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DENERVATED CHANGES IN MUSCLE FIBERS AND MOTOR END-PLATES*)

By

Eiji HATANO, Kenya TSUGE, Yoshikazu IKUTA, Yoshihiro MIYAMOTO, Kaoru YOSHIOKA and Nobuo HIRAMATSU

Department of Orthopedic Surgery, Hiroshima University School of Medicine, Hiroshima 734, Japan

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ABSTRACT

A histochemical study was conducted on the anterior tibial muscle of rats after transection of the right sciatic nerve at the proximal third of the thigh. Fibrillation was most prominent at the 4th week after denervation and even at the 7th month tiny fibrillations were infrequently recognized. The denervated muscle reduced its wet weight rapidly for the first one nonth and thereafter the decrease was gradual. From the 3rd month onwards muscle weight loss reached the plateau where the denervated muscle weighed about 15 to 20% against the control. The decrease of S. D. H. activity in the red muscle fibers resulted in histochemical undifferentiation of muscle fiber types, particularly from the 2nd month onwards after denervation. Motor end-plates stained by Wachstein, Meisel and Falcon's method became less visible in the course of time up to the 2nd month after denervation, but thereafter their staining intensity increased gradually with the abnormal internal structure of the synaptic folds.

INTRODUCTION

Once the peripheral nerve is severed, the muscle loses the nerve stimulated contractibility and the neurotrophic influence on the muscle. As a result the muscle becomes atrophic. Many observations exist in the literature concerning the rate of atrophy of the red and white muscle fibers and the changes in motor end-plates after denervation, but the results are different depending upon the muscle examined. The present study was undertaken in order to investigate how long the denervated skeletal muscle and motor end-plates keep its histochemical properties and how the degenerative process of muscle fibers and motor end-plates develop after denervation. For this aim the period of denervation was extended until the 7th month after denervation.

EXPERIMENTAL METHODS

The experiments were performed on 30 Wistar albino male rats weighing about 200 grams under somnopentil anesthesia with the right sciatic nerve severed at the proximal 1/3 of the thigh. After division of the nerve the ends were treated in order to prevent regenerating axons from approaching the distal stump. The rats were allowed to survive for 1, 2, 3, 4 wks, 2, 3, 4, 5, 6 and 7 months to be prepared for the next procedures.

The electromyography was taken at the middle point of the anterior tibial muscle under somnopentil anesthesia at the respective time.

*) 畑野栄治，津下健哉，生田義和，宮本義洋，吉岡薫，平松伸夫：神経切断後の筋肉と神経筋接合部の変化

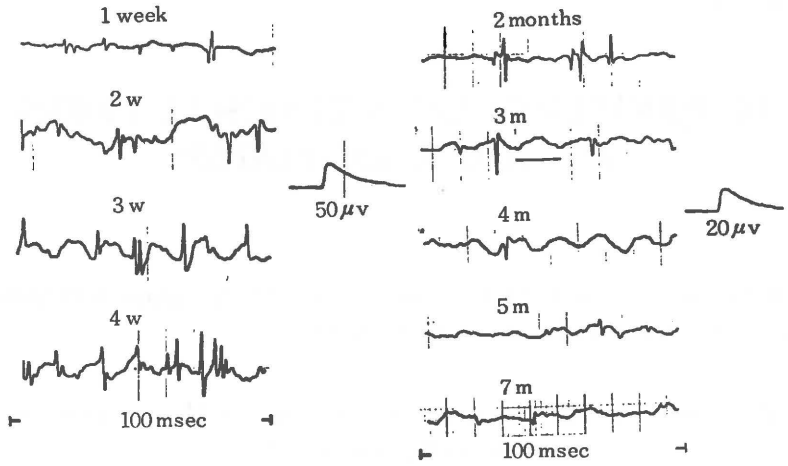

Fig. 1. Electromyographic changes after denervation
Fibrillation increased the amplitude and frequency up to the 1st month after denervation and thereafter decreaased them rapidly. Even at the 7th month after denervation very tiny fibrillations were present infrequently.

The anterior tibial muscles were carefully removed on both sides and were weighed to the nearest milligram. The control side was used in calculating the percentage of wet muscle weight loss.

The appropriate muscle was taken from the respective muscle bellies. They were immersed in n-hexane which was cooled by a mixture of dry ice and acetone for rapid freezing. Ten micra cross-sections were cut in a cryostat at −25°C and then dried in open air at room temperature for 10 minutes. They were stained for succinic dehydrogenase (S. D. H.) by the method of Nachlas et al[1]. A number of photographs with a calibrated scale were taken of the intermediate regions of the muscle to measure the short diameter of 200 muscle fibers for each fiber type. For histochemical study of the motor end-plates, frozen sections which were cut longitudinally 30 micra in thickness were stained by the method of Wachstein, Meisel and Falcon[2].

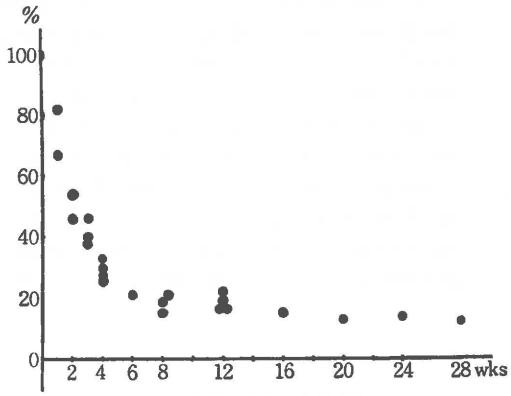

Fig. 2. The changes in wet weight loss after denervation
The percentage of weight loss is plotted against duration in days. The denervated muscles made a rapid loss in wet muscle weight for the first one month and thereafter the decrease was gradual. From the 3rd month onwards the weight loss reached a plateau, where the muscles weighed about 15 to 20% against the control.

OBSERVATIONS

Summarized in Fig. 1 are the results of electromyographic examination of the muscle following nerve transection. The data indicate that fibrillation is seen at the first week after denervation and that even at the 7th month only a tiny fibrillation is somewhat visible. As for the frequency and the amplitude of fibrillation, they increased up to the 4th week after neurectomy and thereafter decreased rapidly.

The changes observed grossly in the denervated muscles were a progressive loss of bulk and changes in colour from red to pale. The shape of the muscle was rod-shaped rather than spindle-shaped. The longer the time after

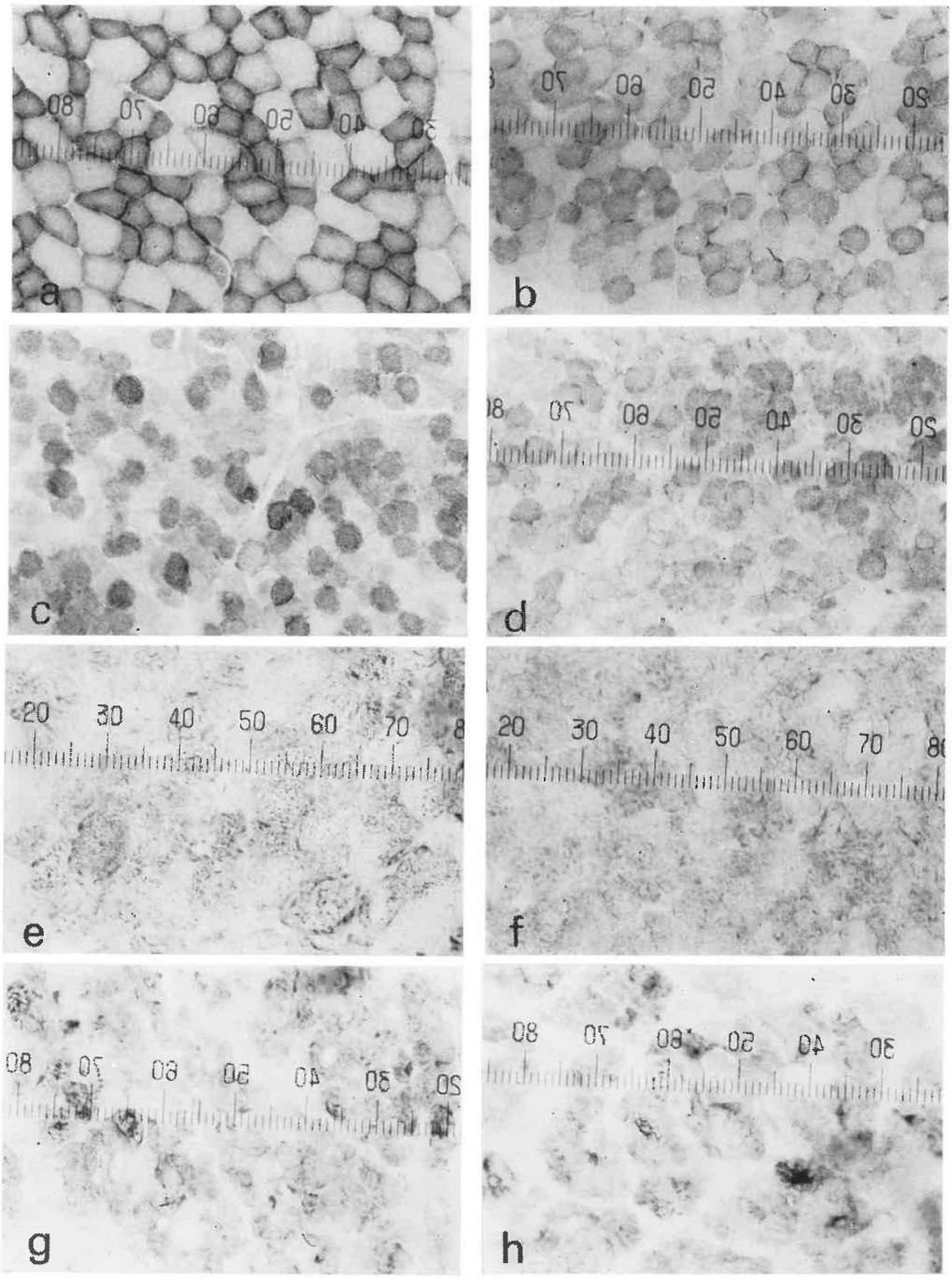

Fig. 3. Histochemical changes in the muscle fibers after denervation

After nerve transection S. D. H. activity decreased gradually in type I fibers, which caused the contrast between the enzymatic activity of the different types of muscle fibers to decrease. From the 3rd month onwards after denervation no difference was recognized between the muscle fiber types. a. normal (×100), b. 2 weeks (×100), c. 3 weeks (×100), d. 4 weeks (×100), e. 2 months (×200), f. 3 months (×200), g. 5 months (×200), h. 7 months (×200) after denervation respectively. Specimens from a to d were incubated for 20 minutes, but those from e to h for 30 minutes due to weak enzyme reaction.

denervation, the more remarkable were these changes.

The changes in wet weight loss are depicted in Fig. 2. Weight loss, expressed as percent of normal, decreased rapidly for the first 4 weeks. The denervated muscle weighed about 50% at the 2nd week after denervation against control, 30% at the 4th week and 15% to 20% from the 3rd month after denervation.

In the normal anterior tibial muscle stained for S. D. H., the dark fibers have a large number of mitochondria and are red (type I), and the light fibers have little oxidative activity and are white (type II). These different muscle fibers were distributed like a checkerboard appearance. The internal structure of the red muscle fibers seemed to be criss-cross in appearance secondary to the enzymatic activity between the myofibrils. Also the red muscle fibers had particulary high activity just at the subsarcolemma. The first change seen at the 2nd week after denervation was a very slight decrease of enzyme activity and the rounding tendency of the fibers. At this time the disorganization of muscle fiber architecture was negligible. At the 3rd week the checkerboard pattern given by the different types of muscle fibers became unclear. This was due to the loss of S. D. H. activity in the red muscle fibers. The muscle fibers became more round in shape and slightly shrank. Subsarcolemmal aggregation of the red muscle fibers began to disappear. Some criss-cross appearances of the internal structure of the red muscle fibers were replaced by parallel arranged diformazan. At the lst month after nerve severance the above described changes were extensive. Most of red muscle fibers became less stainable. Consequently, the contrast between the enzymatic activity of the different types of muscle fibers decreased. The red fibers showed total loss of enzymatic activity of the subsarcolemmal aggregation. The specimens taken after more than 2 months following denervation were incubated for 30 minutes because of low enzymatic activity. At the 2nd month the fibers were of uniform colour and for this reason the checkerboard appearance was not visible. The fibers were very much reduced in size.

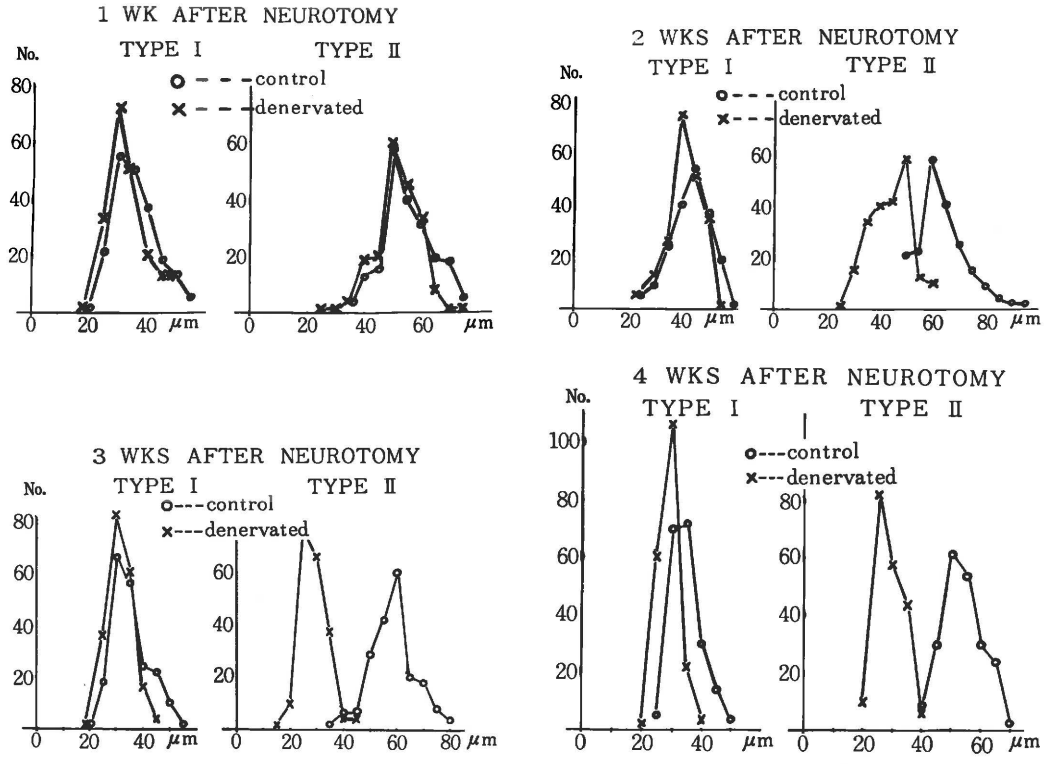

Fig. 4. Histograms of muscle fiber diameter after denervation
Type II muscle fibers were reduced in size much more than type I muscle fibers.

At the 3rd month the activity was very low in all muscle fibers and no difference was present between the muscle fiber types. Less details could be seen because of the extremely decreased enzymatic activity. Only a blurred outline of the muscle fibers was recognized. At the 5th month after denervation the muscle fibers appeared to be debris and fragments of the muscle fibers. The small stained dots were found in some muscle fibers. The above described changes were found to develop more at the 7th month (Fig. 3).

Following denervation there was preferential atrophy of white muscle fibers. These results are shown graphically in Fig. 4. Measurement of each fiber was made across its smallest diameter. At the 2nd week after denervation the atrophy of the white muscle fibers was easily seen, however, as for the red fibers the reduction in size was very small even at the 3rd week and reduction became distinct at the 4th week. These results showed that the degree of decrease in size was greater in white muscle fibers than in red fibers. From the 2nd month onwards the histogram could not be made due to undifferentiated fiber types (Fig. 4).

The normal neuromuscular junctions of the rat stained by the procedure of Wachstein, Meisel and Falcon demonstrated intense staining of the subneural apparatus. Round to oval shaped subneural apparatus had tortuous synaptic folds in the muscular part of the neuromuscular junctions. At the 1st week after denervation a slight decrease was observed in the intensity of enzyme reaction. All end-plates were distinctly outlined. At the 3rd week the decrease in the intensity was moderate and the reduction in size was distinct. The great majority of end-plates had more or less irregular arrangements of the synaptic folds. At the 4th week subsequently the end-plates changed in shape from round to long in relation to its diameter, possibly due to degenerative changes in the muscle fibers. The afore-mentioned disorganization of the internal architecture was more advanced. At the 2nd month an extremely weak enzyme reaction was observed to an extent that the outline of the motor end-plates was not clearly visible. All endplates were long shaped and were completely void of normal internal structure. In the interval of 3 months after denervation a very poor gain of enzymatic activity was recognized, but the internal structure of the end-plates was completely disorganized. At the 5th month most of end-plates exhibited a reaction of almost normal intensity and the increase of number of end-plates was found. However, the end-plates were not morphologically quite similar to the intact ones. All endplates definitely lacked in the normal internal part of synaptic folds. The shape of end-plates were long in relation to the direction of muscle fibers. Seven months' specimens showed that reaction intensity became higher, but that the structure of the subneural apparatus was cpmpletely destroyed compared with the normal end-plate's internal structure (Fig. 5).

DISCUSSION

Romanul[3] identified eight different muscle fibers in the normal muscle fibers of the gastrocnemius and plantaris from the histochemical standpoint, but we differentiated three types of muscle fibers by S. D. H. staining. There are various opinions concerning the preferential muscle fiber atrophy after denervation. Fidzianska[4] concluded that the red and intermediate muscle fibers became atrophied soonest and most markedly and that no structural alterations were observed in the white muscle fibers. Jakubiec-Puka) mentioned that atrophy appeared earlier in the soleus (slow muscle), but from the 15th day onwards the degree of atrophy was equal in both soleus and anterior tibial muscle up to the 90th day by severing the rat sciatic nerve. The results of Jaweed's experiment[6] showed that in the soleus the fiber diameter of red and white muscle fibers atrophied similarly, but that the fiber diameter of white fibers became smaller than that of the red fibers in the plantaris (fast muscle). He concluded that the response to denervation of the red and white muscle fibers might be different in the slow and fast muscles. The intermediate regions of the anterior tibial muscle consisted of approximately 40% of red fibers, 40% of white fibers and 20% of intermediate fibers. Therefore, from the histochemical point of view the anterior tibial muscle seemed to be partly fast muscle and partly slow muscle. For this reason we can not infer a preferential atrophy in the anterior tibial muscle from Jaweed's hypotheses. Practically we demonstrated

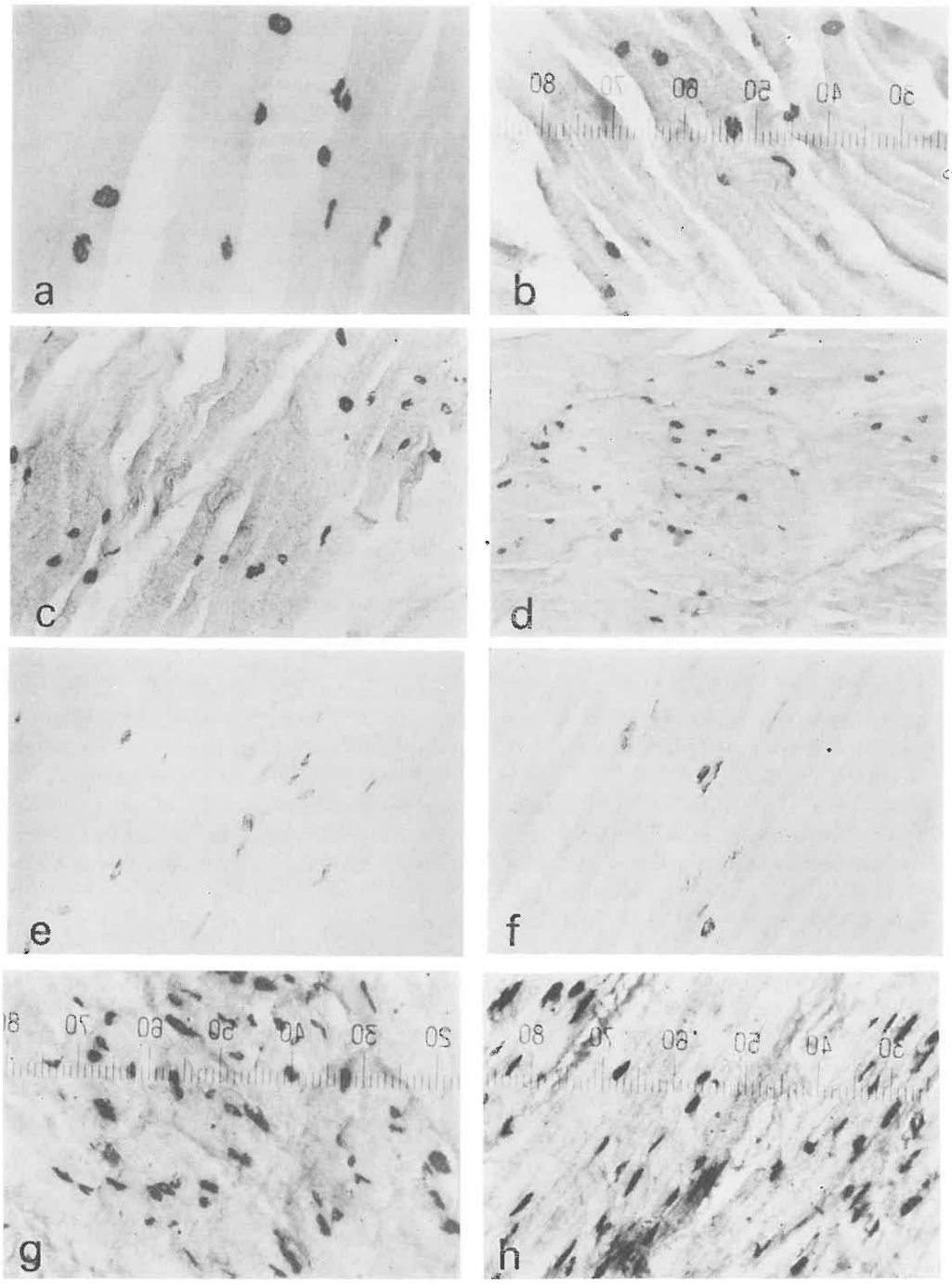

Fig. 5. Histochemical changes in the motor end-plates after denervation

After denervation the motor end-plates lost the enzyme intensity and were reduced in size gradually. At the 2nd month the enzyme reaction was lowest with indistinct end-plate outline. Thereafter the motor end-plates regained the enzyme intensity and increased the size rapidly, but their internal structure was completely disorganized. a. normal (×100), b. 1 week (×100), c. 3 weeks (×100), d. 4 weeks (×100), e. 2 months(×200), f. 3 months (×200), g. 5 months (×100), h. 7 months (×100) after denervation, respectively.

that the white muscle fibers reduced the size more than the red fibers. The decrease of muscle fiber diameter after denervation is thought to be due to a loss of both sarcoplasm and myofibrils. Denervated muscles lose the trophic influence of the nerves on muscle and nerve-stimulated contractibility of muscle. Therefore atrophy occurs after denervation according to the fiber types. However, the concept of trophic nerve function is still vague. Smith[7] mentioned that the histochemical appearance of denervated muscle was similar to cardiac muscle. Hogan[8] reported that following denervation, a selective depletion of the enzyme of the muscles occurs and that there are some enzymes of acid phosphatase and β-glrcuronidase which increase the activity after denervation. These increased proteolytic enzymes might cause an intracellular digestion process in the muscle fibers. Consequently, the enzymatic differences between the various types of muscle fibers gradually decrease and at last disappear.

Various types of choline ester-splitting enzymes have been said to exist. In this experiment the motor end-plates were demonstrated by the method of Wachstein, Meisel and Falcon. According to them thiolacetic acid is hydrolyzed by acetylcholinesterase as well as thiolacetic acid esterase. And the produced hydrogen sulfide can be demonstrated as dark brown lead sulfide[9]. In our experiment we did not use any cholinesterase inhibitor, and therefore the enzymes of acetylcholinesterase and thiolacetic acid esterase were demonstrated at the endplates after denervation. The results showed that there was gradual but greater increase of enzyme activity in the subneural apparatus from the 3rd month after denervation, but the shape and internal structure of the end-plates were completely different from the normal. The present results gave rise to the question of why the motor end-plates after a long period of denervation regain the enzyme activity.

Fibrillation is said to arise 2 to 3 days after nerve transection of rats and Sunderland[10] observed fibrillation 485 days after denervation. We demonstrated the maximum frequency and maximum amplitude of fibrillation at the 4th week after denervation and even at the 7th month following denervation very tiny fibrillations remained infrequently.

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