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Electromyographic and Histological Studies on the Regeneration of Motor Nerve Endings in Dogs

by

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Histological changes in the motor nerve ending after denervation and during the process of reinnervation were extensively investigated by GUTMANN and YOUNG[17] in 1944. In the same year BOWDEN[2] and WEDDELL[22] also made electromyographic studies on denervation and reinnervation of human muscles. Since then many works have been done concerning these subjects.

In 1922 HOFFMANN[9] first made a study of electical activities of monosynaptic reflexes in men by an evoked electromyography. These electrical activities have been further investigated in detail by LIOYD[15], HODES[8], MAGLADERY[16] and many other workers.

The present paper is concerned with comparative studies between electromyographic findings (especially evoked E. M. G.) and histological changes made during six months after nerve section and immediate repair by suture in dogs.

MATERIAL AND METHODS

Twenty nine adult mongrel dogs were used. They were anesthetized with an intravenous injection of pentobarbital in a dose of about 5 mg per kg, and placed in the prone position on a specially designed operating table (Fig. 6). The dorsal aspect of the right hind limb was shaved and disinfected. The tibial nerve was aseptically exposed over a length of 5 to 6 cm just proximal to the popliteal fossa. It was severed about 1.5 cm distal to the junction of the peroneal nerve and the tibial nerve (3 to 4 cm proximal to the calf muscle), and both cut ends were immediately approximated by epineural suture with 6-0 silk thread. The operative wound was then closed.

The sutured nerve was exposed at varying postoperative intervals under an intravenous anesthesia. The peroneal nerve was resected about 2 cm in length to prevent a simultaneous contraction of the extensor muscles by an electrical stimulation of the tibial nerve. Then a copper band covered with warm saline-moistened gauze was applied around the knee joint to minimize the stimulus artifact.

The tibial nerve, immersed in the warmed paraffin pool, was stimulated at the level proximal to the suture site with paired needles which were kept 0.5 cm apart and insulate at their tips and connected with an isolated stimulator. Square waves of 0.5 millisecond duration were applied with increasing intensity from the threshold to supramaximal stimulation at 2 second intervals. Then, evoked electromyograms (hereafter, abbreviated

as evoked E.M.G.) were made through paired electrodes of 1 cm square silver foil which were laid 2 cm longitudinally apart on the prepared skin overlying the lateral belly of the calf muscle. At the same time evoked E. M. G. were recorded from the control side by the same technique used for control. All these experiments were done in a room which was in part electrically shielded.

After the electromyographical examination the animals were bled to death by severing the carotid artery so as to minimize blood content in the muscle specimens. The calf muscles were removed from both legs, weighed and fixed in 10 % buffered Formalin. After fixation 10 to 20 blocks of $1 \times 1.5 \times 1$ cm were obtained from each specimen. They were dehydrated in serial alcoholic solutions and embeded in celloidin. The blocks were sectioned longitudinally in a thickness of 13.5 to 15 microns and stained by SUZUKI's[20] modification of BIELCHOWSKY's silver impregnation technique. The nerve endings in each section was examined under a light microscope.

The animals were divided into seven groups : Group 1 (4 dogs), Group 2 (2 dogs), Group 3 (4 dogs), Group 4 (4 dogs), Group 5 (3 dogs), Group 6 (6 dogs), and Group 7 (6 dogs) were examined 1, 1.5, 2, 3, 4, 5 and 6 months after nerve suture, respectively.

RESULTS

1) Electromyographic Findings

The fibrillation activities appeared in the denervated calf muscles from 5 to 14 days after the operation.

One month postoperatively (Group 1) the nascent motor unit action potential (hereafter, abbreviated as complex N.M.U. potential) was not yet recorded. Small monophasic action potentials were seen in the evoked E.M.G. (Figs. 7 a and b). These action potentials always showed a high threshold value and a markedly increased latency of about 15 milliseconds. However, the nerve stimulation caused a simultaneous contraction of the hamstring muscles, which increased in intensity with stimulation. This fact suggests that these electrical activities were not induced from the muscle being tested but from the hamstring muscles that contracted simultaneously.

One and a half months postoperatively (Group 2) complex N. M .U. potentials were recorded for the first time. In Figs. 8 a and b are shown the patterns of electrical activities in evoked E.M.G. when the response first became detectable. These electrical activities are designated as M waves and consisted of small potential fluctuations, being less than 20% of the control value at the supramaximal stimulation. The M wave showed a latent period of 4.7 to 8 times that of the control and a higher threshold and was not dispersed.

Two months postoperatively (Group 3) complex N.M.U. potentials as well as fibrillation activities were found, but the normal neuromuscular unit potential (hereafter, abbreviated as normal N. M. U. potential) was not yet recorded. The maximal amplitude of M wave increased from 24% to 43% of the control (Figs. 9 a and b).

Three months postoperatively (Group 4) normal N. M. U. potentials were first recorded. The maximal amplitude of M wave increased from 20% to 96% of the control, its latent period became shorter, being 3 to 3.5 times the control value, and its threshold was decreased (Figs. 10 a and b).

Four months postoperatively (Group 5) fibrillation activities continued to be recorded, but complex N.M.U. potential and the normal N.M.U. potential were evoked very easily. The latency of the M wave diminished from 170 to 200% of the control. Its threshold was lower than that in previous groups, and its maximal amplitude markedly increased, reaching from 41 % to 90 % of the control value (Figs. 11 a and b).

Five and six months postoperatively (Groups 6 and 7) the appearance of the fibrillation and complex N.M.U. potentials became less frequent. The normal N. M. U. potential consisting of a complete interference pattern was recorded from all points of the muscle being tested and the reinnervation potentials or giant spikes were seen for the

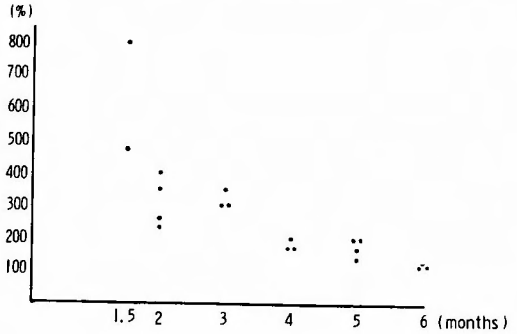

Fig. 1 Latency of response as expressed in percentage of the control value. It gradually decreases from 1.5 months to 6 months postoperatively when it reached the control value.

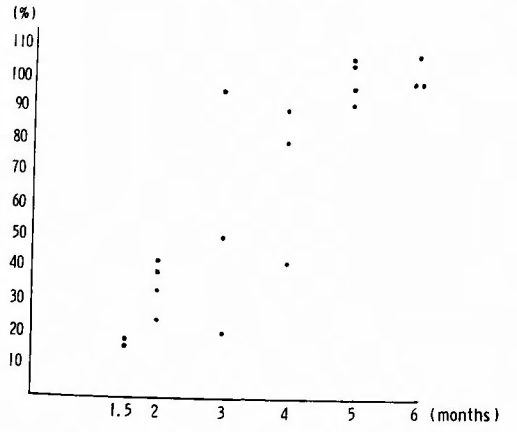

Fig. 2 Amplitude of M wave in response to supramaximal stimulation as expressed in percentage of the control value. It gradually increases in the course of regeneration of the nerve. Two dogs in Group 6 and one dog in Group 7 show higher value than control.

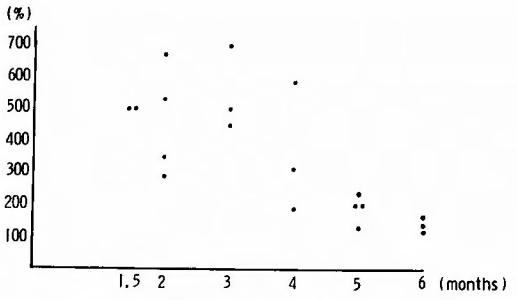

Fig. 3 Maximal stimulation voltage as expressed in percentage of the control value. It falls gradually from 1.5 months to 6 months postoperatively when it is near normal.

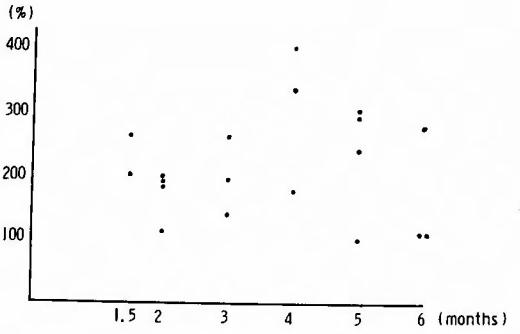

Fig. 4 Strength of threshold as expressed in percentage of the control value. No definite trend is recognized after nerve suture

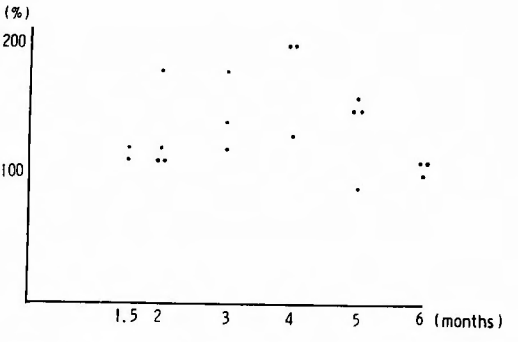

Fig. 5 Temporal dispersion in each group as expressed in percentage of the control value. There are no significant changes after nerve suture.

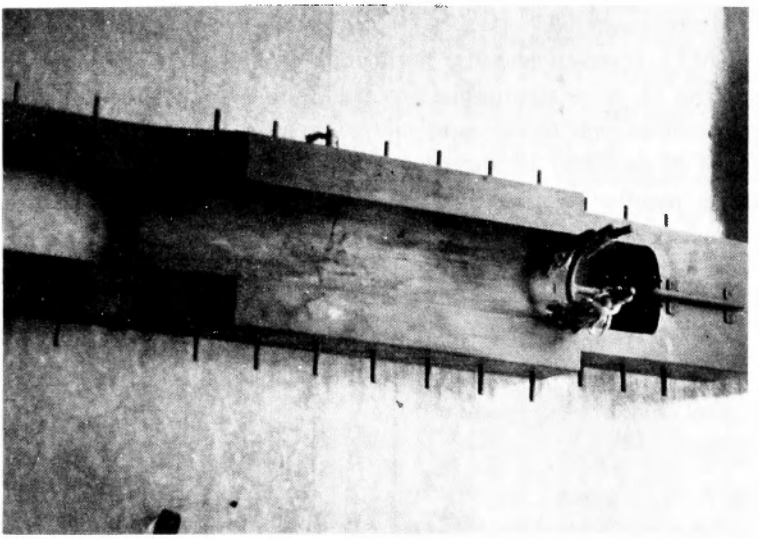

Fig. 6 Operating table designed to place the dog in the prone position.

first time. The threshold and latency of the M wave, especially those of Group 7, were nearly normal, being between 110 % and 120 % respectively of each control value. Also, the maximal amplitude reached or even exceeded the control value, ranging from 92 to 106 % in Group 6 and from 98 to 107% in Geoup 7 (Figs. 12 a and b, 13 a and b).

The results mentioned above are summarized in Figs. 1 through 5. During the postoperative period of 1.5 to 6 months the latency of response, maximal potential of M wave and maximal stimulation voltage gradually decreased and approximated the normal value, whereas the threshold value and temporal dispersion showed no significant changes.

2) Histological Findings

One and a half months after the operation (Group 2), muscular nuclei were multiplied, striations obscure and silver impregnation was not homogenous. Muscle fiber showed marked atrophy. Axons were tapered, smooth and not tortuous. The regenerated fine axis cylinder already reached the end-plate (Figs. 15 a and b).

Two months postoperatively (Group 3) muscular nuclei were proliferated, increased in size and irregularly stained. Muscular striation became clearer than that of Group 2. The regenerated axons increased in diameter. The oval-shaped, elongated end-plate nuclei were grouped at the nerve ending, but not arranged in the shape of a sole (Figs. 16 a and b).

Three months postoperatively (Group 4) muscular nuclei were still proliferating and muscular striations were clearly recognized. The regenerated axis cylinder showed terminal arborization, resembling a matured axon. The end-plate nuclei were oval, uniform in size and shape, more argyrophilic and arranged in the shape of a sole (Figs. 14 a and b, 17 a and b).

Five months postoperatively (Group 6) muscular nuclei were decreased to normal in number and fibers recovered to normal in size. The axons were tortuous, knobby

and thick. The end-plate nuclei were homogenously and densely impregnated and took the sole-shaped arrangement. There was a light halo around the end-plate (Figs. 18 a and b).

Six months postoperatively (Group 7) muscular nuclei were decreased in number and fibers were restored to normal as found in Group 6. The axons wers more matured than those of Group 6, forming terminal arborization. The oval-shaped end-plate nuclei were clearly stained and decreased in size, resembling the normal ones (Figs. 19 a and b).

DISCUSSION

Of the electromyographic activities of the skeletal muscle recorded during the course after the nerve suture, the fibrillation potential serves as the most important electric index in early stages of muscular denervation. The period between the muscular denervation and the onset of fibrillation potential varies from one species to another. Even of the same species it differs from one animal to another. According to WEDELL et al.[21], the onset of fibrillation after denervation ranges from 3.5 days (the peroneal muscles of mouse) to 18 days (human brachioradial muscle) in five different species including mouse, rat, rabbit, monkey and man. INADA et al.[12] described that scattered spike potentials with low amplitude and poor persistency appeared in the m. quadriceps femoris of dogs on the fifth day after nerve section, stating that their observations in dogs were generally in keeping with those made on cats by DENNY-BROWN[4], BROWN[3] and ROSENBLUETH[19]. The author's results obtained in dogs also agree well with those by INADA et al.[12]. The fibrillation potential appeared five to fourteen days after the operation.

An electromyographic study on regeneration of the denervated muscles were performed in 1944 by BOWDEN and GUTMANN[2] on 86 patients with peripheral nerve injury. Their electromyographic description was confined to fibrillation activity and motor unit action potential. WEDDELL, FEINSTEIN and PATTLE[22] observed the regenerative process of the brachioradial muscle branch of the radial nerve after its compression. They stated that dimunition of fibrillation potentials and appearance of highly polyphasic spike of low amplitude were indicative of regeneration of the denervated muscles. They also described the high amplitude waves of longer duration than normal which were thought to develop with the restoration of muscular function and to persist as long as 18 months thereafter.

GOLSETH and FIZZELL[6] (1947) who made electromyographic observations on cats for 90 days after section followed by suture of the sciatic nerve stated that electromyographic findings returned to normal when muscular function was completely restored. In Japan, KIRITA et al.[14] reported that complex N. M. U. potential appeared nearly at the same time as the regenerated axis cylinder reached the end-plate in rabbits.

The H wave was originally described by HOFFMANN[9]. His observation in man was confirmed by LLOYD[15] in cats. In 1955, MAGLADERY[17] analyzed H waves of men in detail. In Japan, since the reports of FUJIMORI[5] and HOMMA[10] H reflex (monosynaptic reflex) have been studied by many investigators. The author's attempt to percutaneously record H wave in dogs failed. The H waves were also not clearly demonstrated by direct electrical stimulation of the tibial nerve, except in two instances in which deflections similar

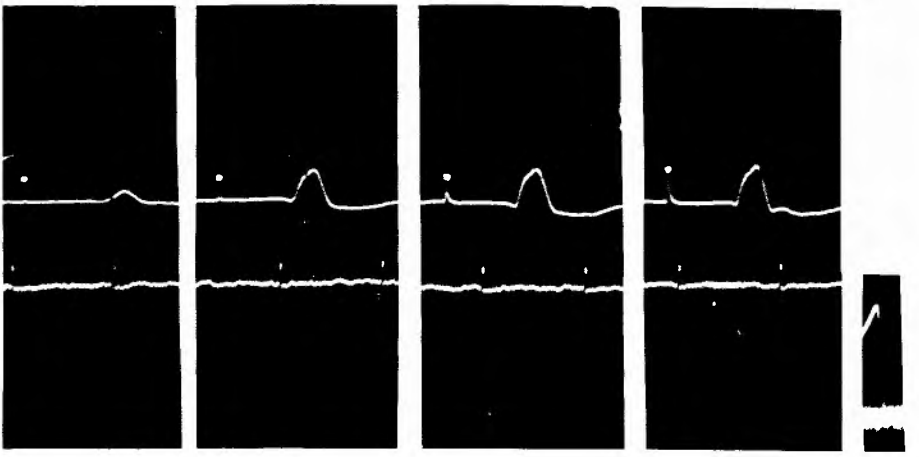

Fig. 7 a

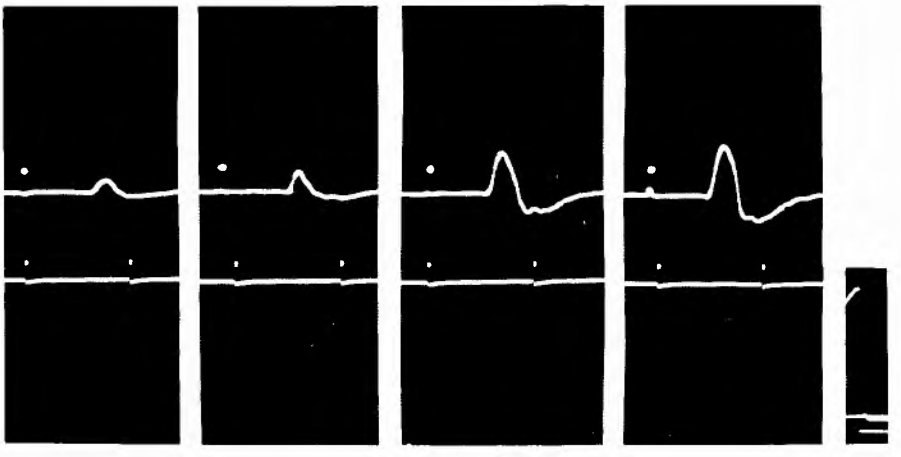

Fig. 8 a

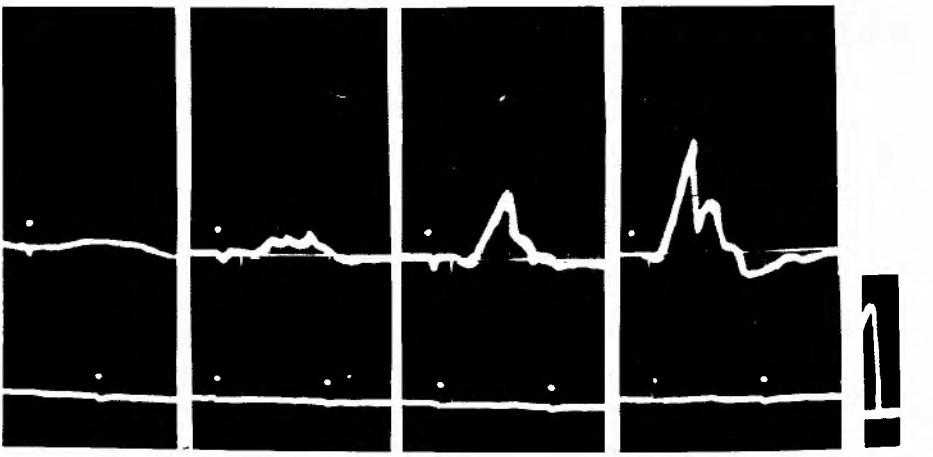

Fig. 9 a

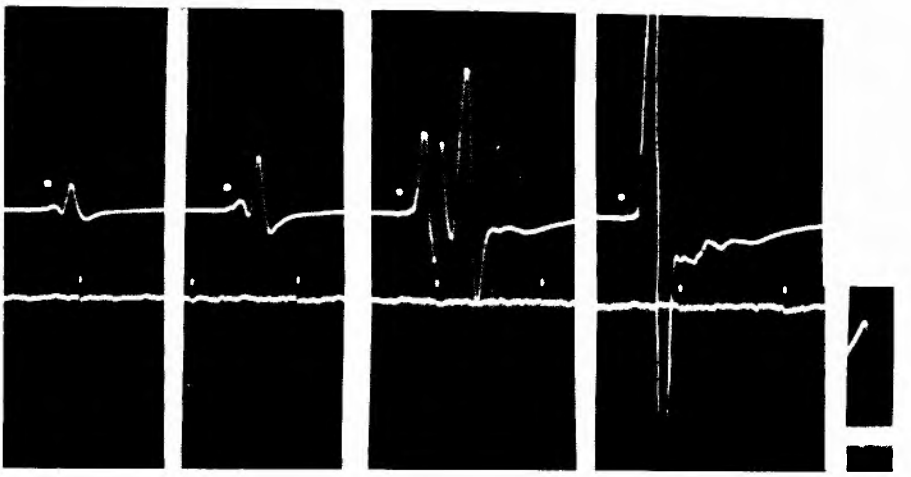

Fig. 7 b

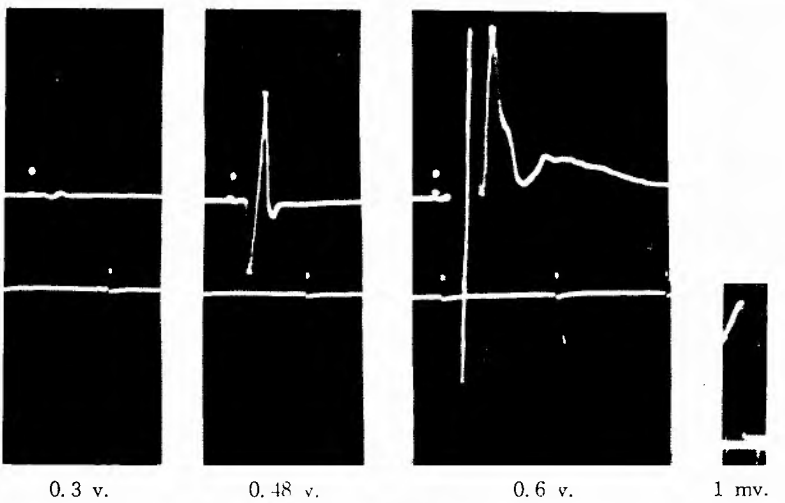

Fig. 8 b

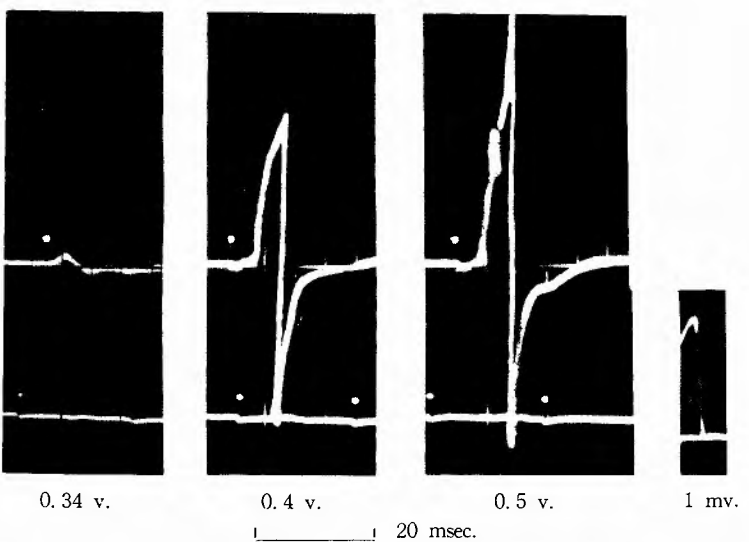

Fig. 9 b

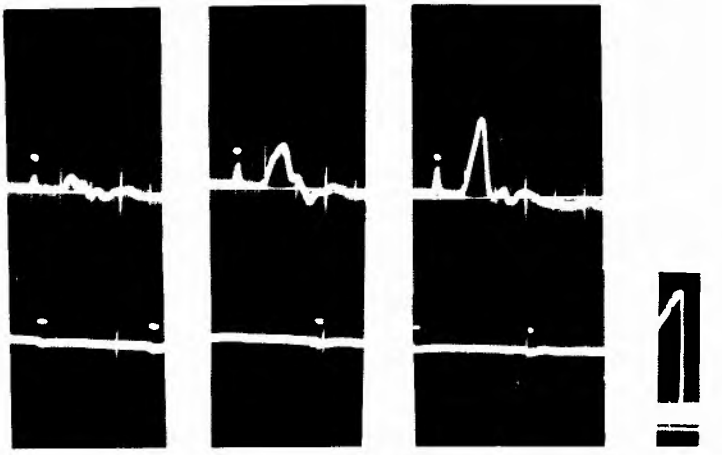

Fig. 10 a

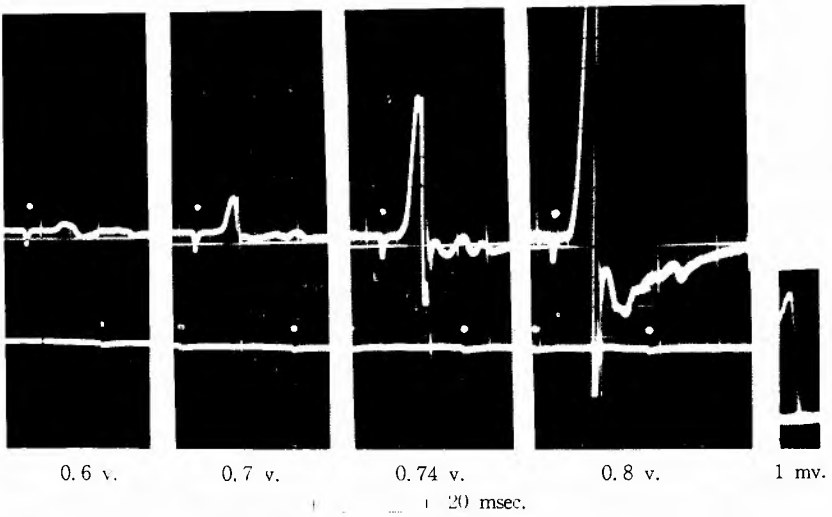

Fig. 11 a

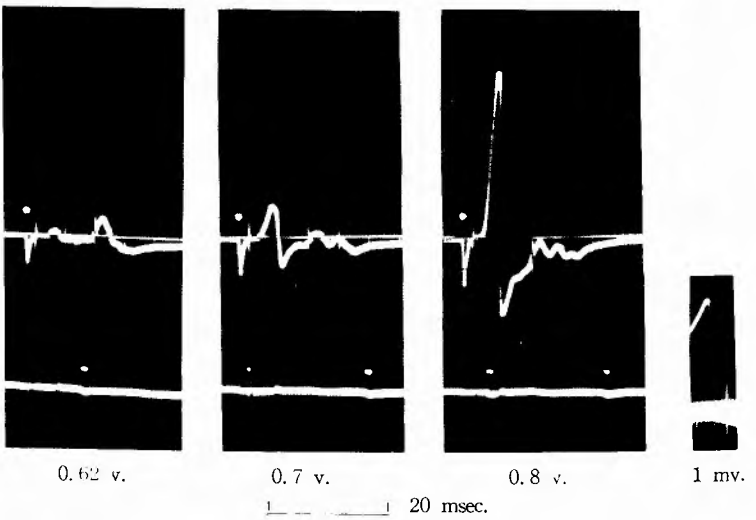

Fig. 12 a

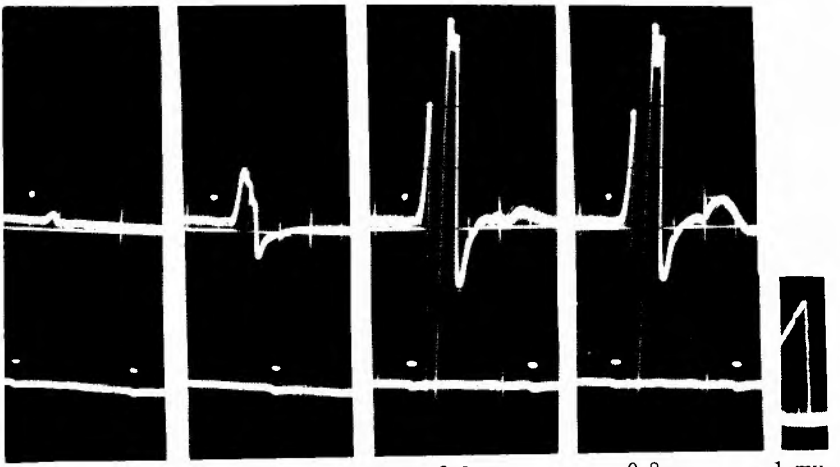

Fig. 10 b

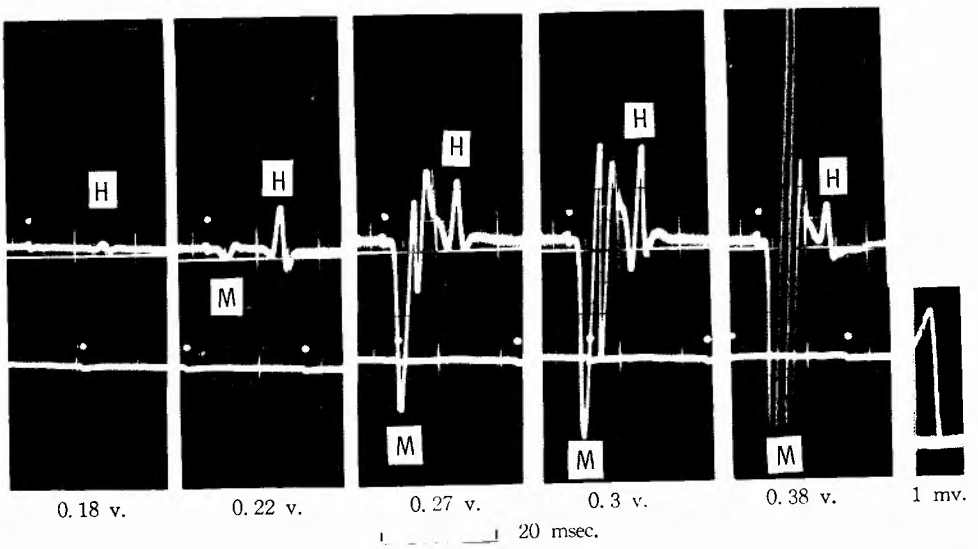

Fig. 11 b

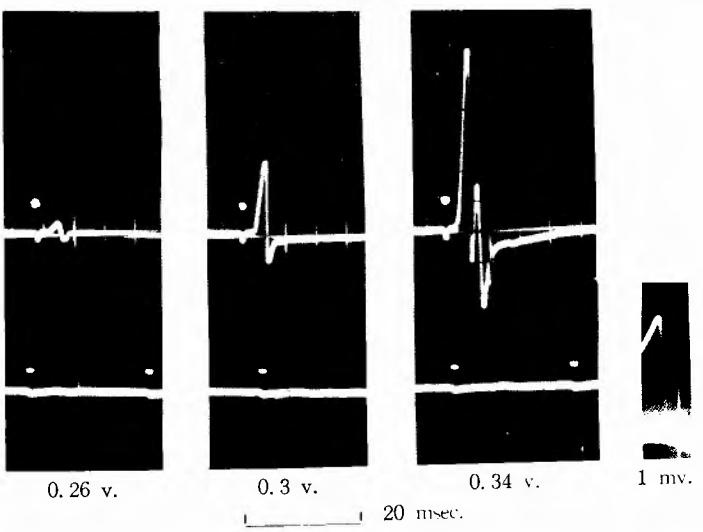

Fig. 12 b

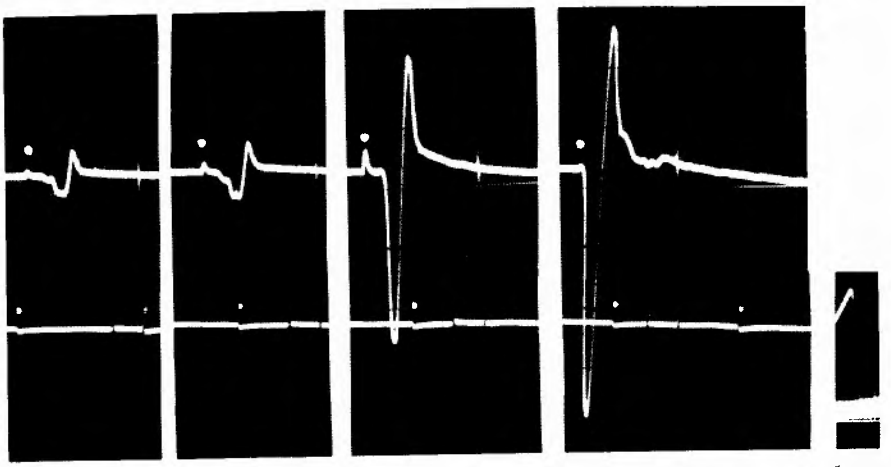

Fig. 13 a

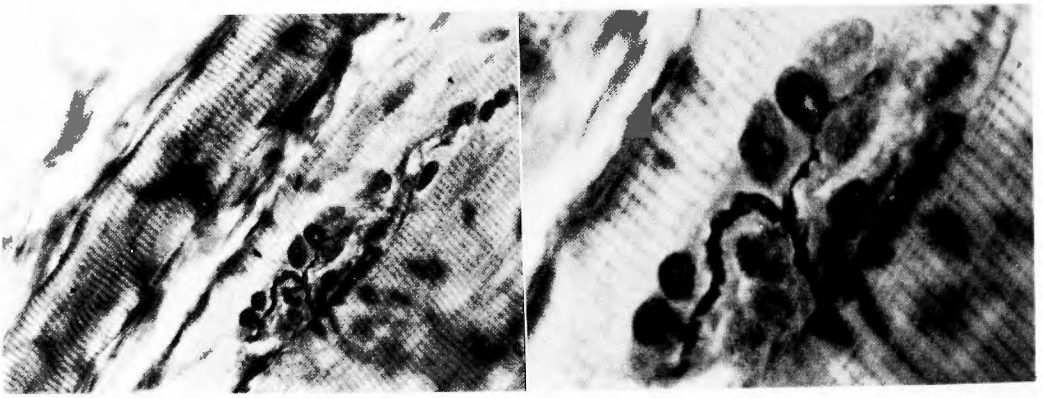

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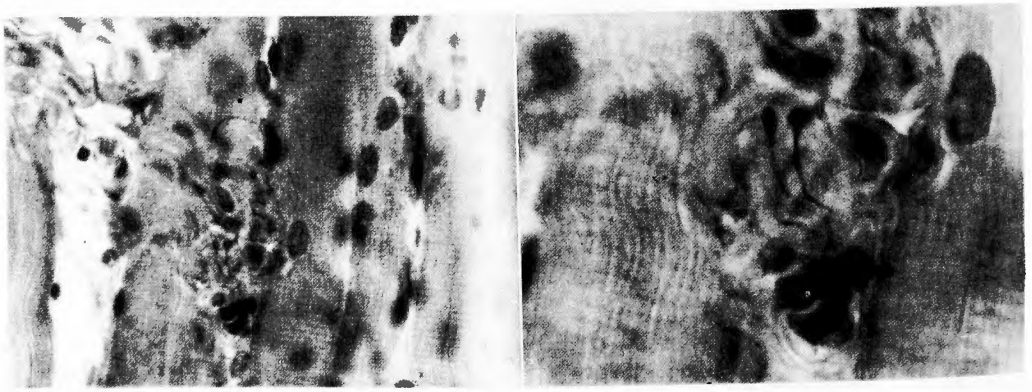
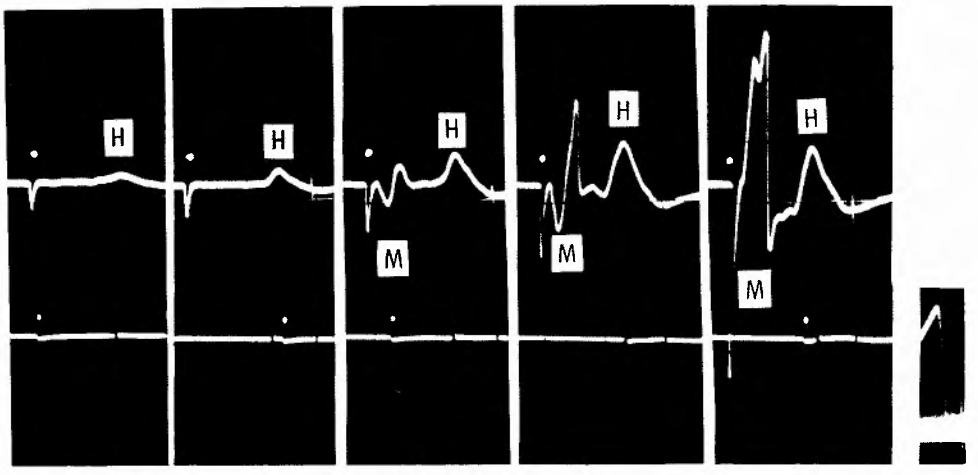
Fig. 14



a b

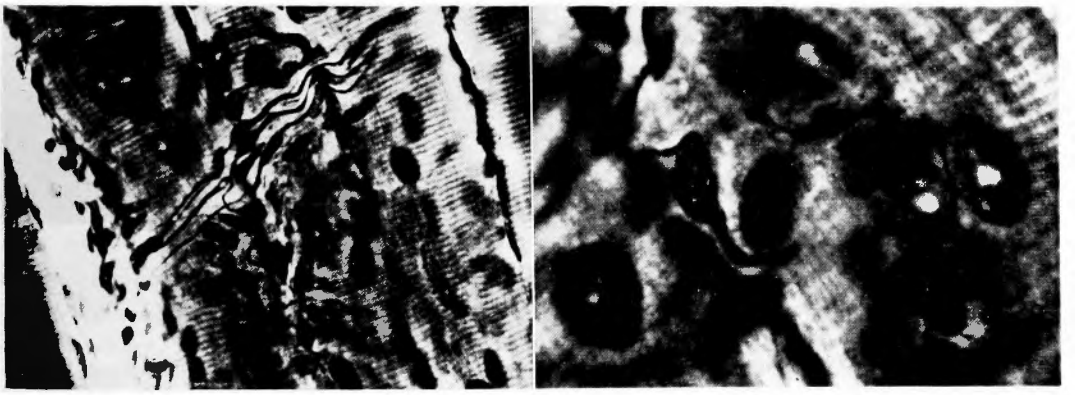
Fig. 15



0.42 v. 0.46 v. 0.58 v. 0.7 v. 0.98 v. 1 mv.

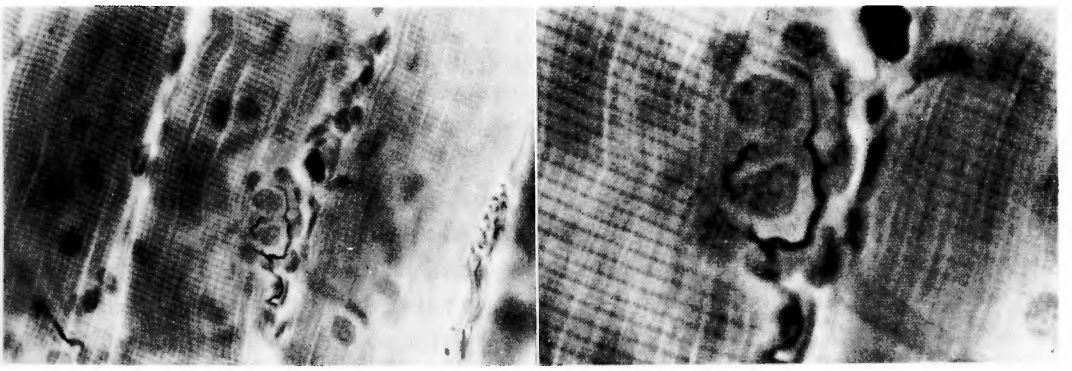
20 msec.

Fig. 13 b



a **b**

Fig. 16



a **b**

Fig. 17

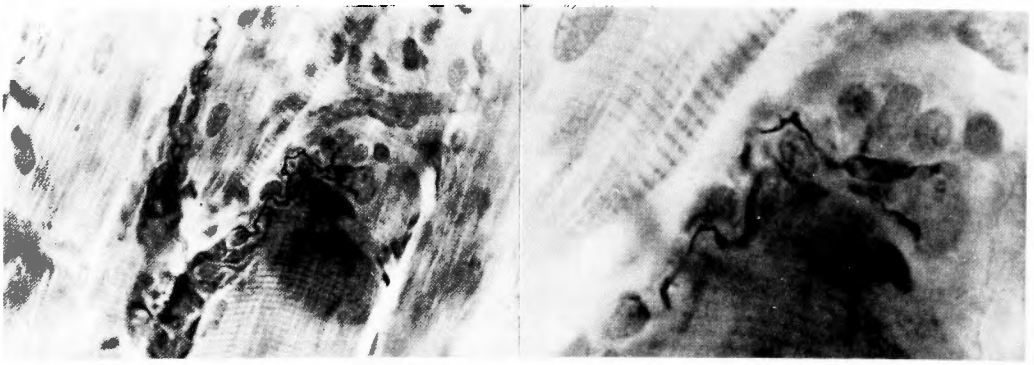


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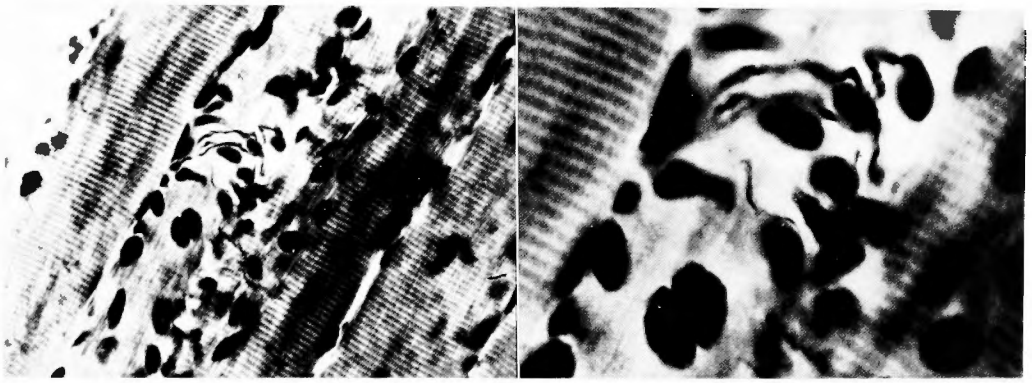
Fig. 18



a b

Fig. 19

LEGENDS FOR FIGURES

Figs. 7 through 13. Action Potentials from calf muscle of experimental side (a) and control side (b), recorded on cathode ray oscilloscope after section and suture of the tibial nerve. Surface electrodes. Single cathodal shocks of progressively increasing intensity to the tibial nerve with direct stimulation. Suprathreshold stimulation voltage is indicated under each record. Maximal amplitudes are shown on the right sides. Stimulus artefact is indicated by a dot. Calibration-1 mv. Time-20 msec. From Fig. 7 to Fig. 13 1, 1.5, 2, 3, 4, 5 and 6 months after nerve section and suture.

In Fig. 7 a action potentials are not considered as M waves, but as those from the hamstring muscle. In Figs. 11 b and 13 b are seen H wave-like deflections. For futher explanation see text.

Fig. 14 Normal motor nerve ending. Silver impregnation. a. × 400. b. × 1000.

Figs. 15 through 19. Motor nerve ending after section and suture of tibial nerve. From Fig. 15 to Fig. 19 1.5, 2, 3, 5 and 6 months after nerve section and suture. Silver impregnation.
a. × 400. b, × 100. For explanation see text.

to H waves were recorded in the control side (Figs. 11 b and 13 b). The reason seems to be that H waves are evoked due to a very small difference in size and, therefore, in the threshold between the sensory and motor nerve fibers, so that the direct electrical stimulation failed to selectively activate the sensory fibers, as pointed out by FUJIMORI[6]. Therefore, the author attempted to compare the findings of E.M.G. and M waves with histological studies of nerve endings for the purpose of investigating the process of rege-

neration of the motor fibers and end-plates following section and repair by suture of the peripheral nerve.

One and a half months postoperatively when complex N. M. U. potentials were first detectable, small potential fluctuations of M wave are recognized. Histological studies revealed that regenerated fine axis cylinder reached the end-plate, although they were tapered and their arborization incomplete. These histological findings were consistent with the electromyographical findings, indicating that regenerated motor fibers reached the neuromuscular junction during this postoperative period. In histological investigation of the regenerating nerve endings in rabbits, INOUE[13] described that the regenerated nerve termination in tapering ending was observed as telodendria, but regenerated axis cylinder did not extend beyond the end of band fibers 41 days after the resuturing of the sciatic nerve. In MURAKAMI's experiments[18], employing the same method and material, the regenerated axon arrived at the end-plate 35 days postoperatively, in which simple terminal arborization was formed.

The author's histological study made one and a half months postoperatively in dogs yielded almost the same results as those of the above reports. As MURAKAMI[18] stated, the reinnervation potentials or giant spikes were first recorded five months after nerve suture. At this stage, maximal amplitude of M wave was nearly normal. Six months after nerve resuturing fibrillation and complex N.M.U. potentials were still recorded, but markedly reduced in rate of appearance, while normal N.M.U. potentials were recorded from any point of the muscle, presenting a complete interference patttern. In evoked E. M. G. maximal stimulation voltage and maximal amplitude as well as latency of M wave were nearly normal. Histologically, muscle nuclei were reduced in number and size, and the muscle fibers showed clear striation and recovery from atrophy. The regenerated axons were matured to form arborization and end-plate nuclei were arranged in the shape of a sole. However, the facts that fibrillation and complex N.M.U. potentials were recorded six months after nerve resuturing imply that during this period the affected muscle was not yet completely restored.

As for the M wave, its maximal amplitude increases while its latency of response decreases progressively during the course of six months after nerve repair These facts can be explained by the increase in number of muscle fibers innervated and acceleration of conduction velocity of the regenerating nerve fibers which is caused by an increase in axonal diameter, as suggested by HODES[8], BERRY[1], and HURSH[11].

HODES[8] observed an abnormal temporal dispersion of response in early regenerative stage of peripheral nerve in men. This phenomenon was explained by the slowed conduction of nerve impulses, which causes an increased duration of potentials. This conduction velocity, he stated, increased progressively during the first year after repair, but probably never regained more than 60 % of normal. However, the author's experiments revealed that latency of response returned almost to normal six months after repair. Moreover, temporal dispersion did not show significant changes during the course of experiment. This discrepancy may be attributed to, 1) difference between the man and dog, 2) direct electric stimulation instead of percutaneous stimulation, and 3) sharp section followed by suture of the nerve in the present experiment.

The threshold of stimulus showed fluctuations during the course of experiment, alth-

ough theoretically it should have gradually decreased to normal. This may be due to the difference in the degree of scar formation at the site of direct stimulation.

Recovery of the maximal amplitude and latent period of M wave does not always indicate a complete restoration of muscular activity. However, the results obtained from the present experiments prove that the maximal amplitude is regarded as a useful index for objective evaluation of neuromuscular function.

SUMMARY

The tibial nerve was severed 3 to 4 cm proximal to the calf muscle and immediately thereafter was approximated by epineural suture in 29 adult dogs. Then, degenerative and regenerative processes of the motor nerve and muscle fibers were studied electromyographically, especially by means of an evoked E. M. G., Furthermore, histological studies on nerve endings were made using silver impregnation technique, and compared with electromyographic findings. The results were as follows:

1) Fibrillation potentials appeared 5 to 14 days after section and repair by suture.

2) Nascent motor unit action potentials and M waves were first detected 1.5 months postoperatively, when early regenerative process of the nerve endings was also seen histologically.

3) Normal neuromuscular unit potentials were first recorded 3 months postoperatively.

4) Maximal potentials of M wave increased nearly to the normal value (control value) 5 months after nerve suture, when reinnervation potentials appeared for the first time.

5) 6 months postoperatively normal N. M. U. potentials showed a complete interference pattern. whereas fibrillation potentials and nascent motor unit potentials were still recorded. However, the maximal potentials and the latent period of M wave showed nearly normal values, and histological examination revealed an almost complete regeneration of the nerve ending. This fact indicates that the neuromuscular function is almost completely restored to normal.

Therefore, muscle action potential in response to supramaximal stimulation in evoked E.M.G. is regarded as a useful index for assessment of regeneraion of peripheral nerve or recovery of the neuromuscular function.

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和文抄録

犬の骨格筋神経終末再生時における筋電図学的及び組織学的研究

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末梢神経損傷後の再生状態，特に神経終末の再生状態をうかがう一方法として，誘発筋電図学的方法を導入し，誘発筋電図と自発筋電図とを比較検討すると共に，その際における神経終末を組織学的に検索した．即ち，成犬29匹を用い，脛骨神経を総腓骨神経との分岐部より約1.5cm遠位部で切断，直ちに縫合し，術後6カ月迄，1，1.5，2，3，4，5及び6カ月の各実験動物群に分け，膝膕部に於いて，健側及び縫合側の脛骨神経を露出し，両電極線の間隔を0.5cm以上に保つた針電極にて，直接電気刺激を行ない，腓腹筋の皮膚上より，その誘発筋電図を誘導して，普通筋電図と比較検討した．この際，腓骨神経を総腓骨神経の分岐部より出来るだけ広範囲に切除して，誘発筋電図に記録される腓骨神経からの波形を除去した．電気的刺激を持続時間0.5m秒，刺激頻度を2秒と一定にした．

組織学的には，腓腹筋をBielschowsky氏　鈴木氏変法を用いて鍍銀染色して，各群毎に神経終末の再生状態を検索した．

実験成績：誘発筋電図においては，神経を直接に電気刺激したためにH波を導出する事が出来なかつた．そのために，M波の刺激域値，潜時及び最大刺激時における振幅の変遷について検討した．

1）神経縫合後5日より14日でfibrillation potentialが見られた．

2）術後1.5カ月の動物群において，始めてnascent motor unit action potential（complex NMU voltage）が誘導され，誘発筋電図ではM波が記録された．同時に，組織学に再生軸索がend-plateに到達していることが証明された．即ち，自発筋電図及び誘発筋電図学的に，始めて神経の再生波形が導出される時期と組織学的所見とが一致した．

3）術後3カ月群において，normal NMU potentialが始めて誘導された．

4）自発筋電図で，giant spike（reinnervation potential）が出現する術後5カ月群では，M波の最大刺激時の振幅は正常以上かほとんど正常値に近づく．

5）術後6カ月では，normal NMU potentialは容易に導出されるが，なお complex及びfibrillation potentialが誘導された．しかしながら，M波の最大刺激時の振幅（maximal amplitude）及び潜時（latency）はほぼ健側と同値を示し，組織学的には正常な神経終末像の所見がみられた．

自発筋電図学的には術後6カ月でもなおfibrillation及びcomplex NMU potentialが出現しているけれども，誘発筋電図学的及び組織学的には，neuromuscular functionがほぼ正常に回復したと考えられる．即ち，自発筋電図では残存する1部のneuromuscular unitの変性所見がfibrillation potentialとして記録され，M波では再生されたneuromuscular unitのより多数が誘導される為と考えられる．

以上の所見から，M波のsupramaximum stimulation時における振幅及び潜時の変遷は末梢神経損傷後の再生状態並びに骨格筋神経終末における nouromuscular functionの回復状態を推定する有用な一方法であると考えられる．