Paraffin sections were stained according to Herlant's tetrachrome method. Mitoses (metaphases) were counted in 1000 cells of each explant. Statistical significance was evaluated by Student's t-test. The adenohypophysial explants exposed to TRH alone showed significantly higher mitotic incidence ($7.44 \pm 0.85‰$) if compared with the control ($4.78 \pm 0.66‰$). SRIF alone had no significant influence on the mitotic incidence of the adenohypophysial explants ($4.2 \pm 0.47‰$). However, SRIF added together with TRH totally blocked the mitogenic effect of the latter ($3.89 \pm 0.74‰$). Vale et al.[6] reported that SRIF blocks thyroropin release induced by TRH. Thus, the effect of SRIF on the cell proliferation seems to be connected with its effect on hormone release. It has been postulated that SRIF acts on the pituitary by lowering the cyclic AMP[7]. Since the cyclic AMP is known to be an inhibitor of mitoses in certain cells, some authors presumed rather a stimulatory than an inhibitory influence of SRIF on the adenohypophysial cell replication[8,9]. However, we demonstrated previously that dibutyryl cyclic AMP does not inhibit, but stimulates adenohypophysial cell proloferation in vitro[3]. It was also shown that estradiol, which is known to stimulate adenohypophysial cell proliferation, increased cyclic AMP concentration in the anterior pituitary[10]. Thus, the inhibitory effect of SRIF on TRH-stimulated adenohypophysial mitotic activity is not in opposition with the effect of SRIF on adenohypophysial cyclic AMP.

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PRO EXPERIMENTIS

A new method of measuring functional recovery after crushing the peripheral nerves in unanesthetized and unrestrained rats

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Summary. The distances between the first and fifth digits and between the second and fourth digits of the rat's hind paw were measured after crushing the sciatic nerve. The distances between the digits recovered significantly faster in weak nerve crushing than in strong crushing, and faster in distal nerve crushing than in proximal crushing. These results suggest that this method is available for evaluating the functional recovery after nerve crushing.

A simple method for evaluating the degrees of nerve regeneration after crushing the peripheral nerve in the unanesthetized and unrestrained animal has been required in pharmacological, physiological and biochemical studies. For example, it is much needed in investigating the time course of functional recovery in the same animal after nerve injuries or in measuring both the biochemical changes of muscle or of nerve and the degrees of the nerve regeneration. This report describes a new method.

Methods. Male Wistar-Imamichi rats (6 weeks after birth) were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The left sciatic nerve was crushed over a length of 2 mm under constant pressure at the level of the hips ('proximal crush') or the thigh ('distal crush') for 5 min with Péan's hemostatic forceps whose contact surfaces had been flattened. The nerve was crushed at a position 10 mm from the tip of the forceps. The forceps have 3 levels (refered to here as the weakest step, the middle step and the strongest step) of compressive strength. In this experiments the middle step ('weak crush') and strongest step ('strong crush') of the forceps were used. The sciatic nerve which is approximately 0.8 mm thick was crushed to approximately 0.3 mm thick in the weak crush and to approximately 0.2 mm thick in the strong crush.

Fig. 1. The rat's hind paw on the day after strong nerve crushing. The 5 digits of the rat's hind paw are spread apart on the intact side, but not on the injured side.

Maximum distances between the digits (DBD) of the rat's hind paw were measured with callipers at the tips by holding the rat's hips from behind and by pushing the paw slightly to the floor. When the first and fifth digits approached and their tips became invisible from the dorsal side, the DBD were gauged from the sole side. The distances between the first and fifth digits (DBD · 1–5) and between the second and fourth digits (DBD · 2–4) were measured 3 times or more and the values were averaged.

Student's t-test was used for statistical comparison between different treatment groups.

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Results. The 5 digits of the rat's hind paw are spread under normal conditions, but closed after nerve crushing. Figure 1 shows the hind paw on the day after strong nerve crushing. The DBD were measured 1, 2, 4 and 7 days, and then at 7-day-intervals up to 70 days after crushing the sciatic nerve. The DBD · 1–5 and 2–4 were at a minimum on the first or the second day after nerve crushing (figures 2–5). The DBD recovered significantly faster in weak nerve crushing than in strong nerve crushing (figures 2 and 3).

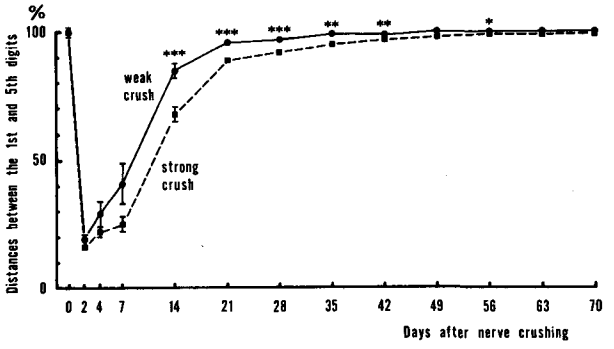

Fig. 2. The distances between the first and fifth digits of the rat's hind paw after weak and strong nerve crushing at a distal level. The distances between the digits of the injured side are expressed as a percentage in comparison with those of the intact side. Each value is the mean ± SEM of 9 animals in weak crushing and 7 animals in strong crushing. *p < 0.05, **p < 0.01, ***p < 0.001 (Student's t-test).

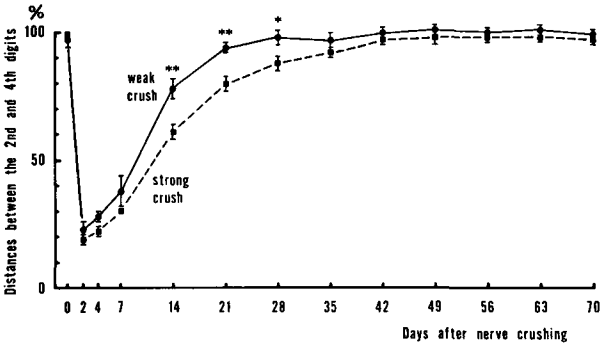

Fig. 3. The distances between the second and fourth digits of the rat's hind paw after weak and strong nerve crushing at a distal level. Each value is the mean ± SEM of 9 animals in weak crushing and 7 animals in strong crushing. Other remarks on data are the same as in figure 2.

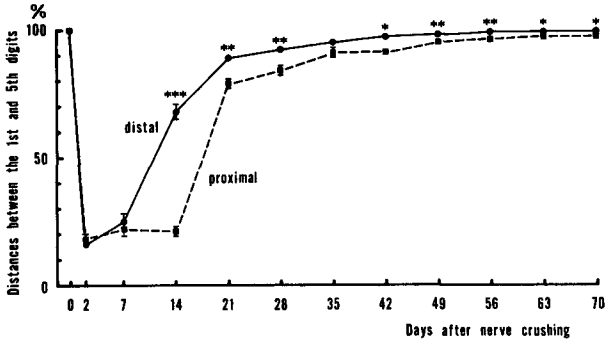

Fig. 4. The distances between the first and fifth digits of the rat's hind paw after strong nerve crushing at distal and proximal levels. Each value is the mean ± SEM of 7 animals. Other remarks on data are the same as in figure 2.

The DBD recovered significantly faster in distal crushing than in proximal crushing after both weak and strong nerve crushing. Figures 4 and 5 show the differences in the recovery rate after strong nerve crushing at different levels. Until 7 days after nerve crushing, the values for the DBD in distal crushing were small and almost the same as those in proximal crushing. However, 14 days after nerve crushing the DBD abruptly began to recover in distal crushing, but not in proximal crushing.

Discussion. The variability of the data observed in this method is very slight, especially in the DBD · 1–5. In the DBD · 2–4 practice makes the variability less. There was a significant difference between weak and strong nerve crushing. Therefore the effects of drugs on the functional recovery after nerve crushing could be examined by this method.

There was also a significant difference in the recovery rate between distal and proximal nerve crushing. The DBD after distal nerve crushing recovered significantly faster than after proximal crushing in both weak and strong nerve crushing. Stopford[2] claimed that the prognosis was more favourable the nearer the suture is to the spinal cord. Young[3], however, suggested that in the rabbit there was no great difference between the rate of advance of the axon tips in different divisions of the sciatic nerve. On the other hand, Gutmann et al.[4] reported that in the rabbit the rate of advance of functional completion was lower after high lesions than after low ones.

Advantages of measuring the DBD · 1–5 and 2–4 of the rat's hind paw are as follows. 1. The methods are simple. 2. Time course of the functional recovery after crushing the sciatic nerve can be observed in an unanesthetized and unrestrained animal. 3. There is a significant difference in the speed of the functional recovery between weak and strong nerve crushing. 4. There is a significant difference of functional recovery between the levels of nerve crushing. 5. The reproducibility of the data obtained by this method is very good.

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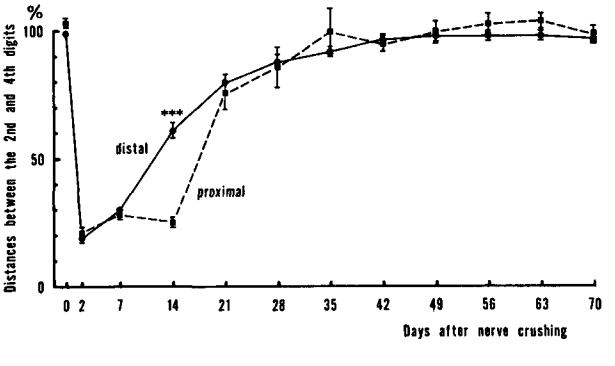

Fig. 5. The distances between the second and fourth digits of the rat's hind paw after strong nerve crushing at distal and proximal levels. Each value is the mean ± SEM of 7 animals. Other remarks on data are the same as in figure 2.