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EXPERIMENTAL REGENERATION IN PERIPHERAL NERVES AND THE SPINAL CORD IN LABORATORY ANIMALS EXPOSED TO A PULSED ELECTROMAGNETIC FIELD

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Abstract. Peripheral nerve section and suture was performed in 132 rats. Post-operatively half the animals were exposed to a pulsed electromagnetic field each day and half were kept as controls. Nerve conduction studies, histology and nerve fibre counts all indicated an increased rate of regeneration in the treated animals. A similar controlled study of spinal cord regeneration following hemicordotomy in cats has been started, and preliminary results indicate that when the animals are sacrificed three months after the hemicordotomy, the pulsed electromagnetic therapy has induced nerve fibre regeneration across the region of the scar.

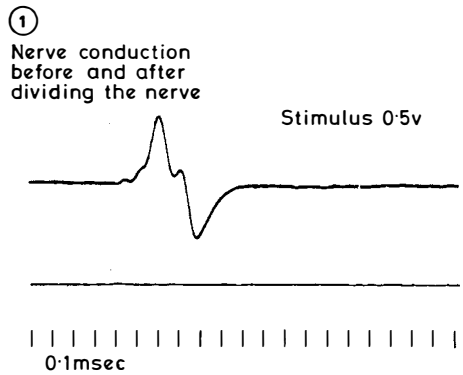
Introduction

DURING recent years there has been an increasing interest in the effect of applying electrical energy to healing tissue. Yasuda (1958) demonstrated the formation of callus in response to a weak direct current passed between needle electrodes inserted into a bone, and Becker (1961) published his pioneer work on the bio-electric factors of amphibian limb regeneration. Among more recent papers, Bassett (1974) reported that pulsing electromagnetic fields of low frequency through a fractured limb increases the organisation and strength of the repair process of the bone. Brighton and his associates (1975) have reviewed the work done on bone healing and reported on 29 patients treated by direct current stimulation for non-union of fractures or for congenital pseudarthrosis.

For the past four years we have been investigating the effects produced by exposing injuries of soft tissue and nerves to a pulsed electromagnetic field. The apparatus we have used in this study is a Diapulse machine—a standard commercial unit made by the Diapulse Corporation of America.[1] The apparatus emits the electromagnetic energy in short bursts, each one lasting 65 microseconds. The number of pulses per second can be varied from 80 to 600 and the average field strength can be carried from 5 to 120 milliwatts per sq. cm.

Initially we conducted a double blind trial on patients with sprained ankles. Pairs of patients were matched for age, weight, sex and degree of injury. One patient in each pair was treated by an active machine and the other by a placebo

* Diapulse Corporation of America, 4 Nevada Drive, Lake Success, New Hyde Park, New York, N.Y. 11040, U.S.A.



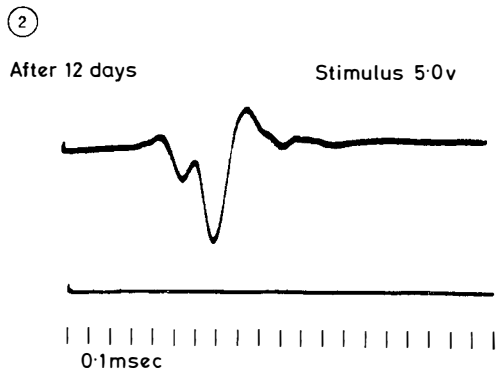


FIG. 1

Nerve conduction in the median ulnar nerve of a rat before and after dividing the nerve.

FIG. 2

Nerve conduction in the median/ulnar nerve of a rat 12 days after division and suture of the nerve. The upper trace is from a diapulse-treated rat, the lower trace from a control rat.

machine. The results showed that diapulse therapy has a definite, beneficial effect in reducing pain, swelling and disability (Wilson, 1972). In a second trial we compared the effects of diapulse with conventional short-wave diathermy and again demonstrated the value of the diapulse treatment and showed that short-wave diathermy is only marginally superior to a placebo (Wilson, 1974).

In an attempt to make a more precise measurement of the action of the diapulse treatment we decided to investigate its effect on the rate of regeneration of peripheral nerves.

Peripheral Nerve Experiments

As an experimental model we divided the median-ulnar nerve in the upper part of the forelimb of a rat and removed a small portion about 2 mm in length. The two parts of the nerve were then reapposed with an epineural suture and the skin wound closed. A series of 132 rats were used for this experiment. After surgery, half the rats were treated for 15 minutes each day with diapulse therapy and the remainder were kept as controls. The rate of regeneration in the sutured nerves was monitored by nerve conduction studies and by histology.

Nerve Conduction Studies

Normal nerve conduction in the forelimb of a rat is shown in Figure 1 in the upper trace and the result of dividing the nerve is seen in the lower trace. A return of nerve conduction can be demonstrated in the diapulse-treated animals 12 days after division and suture of the nerve, but it requires a ten-fold increase in the strength of the stimulus. The control animal shows no response even to this exaggerated stimulus (fig. 2). Figure 3 shows that 30 days after the operation there is a normal biphasic action potential in the treated animal in response to the preoperative level of stimulus. The small flicker of response in the control animal gradually increases in amplitude in tracings taken at 45 and 60 days, but it still does not have the characteristic biphasic shape.

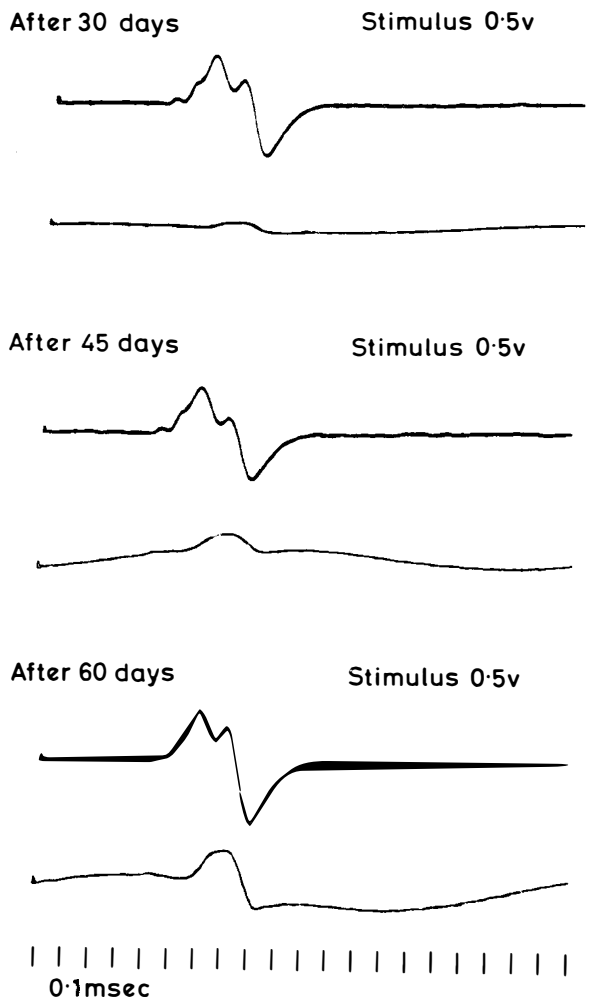


FIG. 3

Nerve conduction in the median/ulnar nerve of diapulse-treated and control rats 30 days, 45 days and 60 days after division and suture of the nerve.

Histology

Rats were sacrificed at regular intervals and the sutured median-ulnar nerve was dissected out for histology. It was notable that while there were adhesions and fibrosis around the site of the nerve suture in the control animals, which made dissection tedious, in the treated animals the dissection was much easier because there were very few adhesions. After fixation, sections were cut and stained with osmium tetroxide to show the myelination of nerve fibres and with a Van Gieson counterstain for the connective tissue. Sections distal to the point of suture in the treated animals 30 days after surgery showed that the nerve contained abundant regenerating nerve fibres, but of a reduced diameter. In a comparable section from an untreated animal 60 days after division and suture of the nerve, there was

TABLE I

Fibre density in normal rat nerves. Average fibres/sq. mm. in nerves from ten rats

	Range	Average
Less than 8·3 μ	4·880- 8·540	6·706
More than 8·3 μ	3·600- 4·420	3·936
Total	8·980-12·480	10·642

TABLE II

Number of myelinated fibres 12 days after section and resuture

Treated with diapulse	Controls
0	0
0	0
1	1
4	2
5	3
8	3
10	3
55	10
*9·440/sq. mm.	15
	58

* All small fibres.

also evidence of regeneration but the degree of recovery was not as advanced as in the treated animal at 30 days. Further confirmation of the action of diapulse therapy in promoting peripheral nerve regeneration was provided by counting the number of regenerating nerve fibres.

Nerve Fibre Counts

The fibre density in a peripheral nerve can be calculated by projecting the microscope image of a stained transverse section on to a screen incorporating a graticule. Table I lists the nerve fibre density in median-ulnar nerves taken from ten healthy rats. The division into small fibres and large fibres was made using 8·3 μ diameter as an arbitrary dividing point. Table II shows the fibre density 12 days after division and suture of the nerves in both treated and control animals. One nerve, from a treated animal, showed evidence of regeneration and the fibres in this specimen were all below 8·3 μ diameter. Table III shows that at 30 days, nine out of ten nerves from treated animals had at least a normal fibre density, and in five nerves the density was more than normal; a feature which is frequently seen during active regeneration of a nerve. In the nerves from control animals, only half of them showed regeneration and in only one of them had the fibre density reached the preoperative level. Table IV indicates that although large-diameter fibres were slower than small fibres in reappearing, nevertheless there was still a marked difference between the treated and the control animals.

Spinal Cord Regeneration

Our observation that there was less fibrosis and scarring around and within the nerve from the diapulse-treated animals, caused us to postulate that it might be

TABLE III

Nerve fibre density/sq. mm 30 days after section and resuture

Treated with diapulse	Controls
*5·626	0
10·260	0
10·346	3
10·906	4
11·120	15
12·700	*5·000
13·100	5·589
14·220	6·960
15·280	7·502
15·333	*13·000

* Regeneration limited to part of the section.

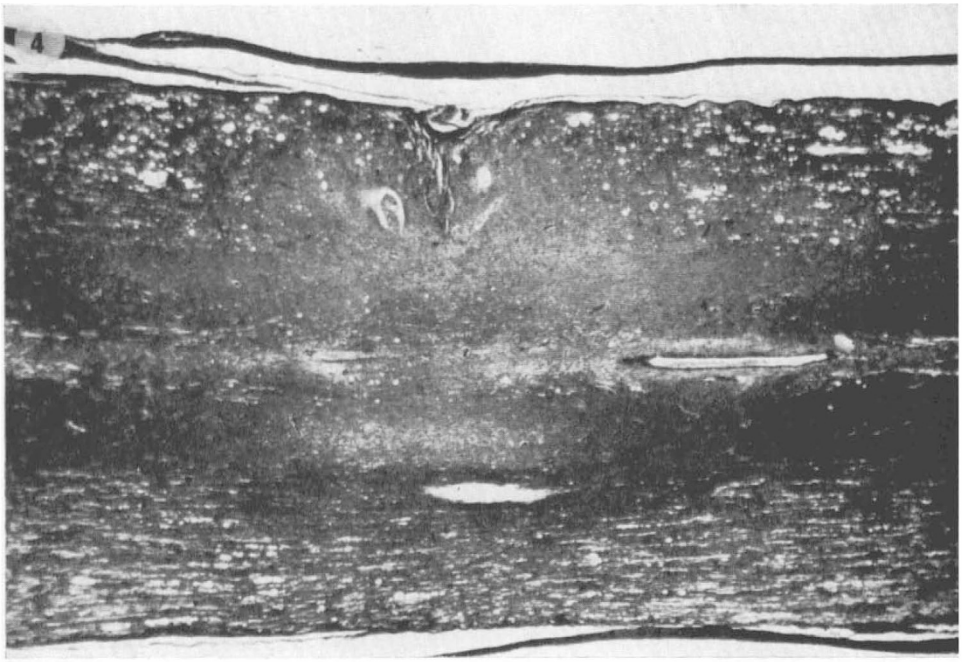
TABLE IV

Large fibre density/sq. mm 30 days after section and resuture

Treated with diapulse	Controls
0	0
0	0
0	0
0	0
26	0
133	0
220	0
240	0
346	0
693	13

possible to promote spinal cord regeneration by this method of treatment. If the proliferation of the neuroglia can be reduced and the growth of axon sprouts accelerated, then it might be possible to re-establish some neuronal continuity across the site of the spinal cord lesion.

As a preliminary investigation of this hypothesis, we have performed hemicordotomies in the upper lumbar cord in cats. 'Treated' cats were then exposed to diapulse therapy for half an hour each day for a month using an average field strength of 50 milliwatts per sq. cm and 400 pulses a second. Then after three months treated and control animals were sacrificed. The dorso-lumbar spine was removed *en masse* and fixed. Subsequently the spinal cord was dissected out and sections cut for histology. Figures 4 and 5 show low-power longitudinal sections taken through the region of the scar caused by the hemicordotomy in control and treated animals respectively. It is notable that the extent of the scar is less in the treated animal (fig. 5). Figure 6 is a high-power section of the hemicordotomy scar from the untreated animal shown in Figure 4. It shows a mass of neuroglia with one axon sprout in the centre of the field. Figure 7 is a comparable high-power section from the treated cord shown in Figure 5. There are abundant regenerating neurones traversing the region of the cordotomy.



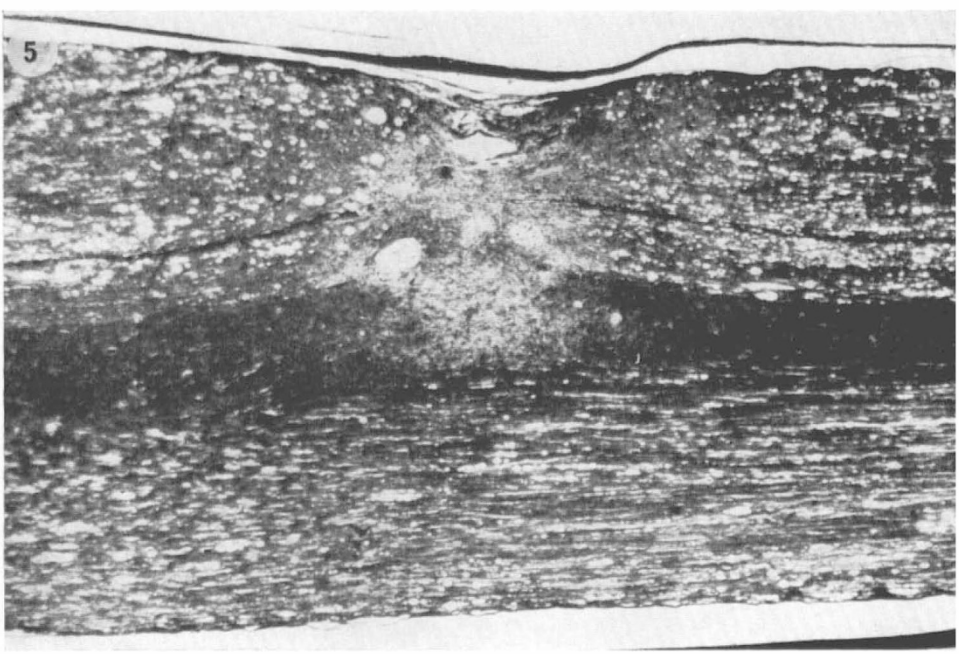
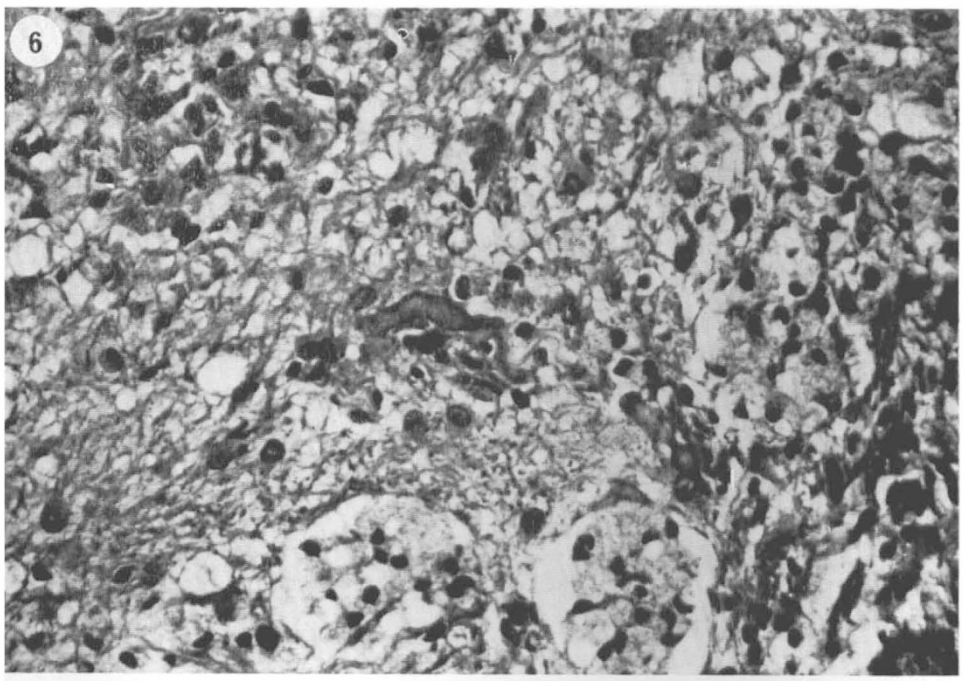


FIG. 4
Longitudinal section of cat spinal cord three months after hemicordotomy (control).

FIG. 5
Longitudinal section of cat spinal cord three months after hemicordotomy (treated with diapulse therapy).



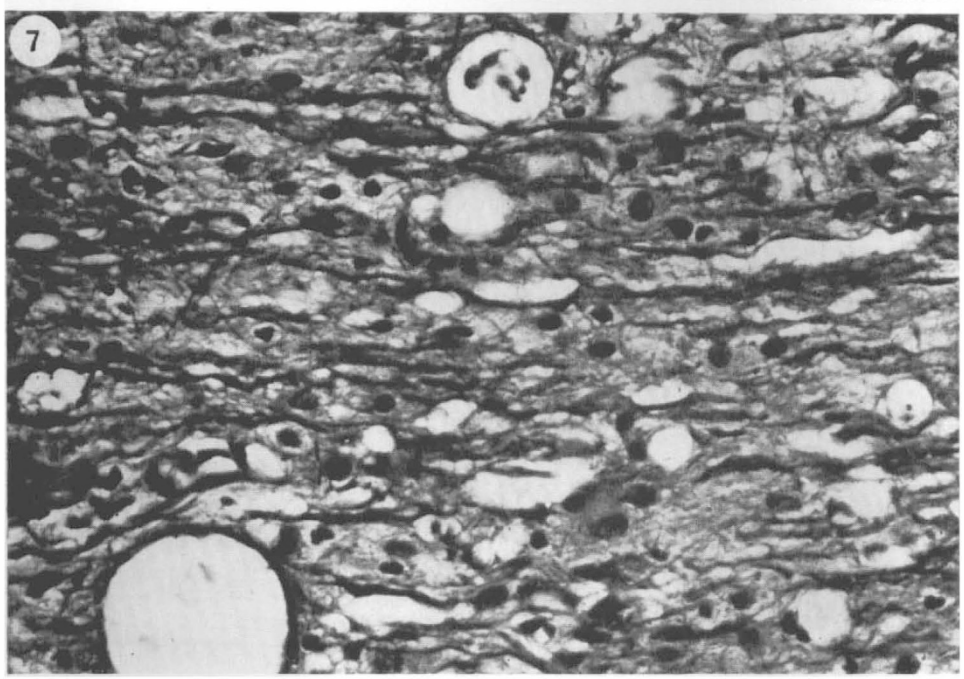


FIG. 6

High-power longitudinal section through the region of the scar in a control cat three months after hemicordotomy.

FIG. 7

High-power longitudinal section through the region of the scar in a diapulse-treated cat three months after hemicordotomy.

CONCLUSIONS

This investigation has shown that exposing injured nerves to a pulsed electromagnetic field speeds up their regeneration. The observations are limited to laboratory animals and to the parameters used by the diapulse machine. So far as the injured spinal cord is concerned, this preliminary report suggests that it might be possible to promote at least partial regeneration by this method of treatment.

RÉSUMÉ

On a procédé sur 132 rats à la section et à la suture d'un nerf périphérique. Durant la phase post-opératoire, la motié des animaux ont été exposés chaque jour à un champ électromagnétique à impulsions, l'autre moitié étant conservée comme témoin. Les études de la conduction nerveuse, l'histologie et la numération des fibres nerveuses indiquent toutes un accroissement de la vitesse de régénération chez les animaux traités. On a aussi entamé une étude analogue, sous controle, de la régénération de la moelle épinière après hémicordotomie chez les chats, et les premiers résultats indiquent que, lorsque les animaux sont sacrifiés trois mois après l'hémicordotomie, la thérapie par impulsions électromagnétiques a provoqué la régénération des fibres nerveuses dans la région de la cicatrice.

ZUSAMMENFASSUNG

Durchschneidung von peripheren Nerven und Nervennaht wurde in 132 Ratten ausgeführt, und die Tiere wurden täglich einem pulsierenden elektromagnetischen Feld ausgesetzt und die regeneration der peripheren Nerven studiert. Eine vermehrte Regenerationsrate wurde bei den behandelten Tieren gefunden. Eine 8 ähnliche kontrollierte Studie wurde bei Katzen nach Hemicordotomie gemacht, und die Autoren glauben, dass die electromagnetische Behandlung Nervenfaser-Regeneration über die Narbe hinaus zur Folge hat.

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Discussion

SIR LUDWIG GUTTMANN (*G.B.*). May I ask what suture material you are using to rejoin the cut nerves?

MR WILSON (*G.B.*). We are using 6/0 Ethilon suture. Our technique was to put in two sutures, make the division and then tie the sutures, *i.e.* an epineural suture.

SIR LUDWIG GUTTMANN. This paper is very interesting, but you will agree one needs more experience about the action of the pulse magnetic field regarding regeneration following transection of the cord. Histological recovery after hemisection of the cord is certainly no evidence, as fibres are growing easily from the opposite side. It would be interesting to find out what will happen after complete transection of the cord. I personally am very doubtful that the Pulse magnetic field has any significant or indeed any effect on the histopathology.

PROFESSOR M. WEISS (*Poland*). I would like to inform you that we have already treated 20 fresh cervical cases with this machine, because we are in the fortunate situation to receive these people within the first four hours. Unfortunately, I cannot report to you any particular neurological improvement. One case who was complete on admission showed a partial return of sensory and motor on one side. The weak point of the clinical research in all fields of this treatment is that our evaluation is based only on clinical evaluation but without active electro-diagnostic evaluation is not precise. We believe in this electro-magnetic field because we can do no further damage. Any swelling of the soft tissue, and also a swelling inside is diminished with this technique. We have said this before on other different cases pre- and post-surgically treated. We have operated on these 20 cases due to instability. We have seen how much cleaner our field was with regard to bleeding, there was much less bleeding. I would have been able to present to you two cases of transections treated with the diapulse for a short time, but unfortunately they were not ready.

DR HACHEN (*Switzerland*). I would like to add that the diapulse has been offered to us by U.S.A. manufacturers for a number of years to study the effect on decubitus lesions, but we were absolutely unable to show any significant improvements.

SPEAKER FROM U.S.A. I hope we can get some insight as to how this works. Some five years ago diapulse appeared on the market in the United States, allegedly as a mechanism to stimulate the reticular system. The list of cures which it will affect would not cover that wall, ranging from urinary tract infection to cancer to asthma to cysts and so on. With this great entry, the United States Government decided something should be done about this, and if my memory serves me correctly, they turned it over to the Veterans Administration for a study. Shortly afterwards it was removed from use in the United States as the Federal Government Administration banned it. It fared almost as badly on the stock market! Anyway, I am interested to hear some of the findings that you have in England, because we cannot duplicate the work as we do not have any apparatus to use.

SIR LUDWIG GUTTMANN. The last speaker reminded me of the confusion which arose many years ago with the effect of electrotherapy on peripheral nerve lesions. There is no question, and I think I have proved it beyond a shadow of doubt, that if electrotherapy is used following nerve division as a substitute for active exercise this has a beneficial effect on the denervated muscles. Unfortunately, electrotherapy, like now the Diapulse, has been used indiscriminately in all sorts of afflictions from tumours, cancer, freckles, and has brought the electrotherapy into discredit. However, I suggest that this study on Diapulse should be continued experimentally in primates with regard to the problem of regeneration following transection of the spinal cord.