Effect of Electrical Stimulation on Healthy Skeletal Muscles[*]

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(Received December 21, 1983)

ABSTRACT

This study was made to obtain a more definite information of adaptive effects of electrical stimulation on healthy skeletal muscle, because the effect of electrical stimulation on the healthy muscle has been still controversial. Right sciatic nerve of Wistar albino rats was electrically stimulated daily 30 minutes at the frequency of 5 Hz for two weeks and they were allowed to survive until the second week, when appropriate leg muscles of both sides were removed for wet muscle weight measurement, succinic dehydrogenase (SDH) stain and microangiogram.

Electrically stimulated muscles gained weight and the diameters of each type of muscle fiber of stimulated muscles became larger comparing with those of nonstimulated muscles. Also each type of stimulated muscle fiber gained SDH activity, that made the difference of individual muscle fiber type indistinct from histochemical point of view.

Microangiography done with India black ink showed the dilatation of vessels as well as the increase of number of capillaries in electrically stimulated muscles.

In view of these results, electrical stimulation can be utilized as a therapeutic procedure to encourage muscle strengthening exercise and also to prevent the muscle wasting in atrophic conditions of the muscles, such as in a cast immobilization.

INTRODUCTION

Orthopaedic surgeons frequently encounter many of the problems concerned with skeletal muscle atrophy and its prevention. It was already demonstrated that electrical stimulation to the denervated skeletal muscles delays the progression of the degeneration of the muscles[17),18),19),22)] and this procedure has been frequently utilized clinically for the patients with peripheral nerve disorder. During the past few years electrical stimulation has become a substitute for the ordinary muscle strengthening exercise program, but as yet research information about the effect of electrical stimulation on the healthy skeletal muscle is still largely lacking. And the effect of electrical stimulation on the healthy muscle has been still controversial, since changes of the muscle induced by electrical stimulation depend on various factors,

such as the type and duration of stimulations applied, the type of muscle stimulated and the position of the joint during electrical stimulation.

Hence, this study was undertaken to obtain a more definite information regarding the adaptive effects of electrical stimulation on the healthy skeletal muscle with respect of the change in the metabolism and in the vascular system.

METHODS

Both the stimulating and reference wire electrodes were placed around right and left sciatic nerves aseptically in Wistar albino rats weighing 250 g to 300 g. These wires were introduced up to the nuchal region through the back subcutaneous tissue to prevent the rats from biting the wires and the wire tips were taken out of the skin to connect the
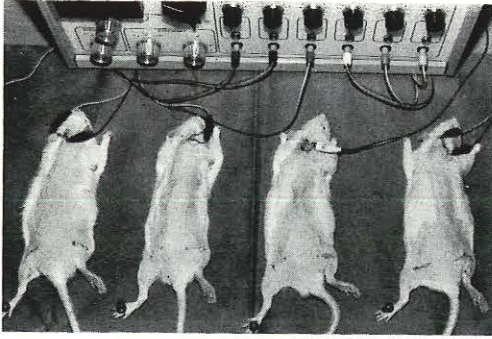
Fig. 1. Method of electrical stimulation
Only right sciatic nerve was stimulated and left sciatic nerve was left nonstimulated.

electrical stimulator (AT-301. Asahidenshi Co. Ltd.). Somnopentil was used to induce general anaesthesia, then only right sciatic nerve was stimulated and left sciatic nerve was left non-stimulated under the identical condition (Fig. 1). Electrical stimulation was given daily for 30 minutes with a supramaximal output voltage, of 0.1 msec pulsed impulse and at a frequency of 5 Hz. Rats were allowed to survive until the second week after electrical stimulation started. Both sides of tibialis anterior muscle, extensor digitorum longus muscle and extensor hallucis longus muscle were compared in the points of macroscopic findings, wet muscle weight, histochemical findings and microangio-

graphic findings.

Carefully removed muscles were weighed precisely and served for histochemical procedure. They were immersed in n-hexane that was cooled by a mixture of dry ice and acetone for rapid freezing. 10 μm thick sections were made in a cryostat at $-25°C$ and then dried in open air for 10 minutes. After this, these cross sections were incubated for 20 minutes for succinic dehydrogenase (SDH) stain by the method of Nachlas et al.[26]. These sections were examined under the microscope and photographs were taken to measure the short diameter of each three different types of muscle fibers.

On the other hand, in some of rats micro-angiography was performed using India black ink, which was perfused through the abdominal artery after heparin with saline was injected in order to prevent coagulation. Black stained leg muscles were taken out and used for fixation by formalin, dehydration and dealcoholization to prepare 300μm thick longitudinal transparent sections by Spaltehorz method[33]. These sections were observed in a 3-D microscope. And also some of these black stained muscles were frozen rapidly in n-hexane, which was surrounded by a mixture of dry ice and acetone and then 10 μm thick cross sections were made in a cryostat. The number of the vessels

Nonstimulated muscle   Stimulated muscle
Fig. 2. Macroscopic findings (Tibialis anterior muscle)
Electrically stimulated muscle was larger and the degree of red hue was definitely deeper.

around muscle fibers was counted using magnified photographs of these sections. All results were analyzed statistically by variance ratio test.

Besides these experiments in order to study the effect of duration of electrical stimulation, some of rats were stimulated daily 15 minutes and 60 minutes for two weeks and wet muscle weight was compared with that of nonstimulated muscles.

RESULTS

1. Macroscopic Findings

The size of the electrically stimulated muscles was larger than that of the nonstimulated muscles and the degree of red hue was definitely deeper in the stimulated muscles than that of the nonstimulated muscles as shown in Fig. 2.

2. Changes in Wet Muscle Weight

Both sides of tibialis anterior, extensor digitorum longus and extensor hallucis longus muscles were weighed precisely in mg unit. As the weight is different in every individual rat, the weight of electrically stimulated muscles was compared with that of corresponding nonstimulated muscles. For this, the weight of stimulated muscle was divided by that of nonstimulated muscle and multiplied by 100 (Table 1).

Table 1. Relative wet muscle weight (%)

$$\left(\frac{\text{wet weight of stimulated muscle}}{\text{wet weight of nonstimulated muscle}} \times 100 \right)$$

Case No.	TA	EDL	EHL
6	104.5	107.0	135.0
7	102.3	112.7	157.7
8	102.1	91.0	109.5
9	116.4	122.7	95.5
10	107.7	110.1	140.1
11	110.9	116.4	177.0
12	100.4	110.6	178.3
13	109.6	108.1	157.1
14	105.0	106.6	116.0
15	104.3	110.9	126.0
26	105.7	117.3	145.0
27	101.7	112.0	134.8
28	109.4	110.2	147.4
29	101.3	107.6	100.0
$\bar{X}$	105.8	110.2	137.1
S E	1.2	1.8	6.7

$\bar{X}$: Mean value, SE: Standard error
TA : Tibialis anterior muscle
EDL: Extensor digitorum longus muscle
EHL: Extensor hallucis longus muscle

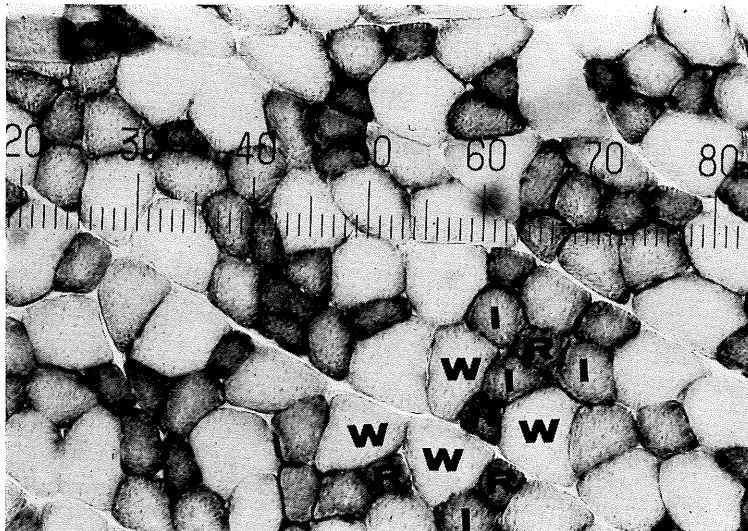This relative wet muscle weight was more than 100 per cent in all muscles of 14 rats

Fig. 3. SDH staining of rats normal muscle (middle layer of tibialis anterior muscle, ×100)
R : Type 1 (Red) muscle fiber
W : Type 2 (White) muscle fiber
I : Intermediate muscle fiber

except for two muscles in No. 8 of extensor digitorum longus muscle and No. 9 of extensor hallucis longus muscle. The extensor hallucis longus muscle of No. 12 showed the highest value of gain. The mean relative wet weight of stimulated tibialis anterior, extensor digitorum longus and extensor hallncis longus muscle was 105.8±1.2%, 110.2±1.8% and 137.1±6.7% (Mean±Standard Error), respectively. The mean relative weight was highest in extensor hallucis longus muscle and was lowest in tibialis anterior muscle. All of these findings definitely suggested that electrically

stimulated muscle gained wet weight. The difference of wet weight between electrically stimulated muscles and nostimulated muscles were found to be statistically significant (p< 0.01).

3. Histochemical Findings

The normal muscles stained for SDH show three kinds of muscle fibers distributing like mosaic pattern. Type 1 (Red) muscle fiber is smallest in size and thickest in staining, type 2 (White) muscle fiber is largest in size and thinest in staining, and intermediate muscle fiber is between the two in size and staining
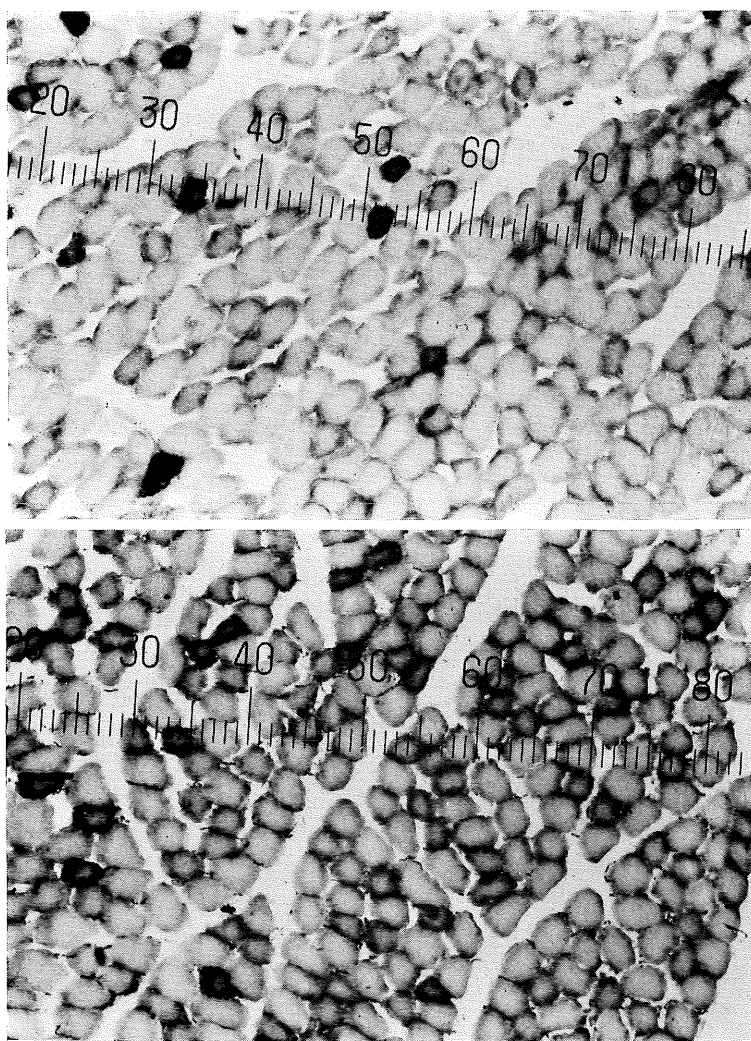

Fig. 4. SDH staining of tibialis anterior muscle (superficial layer, ×40)
Above: Nonstimulated muscle   Below: Stimulated muscle
In the electrically stimulated muscle, all muscle fibers became thickly stained, therefore difference of staining between each individual muscle fiber became indistinct.

(Fig. 3). The distribution of these three types of muscle fiber is not homogeneous in all layers of muscles. In the superficial layer of muscles, type 2 muscle fibers are predominant, whereas in the deep layer, type 1 muscle fibers are predominant[5,24]. Type 1 muscle fibers show a high SDH activity particularly around subsarcolemmal region (subsarcolemmal aggregation) and internal structure of criss-cross appearance in the sarcoplasm secondary to SDH activity between the myofibrils[6,21]. And the difference of each type of muscle fiber can be easily recognized.

Fig. 4 shows the superficial layer of the electrically stimulated and nonstimulated tibialis anterior muscles, which were stained in the completely identical conditions. In the electrically stimulated muscles, all muscle fibers became thickly stained, therefore difference of staining between each individual muscle fiber became indistinct. Some of type 2 and intermediate muscle fibers in the stimulated muscles seemed to have slight degree of subsarcolemmal aggregation of SDH staining as well as criss-
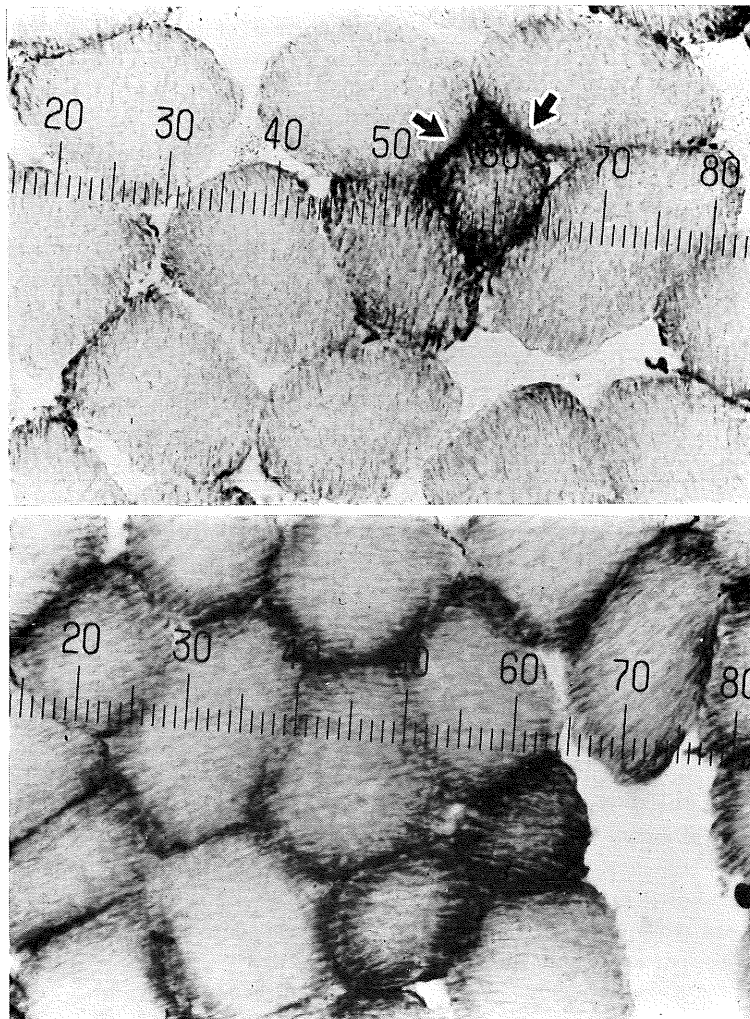
Fig. 5. SDH staining of tiblalis anterior muscle (superficial layer, ×200)
Above: Nonstimulated muscle   Below: Stimulated muscle
Type 1 fiber in nonstimulated muscle showed subsarcolemmal aggregations (arrows).
Type 2 and intermediate fibers in stimulated muscle seemed to have slight degree of subsarcolemmal aggregation as well as criss-cross appearance.

Table 2. Composition of each fiber type (%)

Case No. fiber type		8	10	13	14	15	29	$\overline{X} \pm SE$
Nonstimulated	Type 1	29.6	35.8	30.9	32.7	28.3	23.3	30.0±1.5 %
	Type 2	40.8	38.2	41.8	38.1	41.7	44.5	40.9±0.9 %
	Intermediate	29.6	26.5	27.3	29.2	30.0	32.2	29.1±0.8 %
Stimulated	Type 1	37.6	42.0	42.0	36.1	29.7	27.4	35.8±2.3 %
	Type 2	35.0	29.2	31.6	33.0	37.9	43.8	35.1±1.9 %
	Intermediate	27.4	28.8	26.4	30.9	32.4	28.8	29.1±0.8 %
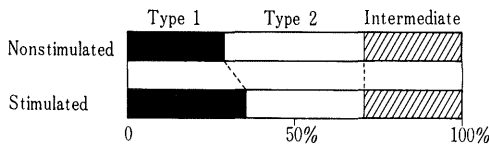
Fig. 6. Change of composition of each fiber type
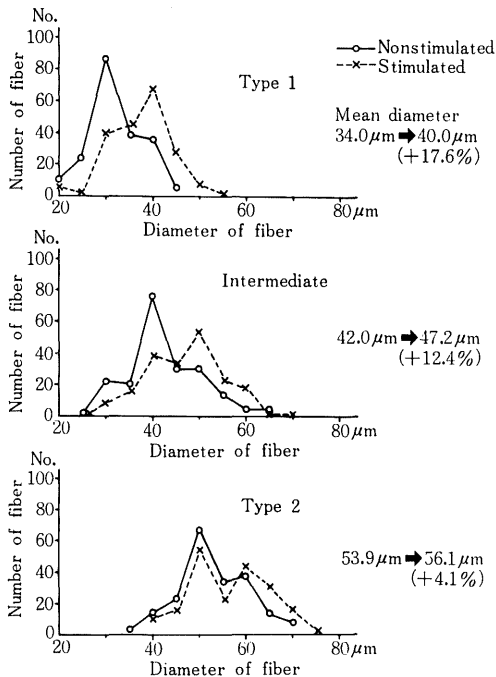
Fig. 7. Histograms of muscle fiber diameter

cross appearance as shown in Fig. 5.

These findings were recognized in every layer of stimulated muscles, particularly in the superficial layer, and these results suggest that electrical stimulation to sciatic nerve at the frequency of 5 Hz increases the activity of SDH in each type of muscle fiber.

4. Composition of Each Type of Muscle Fiber

300 muscle fibers were classified and counted in microscopic photographs of middle layer of tibialis anterior muscles to demonstrate the composition of each fiber type (Table 2). The proportion of type 1, type 2 and intermediate muscle fibers in the nonstimulated muscles was 30.0±1.5%, 40.9±0.9% and 29.1±0.8%, respectively and 35.8±2.3%, 35.1±1.9% and 29.1±0.8% (Mean ± Standard Error), respectively in the electrically stimulated muscles. This suggests that by electrical stimulation to the healthy muscles the proportion of type 1 muscle fiber increased, whereas that of type 2 muscle fiber decreased from the histochemical point of view. And the proportion of intermediate muscle fiber remained almost the same as that of nonstimulated muscles (Fig. 6). The changes of composition of type 1 and type 2 muscle fibers by electrical stimulation were statistically significant ($p < 0.05$).

5. Muscle Fiber Diameter

The short diameters of 200 muscle fibers were measured for each fiber type using 100 times enlarged photographs of the middle layer of tibialis anterior muscle to make histograms of both nonstimulated and stimulated muscles (Fig. 7). The number of muscle fibers was plotted against the diameter of muscle fiber. Stimulated muscle fibers were shifting towards the larger diameter in each fiber type, which are shown in dotted lines comparing with straight lines of nonstimulated muscles. The gain in the mean diameter of stimulated type 1, intermediate and type 2 fibers was 17.6%, 12.4% and 4.1%, respectively.

6. Microangiogram

Fig. 8 indicates transparent longitudinal sections of extensor digitorum longus muscles perfused with India black ink. Two types of vessels were observed in the muscle. Vessels of the first type were thick and running trans-
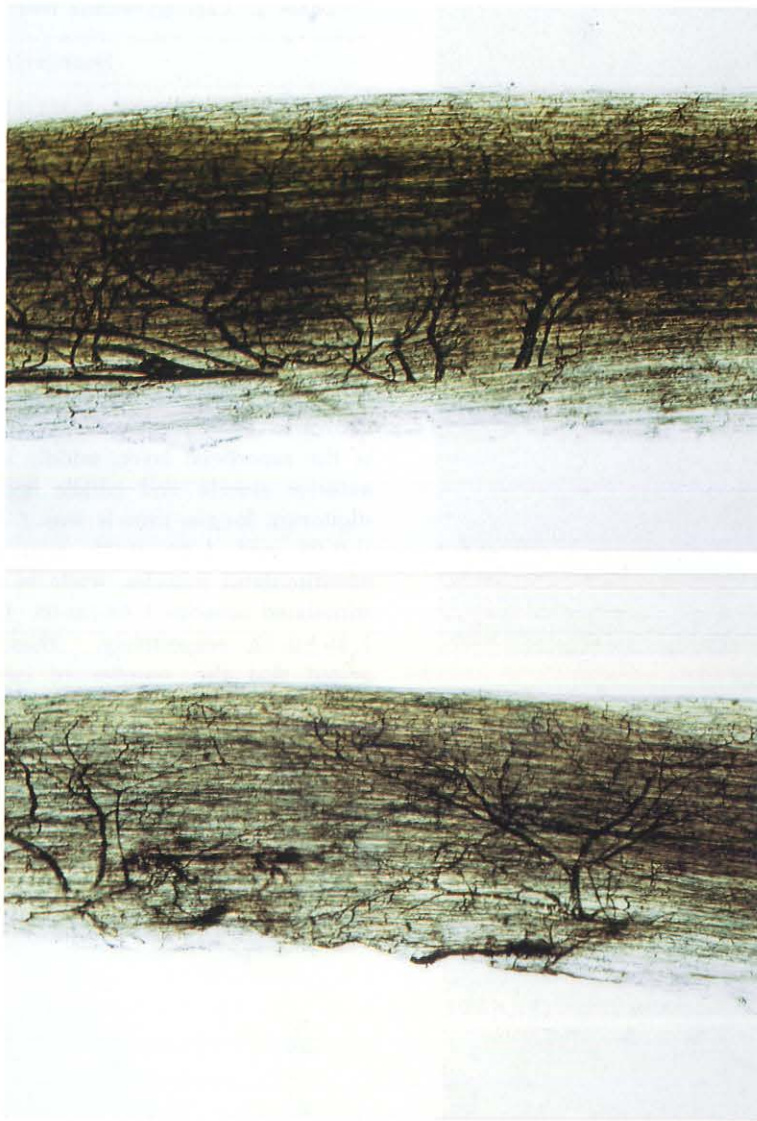
Fig. 8. Longitudinal section of microangiogram (Extensor digitorum longus muscle, 300 μm thick)
Above: Nonstimulated muscle   Below: Stimulated muscle
Vessels of the stimulated muscle were more abundant and thicker than those of the nonstimulated muscle.

versely or obliquely in branches, while vessels of the second type were thin and running longitudinally along the muscle fiber. These vessels of the stimulated muscles were more abundant and thicker than those of the nonstimulated muscles.

In order to evaluate the number of vessels quantitatively, frozen transverse sections were made after the microangiography. Fig. 9 shows the magnified view of the capillaries filled with

India black ink running among the muscle fibers in different directions. This figure also demonstrated that the capillary number and diameter of the stimulated muscles were larger than those of the nonstimulated muscles. The number of capillaries around a muscle fiber (capillary/muscle fiber ratio) was calculated precisely by dividing the number of capillaries by the number of muscle fibers in order to know how
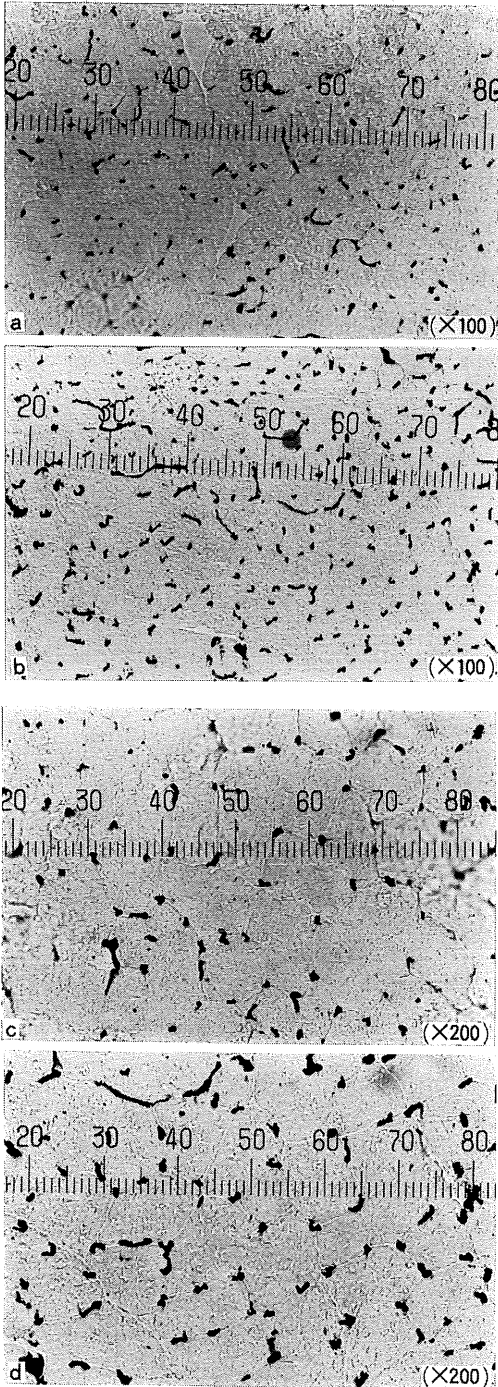
Fig. 9. Transverse section of microangiogram (Tibialis anterior muscle, 10 μm thick)
a, c: Nonstimulated muscle
b, d: Stimulated musele
Capillaries filled with India black ink were observed among the muscle fibers. The number and diameter of capillaries of the stimulated muscles were larger than those of the nonstimulated muscles.

Table 3. Capillary/muscle fiber ratio ($\overline{X} \pm SE$)

		Nonstimulated	Stimulated
TA	Superficial layer	1.32±0.06	1.68±0.08
	Middle layer	1.35±0.06	1.88±0.13
EDL	Middle layer	1.00±0.03	1.55±0.12

TA: Tibialis anterior muscle
EDL: Extensor digitorum longus muscle

many capillaries were increased by electrical stimulation. The average number of capillaries calculated from 600 to 900 muscle fibers are shown in Table 3. Capillary/muscle fiber ratio of the superficial layer, middle layer of tibialis anterior muscle and middle layer of extensor digitorum longus muscle was 1.32±0.06, 1.35 ±0.06 and 1.00±0.03, respectively in the nonstimulated muscles, while in the electrically stimulated muscles 1.68±0.08, 1.88±0.13 and 1.55±0.12, respectively. These results suggested that the number of capillaries filled with India black ink was significantly increased by electrical stimulation ($p < 0.01$).

7. Influence of Duration of Electrical Stimulation on Wet Muscle Weight

In order to study the effect of stimulation duration on the wet muscle weight, right sciatic nerve was also stimulated 15 minutes and 60 minutes daily for two weeks in the same way as
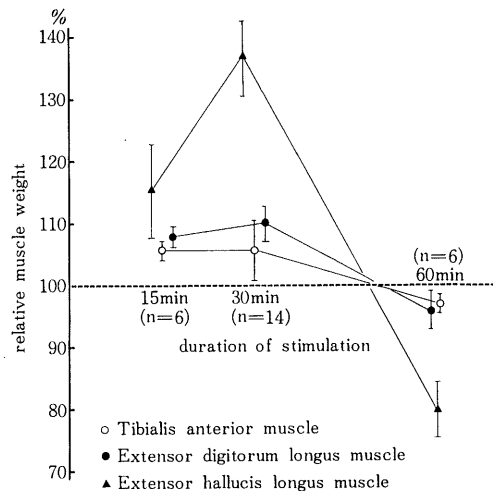

Fig. 10. Relation between the relative wet muscle weight and the duration of electrical stimulation

described in the methods. Relative wet muscle weight of the stimulated muscle calculated as described above was plotted against the duration of daily stimulation (Fig. 10). The relative wet weight of tibialis anterior muscle, extensor digitorum longus muscle and extensor hallucis longus muscle was averaged $105.7\pm1.0\%$, $107.6\pm2.2\%$ and $115.5\pm8.0\%$, respectively in 15 minutes stimulated muscles, and $97.0\pm1.9\%$, $96.5\pm2.8\%$ and $80.0\pm4.4\%$, respectively in 60 minutes stimulated muscles. Gain of wet muscle weight was observed in 15 and 30 minutes stimulated muscles, whereas wet muscle weight decreased in 60 minutes stimulated muscles.

DISCUSSION

Many articles have been published on the effect of electrical stimulation on the denervated muscles. Gutmann et al. (1944)[17] and Kosman et al. (1946)[22] suggested the clinical applicability of electrical stimulation to the treatment of peripheral nerve injury. Recently Hatano et al. (1981)[19] and Harada et al. (1983)[18] have also demonstrated in rats that electrical stimulation can delay the process of muscle degeneration which rapidly develops after sciatic nerve damage. In these experiments muscle contraction can be seen only when electrical stimulation is given to the denervated muscles, whereas in my study muscle contraction can be seen any time even when electrical stimulation is not given. Therefore, when I started my study, there was some possibility that the short duration of electrical stimulation would not give much influence on the healthy muscles. However, after the intact sciatic nerve was stimulated 30 minutes daily for two weeks, remarkable changes were observed on the leg muscles of rats. The red hue of the muscles became much deeper than that of the nonstimulated muscles and muscle weight was found to be increased significantly. These results suggest clinical confidence in applying this procedure to the nondenervated muscles as well as the denervated muscles.

In the following, the effect of electrical stimulation on the healthy muscles are discussed from histochemical and angiographic points of view.

1. Histochemical Findings

Mammalian skeletal muscles have been classified into red and white muscle only by the muscle hue, however the recent advent of a wide variety of physiological and histochemical procedures brought classification of the muscle at the level of muscle fiber cell unit. Padykura (1952)[29] classified the muscle fiber into red and white muscle fibers by SDH stain and on the other hand, Engel (1962)[13] proposed that red and white muscle fibers were regarded as type 1 and type 2 muscle fibers, respectively by adenosine triphosphatase stain. The reports of Ranvier (1874)[30], Denny-Brown (1929)[7] and Edström et al. (1968)[12] demonstrated that red (type 1) muscle fiber contracts slowly and plays an important role in tonic movements, and that white (type 2) muscle fiber contracts fast and contributes to phasic movements. As described above, muscle fibers have been classified into type 1 and type 2 muscle fibers generally from both morphological and functional aspects. In this study I used the classification by Ogata (1958)[27] or Dubowitz et al. (1960)[8], which classified into type 1, type 2 and intermediate muscle fibers by SDH stain.

Eccles et al. (1958)[10] suggested that muscle fiber type is controlled and influenced by its own motoneurone and this concept was confirmed by cross-innervation experiments of Buller et al. (1960)[3,4] and Dubowitz (1967)[9]. According to Eccles et al. (1958)[10], the slow and fast muscle fibers are fired at the frequency of 10 to 20 Hz and 30 to 60 Hz by peripheral nerve impulse respectively. In this study the impulse of low frequency of 5 Hz, which is nearly equivalent to the firing pattern of slow muscle fiber, was given to the right sciatic nerve by electrical stimulation. And as the result, each type of muscle fiber gained SDH activity similarly and some of type 2 and intermediate muscle fibers showed criss-cross appearance in the sarcoplasm as well as subsarcolemmal aggregation, that are characteristic of type 1 muscle fiber. In addition, the composition of each type of muscle fiber classified by SDH stain demonstrated that the proportion of type 1 muscle fiber increased, whereas that of type 2 muscle fiber decreased.

Lømo et al. (1974)[23] electrically stimulated denervated soleus muscle of rats at high frequency of 100 Hz and found that soleus which is originally slow muscle became fast muscle from histochemical and electrophysiological points of view. On the other hand, in this

experiment the healthy muscles also showed the tendency of fiber type transformation by electrical stimulation at low frequency of 5 Hz. These results might suggest that the frequency of electrical impulse, in other words, the contraction pattern of muscle, may be one of the important factors to differentiate the type of muscle fiber. Therefore some of the exercise programs may have almost the same effect as electrical stimulation on the muscle. The work of Edgerton et al. (1969)[11] showed an increase in the percentage of type 1 muscle fibers in plantaris muscle from rats after a 52-day training period consisting of swimming. Barnard et al. (1970)[2] put guinea pigs on the training program of treadmill for 18 weeks and also found an increase in the percentage of type 1 fibers in the medial head of the gastrocnemius muscle. However, in view of the concept that muscle fiber is originally innervated by its own motoneurone, on which the pattern of muscle contraction depends[10], it seems extremely difficult for temporary electrical stimulation or muscle training to change the muscle fiber type permanently. Accordingly it can be considered that the composition of muscle fiber type will return to be normal sooner or later after the end of electrical stimulation or muscle training.

In this study an increase in the diameter of each muscle fiber type was observed and the degree of increase was different among each type of muscle fiber. The gain of diameter was largest in type 1 muscle fiber and smallest in type 2 muscle fiber (Fig. 7). The reason for this result is considered that electrical stimulation with low frequency of 5 Hz, which resembles the discharging pattern of type 1 fiber motoneurone, gave the greatest influence on type 1 fibers.

This also accounts for the difference of degree in wet muscle weight gain among three leg muscles. The gain of wet muscle weight was largest in extensor hallucis longus muscle, which has the largest proportion of type 1 fiber[5], whereas the gain of muscle weight was smallest in tibialis anterior muscle, which has the smallest proportion of type 1 fiber[5]. And the gain of weight of extensor digitorum longus muscle and its proportion of type 1 fiber were between the two (Table 1). From this point of view, low frequency of electrical stimulation should be recommended clinically for the muscles in which type 1 muscle fibers are predominant and it is also assumed that high frequency of electrical stimulation should be recommended for the muscles, in which type 2 muscle fibers are predominant.

2. Microangiographic Findings

Vital microscopic study has been frequently used to demonstrate the vessels of muscles, because the structure and function of the vessels can be seen directly. However, in this method only thin muscle can be observed, therefore, Spaltehorz (1888)[33] method, in which the transparent muscle sections perfused with India black ink can be cut up to the thickness and the level we want, has been utilized generally. Recently the histochemical techniques were introduced in this field, and Romanul (1965)[31] and Eriksson et al. (1972)[14] observed the capillary distribution around each type of muscle fiber. In this study, I used Spaltehorz method first and two types of vessels were observed in the rat muscles. One is comparatively large vessels running transversely or obliquely, in branches into the muscle and the other is small vessels running longitudinally along the muscle fiber (Fig. 8). According to Eriksson et al. (1972)[14], the former vessels are the transverse arterioles and venules deriving from the central vessels and the latter are chiefly the capillaries. In addition, in order to observe the relationship between the vessel and the muscle fiber, muscles perfused with India black ink were immediately frozen to avoid tissue shrinkage artefact and 10 μm thick transverse sections were made (Fig. 9).

Several authors reported that the number of capillaries in the muscle increased after long period of exercise[31]. The work on skeletal muscle biopsy of Saltin et al. (1977)[32] showed that athletes who had long period of exercise revealed the increase in number of capillaries. In my study I applied electrical stimulation instead of muscle exercise, and observed the increase in number of capillaries. Myrhage (1977)[25] also electrically stimulated rats leg muscles at the frequency of 10 Hz and noticed that new capillaries were growing and the number of capillaries increased. The reason why the number of capillaries in the muscle increased have not been elucidated as yet, but one of the reasons might be a relative hypoxia in the muscle due to the increase of oxygen consumption during muscle contraction.

3. General Discussion

Romanul (1965)[31] described that the number of capillaries surrounding every muscle fiber is directly proportionate to its oxidative enzyme activity, such as SDH. In the present study, electrical stimulation was found to increase the number of capillaries in the muscle as well as SDH activity of the muscle fiber. This phenomenon can be considered as a reasonable biological reaction of muscle fiber metabolism and circulation. The muscle owes its red hue to the myoglobin content, which is said to be related to activity of SDH[31], and also the capillaries surrounding the muscle fibers. Therefore the increase of red hue of muscles by low frequency of electrical stimulation is thought to be due to the increase of SDH activity and the number of capillaries, that were obseved microscopically.

Besides qualitative change of histochemical character mentioned above, quantitative changes of the number of capillaries and wet muscle weight were recognized by electircal stimulation in my study. In other words, electrical stimulation can be used to help muscle contraction to prevent the disuse muscle atrophy. Also this procedure can be applied for muscle strengthening exercise, which has already been tentatively utilized, such as for treatment of idiopathic scoliosis[16]. Eriksson et al. (1979)[15] reported that they prevented the atrophy of quadriceps femoris muscle after anterior cruciate ligament repair by electrical stimulation. Onozawa et al. (1983)[28] were satisfied with the effect of the electrical stimulation on muscle strengthening exercise for the patients with osteoarthritis of the knee joints. As electrical stimulation was found to have many benefits, this procedure should be widely utilized in the clinical field.

However, we have to keep this in mind that electrical stimulation might substitute for voluntary muscle contraction, but this procedure is artificial impulse to the last and not natural. Abe (1981)[1] stimulated the rabbits femoral nerve in order to investigate the effect of various levels of stimulation frequency on the quadriceps muscle blood flow and suggested that at the levels of frequency (1-17 Hz), muscle blood flow during contraction increased continuously, but when the stimulation frequency or stimulation duration was increased, muscles became temporarily ischemic, because muscle contraction by electrical stimulation was much stronger than that of physiological stimulation. Hughes et al. (1981)[20] electrically stimulated the sciatic nerve of mice and pointed out that the pulsed current stimulator produced nonspecific changes no worse than those found in the nonstimulated control group, while the direct current stimulator produced frequent myelin degeneration and occasional axon degeneration, particularly with prolonged stimulation.

I have used pulsed current stimulator, but muscle contraction by this apparatus is artificial, as such we might damage the peripheral nerve when we stimulated 60 minutes daily for two weeks. These above mentioned factors may be the cause of weight loss in the daily 60 minutes stimulated muscles.

The effect of stimulation frequency, stimulation duration and various kinds of current waves on the skeletal muscles should be more precisely investigated in the future.

As there are many things unknown to resolve, this study has to be continued to elucidate these points.

ACKNOWLEDGEMENT

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