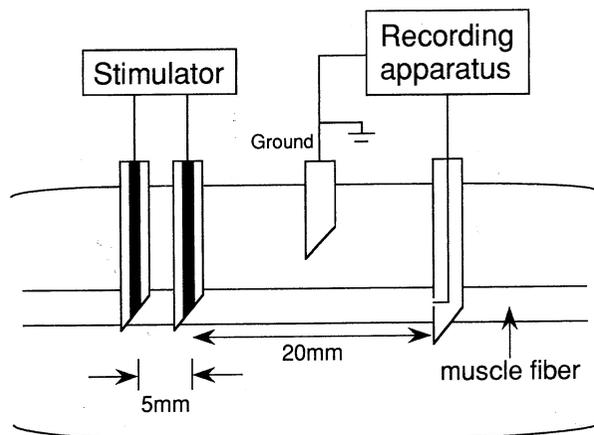

Fig. 2. Diagram of the arrangement for stimulation of muscle fibers and recording of action potentials.

1. SFEMG findings

(1) The tenotomized group

Changes in the SFEMG waves of the tenotomized animals are shown in Fig. 3a. The amplitude of these waves showed a tended to gradually decrease and the duration gradually increased.

The average amplitude and duration of the SFEMG are shown in Fig. 4a. The average amplitude decreased gradually after the superficial pectoral muscle as tenotomized, and there was a significant difference from that of the control $(2.52 \pm 0.51$ mV$)$ after one week $(1.05 \pm 0.44$ mV$)$, and between two weeks $(1.15 \pm 0.45$ mV$)$ and four weeks $(0.88 \pm 0.28$ mV$)$. The average duration showed prolongation with a significant difference from the control $(1.51 \pm 0.28$ msec$)$ after one week $(1.98 \pm 0.59$ msec$)$. The averages of both amplitude and duration showed maximal change one week after tenotomy.

Fig. 5a shows the amplitude and duration of the SFEMG between the control and the tenotomized leghorns after one week and four weeks. As observed, the amplitude is found decreased on the whole, while the duration widely distributed. Especially, the amplitude after tenotomy showed waves of under 2.4 mV, while the duration showed waves of over 2 msec. These waves were not observed in the control. Thus the findings were considered to be similar to those of the SFEMG of type I muscle fibers.

control   1 week   control   1 week

2 weeks   4 weeks   2 weeks   4 weeks

500μV
1ms

500 μV
1ms

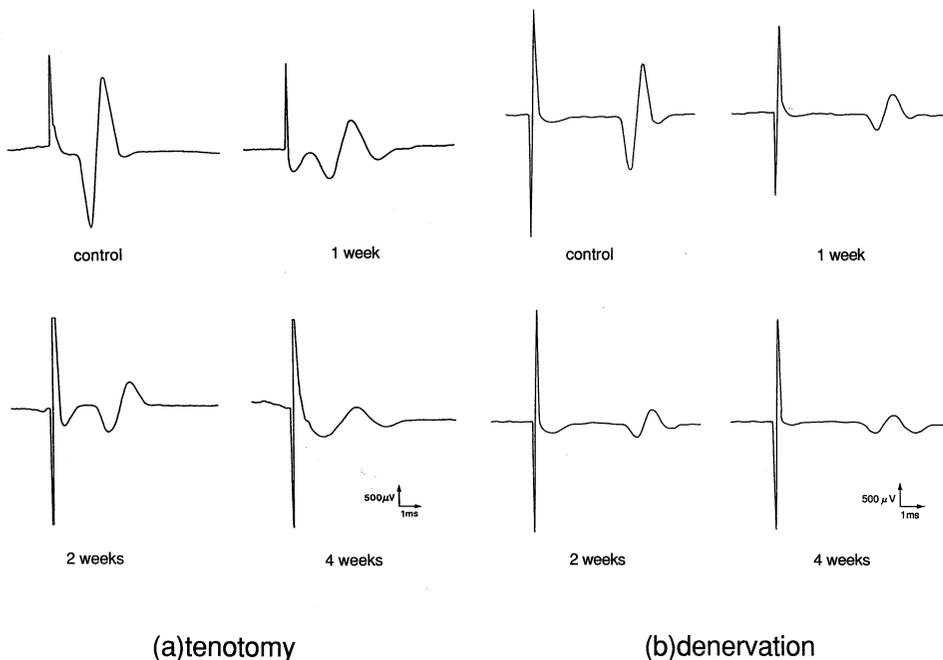**(a)tenotomy**   **(b)denervation**

Fig. 3. Changes in SFEMG waves. The amplitudes of the tenotomized muscle (a) and the denervated muscle (b) tended to gradually decrease, while the duration gradually increased.
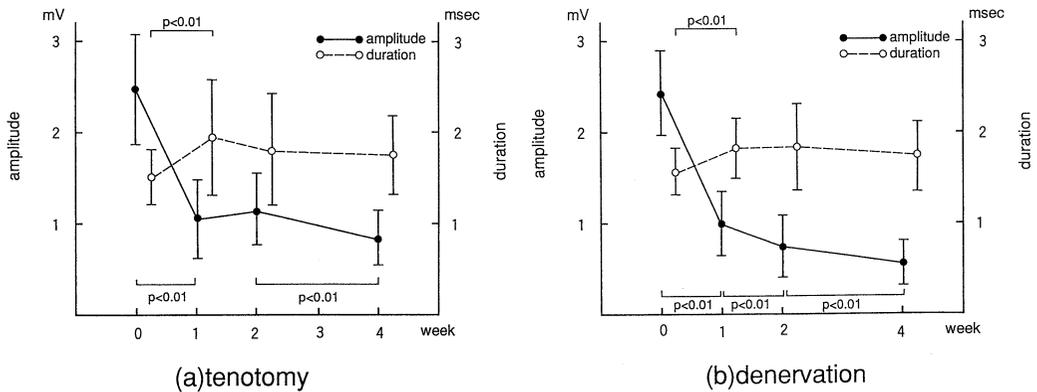
Fig. 4.   Changes in the averages of the amplitude and the duration in SFEMG. The average
amplitude decreased gradually after the superficial pectoral muscle was tenotomized (a)
and denervated (b).   The average duration showed prolongation.

(2) The denervated group

Fig. 3b shows changes in the SFEMG waves following denervation. Both
the amplitude and the duration showed the same tendency as in the tenotom-
ized group.

Fig. 4b shows changes in the amplitude and the duration of SFEMG. The
average amplitude decreased gradually after the superficial pectoral muscle was
tenotomized, and there was a significant difference from that of the control
$(2.43 \pm 0.46$ mV) after one week $(1.00 \pm 0.38$ mV), between one week and two
weeks $(0.75 \pm 0.35$ mV), and between two weeks and four weeks $(0.55 \pm 0.25$
mV). The average duration showed prolongation with a significant difference
from the control $(1.54 \pm 0.23$ msec) after one week $(1.83 \pm 0.39$ msec). However,
neither the amplitude nor the duration showed a significant difference from the
controls of the two groups.   The averages of both amplitude and duration
showed maximal change as in the tenotomized group, one week after
denervation.

Fig. 5b shows the amplitude and the duration of SFEMG between the
control and the denervated leghorn after one week and four weeks. As in the
tenotomized group, the amplitude showed the same decrease on the whole,
while the duration was found widely distributed.   The amplitude after
denervation showed a wave of less than 2.4 mV and the duration of over 2
msec, both of which were not observed in the control. These findings were
similar to those of type I muscle fibers.

2. Histological findings

(1) The tenotomized group

Fig. 6a shows change in the ATPase staining (pH 4.3) of superficial
pectoral muscle.   The diameter of the muscle fibers tended to decrease
gradually as in ATPase staining (pH 10.3). In addition it was found that the
superficial pectoral muscle always remained pure type II fiber, in other words,
it did not change its type.

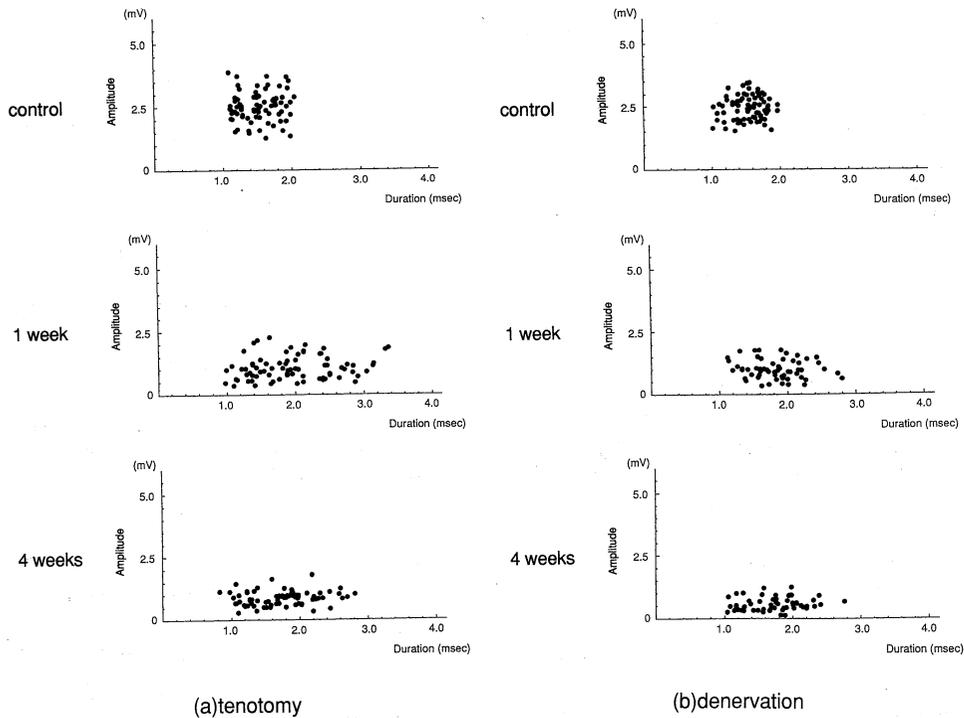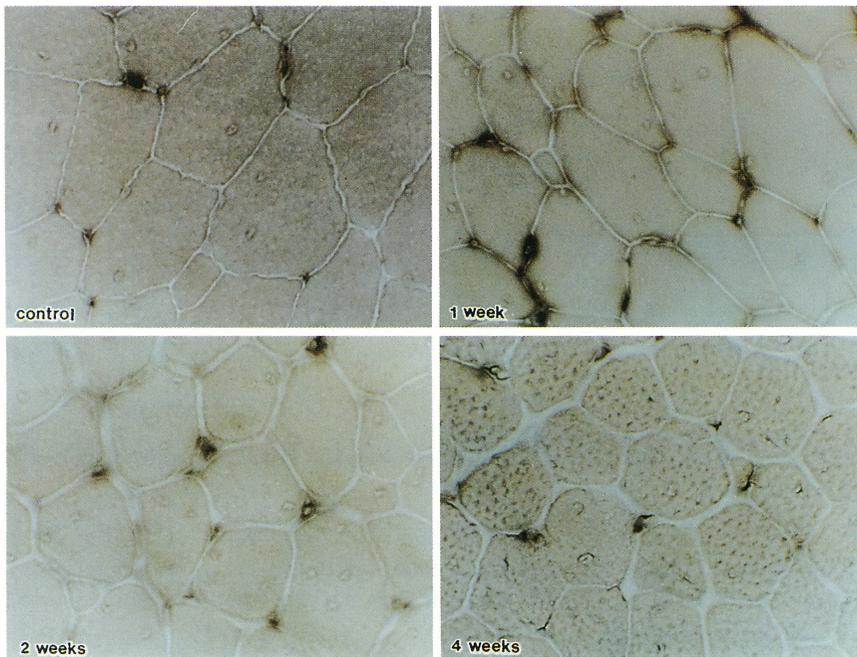(a)tenotomy   (b)denervation

Fig. 5.   The amplitude and duration of SFEMG between the control and the tenotomized (a) and denervated (b) muscles after one week and four weeks.   The amplitude of SFEMG was decreased on the whole, while the duration were widely distributed.   Waves like those of type I fibers found with amplitudes of less than 2.4 mV and a duration of over 2 msec.   The amplitude was smaller in denervated muscle than in tenotomized muscle. The duration of SFEMG in the tenotomized muscle was more uneven than that in denervated muscle.

Fig. 7a shows change in the average diameter of the muscle fiber.   There was a significant difference between the control (43.89 ± 6.91 μm) and the tenotomized muscle after one week (32.35 ± 7.81 μm), and between two weeks (31.05 ± 7.40 μm) and four weeks (25.59 ± 3.69 μm).   The diameter of the muscle fiber showed maximal decrease one week after tenotomy, and the courses indicated by the historical and SFEMG findings were similar.

(2) The denervated group
Fig. 6b shows change in the ATPase staining (pH 4.3) of the superficial pectoral muscle.   The diameter of the muscle fiber tended to decrease gradually as in the tenotomized group.   In addition it was found that the superficial pectoral muscle always remained pure type II fiber, in other words, it did not change its type.
Fig. 7b shows change in the average diameter of muscle fiber.   There were significant decreases between the control (43.71 ± 6.23 μm) and the denervated leghorn after one week (34.70 ± 7.72 μm), between one week and two weeks

(b)denervation
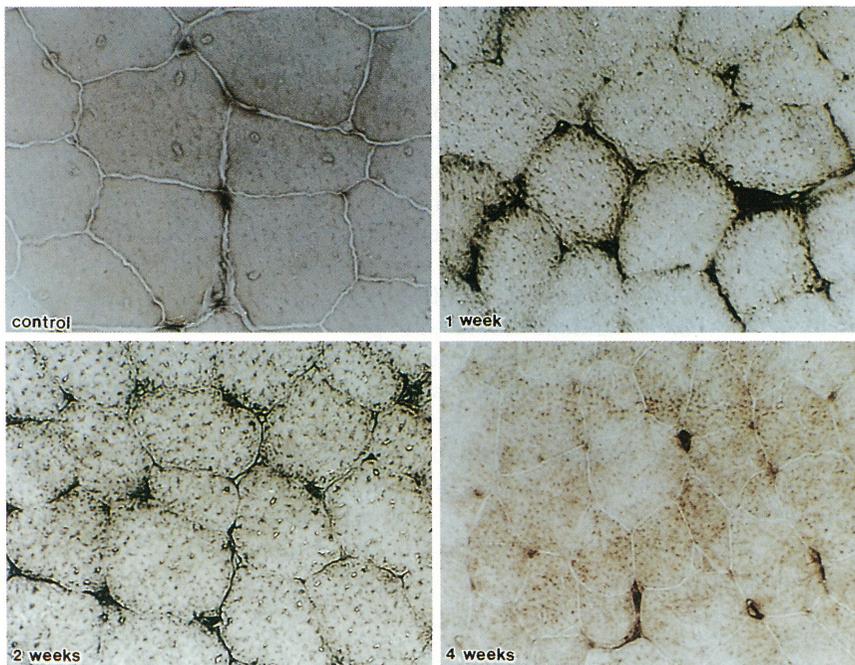
(a)tenotomy

Fig. 6. Change in ATPase staining (pH 4.3) of superficial pectoral muscle after tenotomy (a) and denervation (b). Muscle fibers gradually became atrophic, but the type of muscle fibers did not change. (ATPase staining (pH 4.3)× 400)
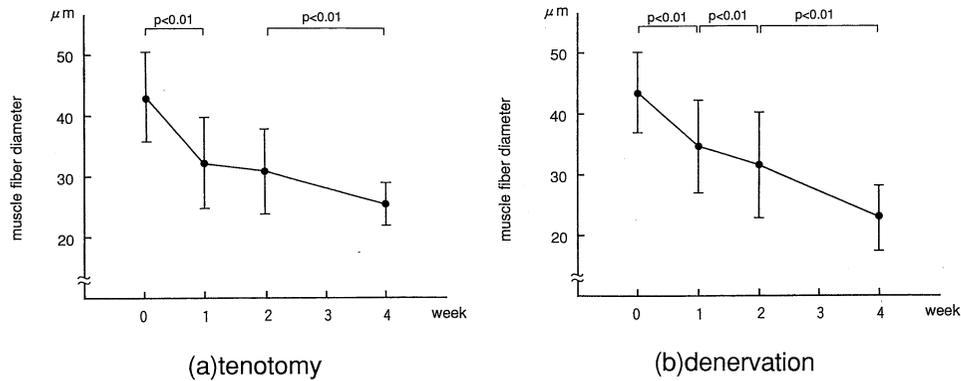
Fig. 7. Change in average diameter of muscle fibers.   Muscle fibers gradually became
atrophic.

(31.30±8.95 μm), and between two weeks and four weeks (22.70±5.29 μm).
The diameter of muscle fiber showed maximal decrease as in the tenotomized
group one week after tenotomy, and the courses indicated by the histological
and the SFEMG findings were similar.

DISCUSSION

1. Experimental Methods Used for This Study

Muscle atrophy and muscle weakness are remarkable symptoms as
deuteropathy clinically caused by a long confinement to bed or rest in bed.
This is called disuse muscle atrophy, and in the field of rehabilitation
medicine, is an extremely serious problem affecting motor function, and its
studies have been carried out as basic experiments through histological
approach.[2-4]   However, there have still been a few who have studied from an
electrophysiological point of view especially, there have been no reports on
disuse change of pure type II fiber muscle based on SFEMG and tissue
findings.   This is why the author decided to pursue his studies into this field.

Although gypsum bandage fixing and pin fixing have often been used as
experimental methods for disuse muscle atrophy, the author employed
Lippmann[5] *et al*.'s tenotomy for his studies because it was difficult to fix a
leghorn's scapulohumeral joint.

In due to loss of trophic action by the nerve, and it is different, in the
strict sense of the word, from disuse induced by a decrease in activity.   But it
is admitted that a gradual decrease in the activity of the muscle is liable to
bring about disuse muscle atrophy,[6] so that the author used denervated muscle
as disuse muscle atrophy.

2. SFEMG

SFEMG was developed by Ekstedt and Stålberg[7] in 1963, which enabled
us to obtain information on various activities of muscle fiber that had not been
observed on conventional electromyogram with a needle electrode.   The author,
therefore, performed the experiments with the hypothesis that SFEMG may
enable muscle fiber type to be classified.[8]   It was found that type I muscle fiber

showed an short amplitude of less than 2.4 mV, and a long duration of over 2 msec, on one hand; type II muscle fiber showed a large amplitude of over 2.4 mV and a short duration of less than 2 msec, on the other hand.   These findings supported my hypothesis.

The author will discuss the significance of the items be measured by SFEMG.   The amplitude is determined by the diameter[9] of muscle fiber and the distance[10] between an excitable muscle fiber and an electrode.   When the recording electrode is imperceptibly inched away from the power supply, the measurement radially declined,[10] and the degree of the decline is determined by the surface size of the electrode.[11]   Since the SFEMG needle electrode whose surface is extremely small makes this phenomenon sharp, great care was taken of as stated in the Method when the position for the needle was determined. The duration is determined mainly by ATPase activity[12] and conduction velocity in muscle fiber.   Construction velocity is said to be proportional to ATPase activity.   Therefore, the duration of type II fiber (with higher ATPase activity[13]) is shorter than that of type I muscle fiber.   Since the conduction velocity in muscle fiber is faster in type II muscle fiber.[14]   Therefore, it is conceivable that the duration will be shorter in type II fiber.

3.   Evoked Electromyography by Direct Stimulation of Muscle Fiber

In the preliminary experiments, I tried to measure the waves of the evoked electromyography for denervated muscle by nerve stimulation, but muscle contractions by nerve stimulation did not occur 3 or 4 days after denervation, making SFEMG measurement impossible.   Then I tried of measure it by direct fiber stimulation; inserting two ordinary stimulation needles at two points of the muscle and giving the needles electrical stimulation.   And then the first shock artifact became large enough for the target action potential to enter it, indisposing the potential for measurement.   Considering that the whole muscle in contact with the exciting needle worked as volume conduction, I used an insulated electrode, which led out the target action potential.   Troni et al.[15] used two stimulating monopolar needle electrodes for human brachial biceps muscle fiber, but they raised a question in their report that when neuromusclar junction was stimulated they could not measure the target evoked potential by direct muscle stimulation because there appeared various evoked potentials by indirect muscle stimulation through the junction.   Their report let me to avoid measuring around the neuromusclar junction.   In anticipation of a possible difference between action potentials through nerve stimulation and direct muscle stimulation, I examined the tenotomized and the denervated controls and found that there was no significant difference between the amplitude and the duration.

Now I refer to my hypothesis I put forward at the beginning of this paper.

4.   Hypothesis (1)

From this experiment both the tenotomized and the denervated muscles showed in SFEMG that the amplitude decreased and that the duration prolonged.

The decrease of the amplitude :

Although Noda[16] reported on the amplitude that he used a needle with its tip exposed by 10 mm for the denervated muscle, the amplitude of single fiber action potential was measured for the first time in my experiment.   The tenotomized group and the denervated group were found to be very similar in the courses of the amplitude and the diameter of muscle fiber.   Hakansson[8] reported that in normal muscle, the diameter of muscle fiber was in proportion to the amplitude.   It is very interesting to note that in disuse muscle atrophy, the courses of the amplitude and the diameter of muscle fiber are closed related to each other.   In addition, a decrease of the amplitude can be caused by a change of electrical characteristics of muscle fiber membrane.[17]

The prolongation of the duration :

In this experiment, the action potentials of single muscle fiber showed a prolonged duration, that is, a prolongation of fiber contraction time on the SFEMG.   Cooper[18] and Davis[19] reported that contraction time was found to be prolonged when tension-length diagram of each atrophic muscle was measured by fixing joints.   I could prove their finding from my experiment based on single muscle fiber level.   As it has been reported that fiber conductivity decreased in denervation,[16,20] the duration of single fiber action potential should be prolong if the fiber conduction velocity drops.   Kuno[21] and Kameyama[22] reported that muscular metabolism is maintained by muscular tone and contractile activity.   Changes in the duration due to tenotomy and denervation suggests that changes in muscular activity are brought about changes in specific fiber contractility ; that is, in the SFEMG of pure type II fiber muscle a wave with a small amplitude and a prolonged duration like that of type I muscle fiber appeared.

The courses of the amplitude of the tenotomized and the denervated muscles clearly reflected the course of the diameter of muscle fiber the amplitude of the denervated muscle was significantly smaller than that of the tenotomized muscle after two weeks and four weeks respectively, while the duration of a action potential of the tenotomized muscle tended to be more uneven than that of the denervated muscle.

5.   Hypothesis (2)

In this experiment, the diameter of muscle fiber decreased up to 58.3% of that of the control in the tenotomized group, and up to 51.7% of the control in the denervated group.   Muscle artophies in both groups had occurred rapidly before one week passed, and they gradually advanced after that.   Cooper[18] reported that a cat's muscle atrophy appeared rapidly 3 or 4 weeks after fixing joints, which was different from the results of my experiments.   However, the reports made by Tomanek[23] and Sukegawa,[24] who used guinea pigs and rats, respectively that muscle atrophies appeared 5 days after fixing joints were identical with the results of my study.   On the other hand, Engel *et al.*[25] reported that a cat's tenotomized muscle showed a marked atrophy of type I fiber, hypertrophied type II fiber, and that both fibers in a denervated muscle showed marked atrophies, which is partly different from the results of my study.   But Herbinson *et al.*[26] reported that after fixing joints, a rat's disuse atrophied soleus muscle showed a marked atrophy in both fibers.   Generally, there have been a lot of reports that disuse atrophied muscle shows atrophy in

both types of fibers, and that type I fibers change into type II fibers.[23,27,28] Mizutani[2] reported, however, that the type of muscle fiber does not change. Jirmanobá et al.,[29] reported that young leghorns would show a conditional change in muscle fiber type, which would be difficult to occur in a mature leghorn.

In this experiment, disuse atrophied muscle did not histologically show a marked change in fiber type. But in SFEMG there appeared in pure type II fiber a wave with action potential like that in type I fiber. What does this mean? Booth[30] reported that muscle protein synthesis decreased by 37% only six hours after a rat's hindlimb immobilization, and that biochemical change appeared much earlier than histological change did. Likewise, there seems to be a fair possibility that electrophysiological change appears earlier than histological change. I strongly hope that the investigation of this possibility will be the subject of future research.

ACKNOWLEDGMENT

REFERENCES

1) Ueda, S. : Disuse syndrome and rehabilitation : Introduction to special issue. Sogo Rehabilitation **19** : 773-774, 1991
2) Mizutani, K. : Histochemical study on disuse atrophy of skeletal muscle in rabbit. J. Jpn. Orthop. Ass. **55** : 1673-1691, 1981
3) Lipschütz, A. and Audova, A. : The comparative atrophy of the skeletal muscle after cutting the nerve and after cutting the tendon. J. Physiol. **55** : 300-304, 1921
4) Sargent, A.J., Davies, C.T.M. and Edwards, R.H.T. : Functional and structural changes after disuse of human muscle. Clin. Sci. Mol. Med. **52** : 337-342, 1977
5) Lippmann, R.K. and Selig, S. : An experimental study of muscle atrophy. Surg. Gynecol. Obstet. **47** : 512-522, 1928
6) Gutman, E. : Denervation and disuse atrophy in cross striated muscle. Rev. Can. Biol. **21** : 353, 1962
7) Ekstedt, J. and Stålberg, E. : A method of recording extracellular action potentials of single muscle fibers and measuring their propagation velocity in voluntarily activated human muscle. Bull. Am. Assoc. Electromyogr. Electrodiagn. **10** : 16, 1963
8) Mizuno, M. : The single fiber EMG of type I and type II muscle fiber : Classification of muscle fiber type of hen. Jpn. J. Rehabil. Med. **29** : 833-835, 1992
9) Håkansson, C.H. : Conduction velocity and amplitude of the action potential and related to circumference in the isolated fiber of frog muscle. Acta Physiol. Scand. **37** : 12-16, 1956
10) Gath, I. and Stålberg, E. : The calculated radial decline of the extracelullar action potential compared with in situ measurements in the human branchial biceps. Electroenceph. Clin. Neurophysiol. **44** : 547-552, 1978
11) Ekstedt, J. and Stålberg, E. : How the size of the needle electrode leading-off surface influences the shape of the single muscle fiber action potential in electromyography. Comput. Prog. Biomed. **3** : 204-212, 1973
12) Bárány, M. : ATPase activity of myosin correlated with speed of shortening. J. Gen. Physiol. **50** : 197-218, 1967

13) Sreter, F.A. : Temperature, PH and seasonal dependence of Ca–uptake and ATPase activity of white and red muscle microsomes. Arch. Biochem. Biophys. **134** : 25–33, 1969

14) Yoshikawa, N. : Clinical studies of single fiber electromyography I. Amplitude and propagation velocity of single muscle fiber action potentials in patients with neuromuscular diseases. Jpn. J. EEG EMG **3** : 414–424, 1975

15) Troni, W., Cantello, R. and Rainero, I. : Conduction velocity along human muscle fibers in situ. Neurology **33** : 1453–1459, 1983

16) Noda, Y. : Direct study of muscle condition and its clinical application in health and disease. Jpn. J. Rehabil. Med. **24** : 153–162, 1987

17) Hubbard, S.J. : The electrical constants and the component conductances of frog skeletal muscle after denervation. J. Physiol. **165** : 443–456, 1963

18) Cooper, R.R. : Alterations during immobilization and regeneration of skeletal muscle in cats. J. Bone Joint Surg. **54-A** : 919–953, 1972

19) Davis, C.J.F. and Montgomery, A. : The effect of prolonged inactivity upon the contraction characteristics of fast and slow mammalian twitch muscle. J. Physiol. **270** : 581–594, 1977

20) Buchthal, F. and Rosenfalck, P. : Rate of impulse conduction in denervated human muscle. Electroencephalogr. Clin. Nerophysiol. **10** : 521–526, 1958

21) Kuno, M. : Trophic interaction between nerve and muscle. Jpn. J. Neuropsychopharmacol. **2** : 549–555, 1980

22) Kameyama, T. and Etlinger, J.D. : Calcium dependent regulation of protein synthesis and degradation in muscle. Nature **279** : 344–346, 1979

23) Tomanek, R. and Lund, D.D. : Degeneration of skeletal muscle fibers. J. Anat. **118** : 531–541, 1974

24) Sukegawa, T. : A pathological study on disuse atrophy of skeletal muscle : With special reference to single muscle fiber function and its minute structure. J. Jpn. Orthop. Assoc. **57** : 779–787, 1983

25) Engel, W.K., Brooke, M.H. and Nelson, P.G. : Histochemical studies of denervated or tenotomized cat muscle. Ann. N. Y. Acad. Sci. **138** : 160–185, 1966

26) Herbison, G.J., Jaweed, M.M. and Ditunno, J.F. : Muscle fiber atrophy after cast immobilization in the rat. Arch. Phys. Med. Rehabil. **59** : 301–305, 1978

27) Booth, F.W. and Kelso, J.R. : Effect of hindlimb immobilization in contractile and histochemical properties of skeletal muscle. Pflugers Arch. **342** : 231–238, 1973

28) Karpati, G. and Engel, W.K. : Histochemical investigation of fiber type rations with the myofibrillar ATPase reaction in normal and denervated skeletal muscles of guinea pig. Am. J. Anat. **122** : 145–156, 1968

29) Jirmanová, I., Hník, P. and Zelená, J. : Implantation of "Fast" nerve into slow muscle in young chickens. Physiol. Bohemoslov. **20** : 199–204, 1971

30) Booth, F.W. and Seider, M.J. : Early change in skeletal muscle protein synthesis after limb immobilization of rats. J. Appl. Physiol. **47** : 974–977, 1979