Identification of Myelinated Motor and Sensory Axons in a Regenerating Mixed Nerve

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The common peroneal nerves of Wistar rats were transected and repaired to compare the sequential changes in the numbers of regenerating motor and sensory myelinated axons in a single mixed nerve. At sequential intervals (2, 4, and 12 weeks) after nerve repair, 3 kinds of staining were performed: cholinesterase staining (Karnovsky's staining) for motor axons, carbonic anhydrase staining for sensory axons, and antineurofilament immunohistochemical staining for all axons. At 2 weeks there was a large number of carbonic anhydrase-positive axons (600 ± 98; mean ± SD) and cholinesterase-positive axons were occasionally seen. Subsequently, there was a striking increase of cholinesterase-positive myelinated axons, reaching to 302 ± 50 at 12 weeks. The results suggest that the myelimated sensory axons regenerate faster in the early stage of nerve regeneration and that regeneration of the myelinated motor axons is prominent in the subsequent stage. (J Hand Surg 2000;25A:104–111. Copyright © 2000 by the American Society for Surgery of the Hand.)

Key words: Rat, peripheral nerve, regeneration, cholinesterase, carbonic anhydrase, neurofilament.

Differences in regeneration between muscle nerves and purely sensory nerves have been investigated in several ways.[1–7] A muscle nerve, defined as a nerve branch to a muscle, contains a certain number of sensory axons (for proprioception).[8] Moreover, nerve regeneration after focal nerve injury varies according to species, age, type of lesion, type of repair, and the individual nerve.[1,3–5,9–11] It remains unclear how the regeneration of motor and sensory axons differs after surgical nerve repair and whether regenerating motor or sensory axons grow faster. A comparison using a single mixed nerve, in which every axon regenerates in the same condition, would be ideal for investigating the differences in nerve regeneration between motor and sensory axons. An understanding of the early events seen in the regeneration of motor and sensory axons would have clinical implications for successful rehabilitation and re-education of motor and sensory function. It also would provide information useful for the clinical application of a mixed nerve as a recipient nerve for free muscle or sensory flap transfer.

Riley and colleagues[6,7] described the identification of motor and sensory myelinated axons in the developing neuroma using cholinesterase (CE) staining and carbonic anhydrase (CA) staining and stated that sensory axons regenerated earlier and to a greater degree than motor axons in the developing neuroma. Insufficient attention, however, was given to histoquantitative and sequential comparisons. These histochemical stainings have been used as an aid for

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Received for publication March 5, 1999; submitted for publication September 29, 1999.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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0363-5023/00/25A01-0028$3.00/0

identifying the axonal type of injured nerve fascicles during surgery.[12,13]

Carbonic anhydrase is a histochemical marker for sensory nerve fibers.[14–18] It catalyzes the hydration of carbon dioxide and the dehydration of bicarbonate ions. In general, it is found at sites of gas exchange and ion transport across membranes.[15] Recently, an electrophysiologic study showed different characteristics between CA-positive neurons and CA-negative neurons in dorsal root ganglia.[19] It is assumed that CA plays a major role in the regulation of the intracellular pH and carbon dioxide.[16] On the other hand, the activity of CE is markedly higher in myelinated motor axons than in myelinated sensory axons.[20–22] Karnovsky's method and several modifications thereof using acetylthiocholine iodide as a substrate have been used extensively for the identification of motor axons.[12,13,21–23]

In the present study we investigated the sequential changes in the number of myelinated motor and sensory axons in a regenerating mixed nerve after transection and immediate repair using CE staining, CA staining, and an immunohistochemical staining for neurofilament protein. Our null hypothesis is that both motor and sensory myelinated axons regenerate according to the same sequential pattern.

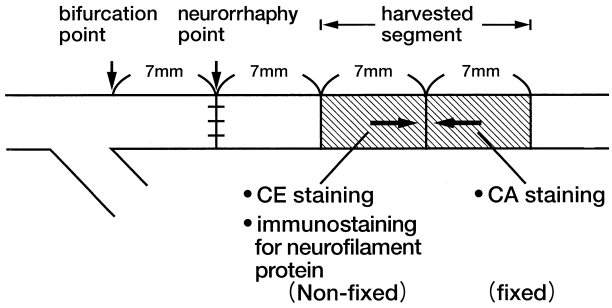

Figure 1. The surgical procedure. The common peroneal nerve was transected and immediately sutured approximately 7 mm distal to the bifurcation of the sciatic nerve. At 2, 4, and 12 weeks after surgery a 14-mm segment of the common peroneal nerve was harvested (7 to 21 mm distal to the nerve repair site). The harvested sample was divided equally into 2 segments. The distal segment was immediately quick-quenched without fixation for CE staining and antineurofilament immunohistochemical staining. The proximal segment was treated in the same manner as the distal segment for CA staining following fixation in 2.5% glutaraldehyde for 2 hours.

Materials and Methods

Animal

Twenty-one male adult Wistar rats (10 weeks old) were used. They were allowed unrestricted cage activity and were given ordinary laboratory food and water *ad libitum.*

Surgical Procedure

All surgical procedures were performed under sterile conditions, with microscope and microsurgical instruments, by one of the authors. Surgery was performed under full sedation with intraperitoneal pentobarbital sodium (50 mg/kg body weight). The right common peroneal nerve was exposed through a skin incision in the posterior thigh. The common peroneal nerve was transected 7 mm distal to the bifurcation of the sciatic nerve and immediately repaired with 3 epineural sutures (10–0 nylon) under a microscope. The experimental rats were randomly distributed into 3 groups: a 2-week group, a 4-week group, and a 12-week group, which comprised rats that were put to death with an overdose of intraperitoneal pentobarbital at 2, 4, and 12 weeks after surgery, respectively. Segments of the common peroneal nerve (14 mm long, 7–21 mm distal to the nerve suture; Fig. 1) were harvested. For controls, the nerve segments of the left common peroneal nerve were harvested in the same way. Since different preparations of samples were required to obtain the best visualization for CA, CE, and neurofilament protein,[6,13,14,22,23] the nerve samples were divided at the middle into 2 samples and processed as follows. The proximal segments were immediately mounted in OCT compound (Miles Inc, Elkhart, IN) and frozen in 2-methylbutane (isopentene) cooled with liquid nitrogen. The distal segments were fixed in 2.5% glutaraldehyde and 0.1 mol/L phosphate-buffered saline (pH 7.4) by immersion for 2 hours. Following fixation, these specimens were frozen in the same manner as the proximal specimens. The proximal ends of the distal specimens and the distal ends of the proximal specimens were cross-sectioned (10 μm) with a cryostat microtome. Serial sections were collected on APS-coated glass slides (Matsunami, Tokyo, Japan). Frozen serial sections of the proximal ends of the distal specimens were used for CA staining; those of the distal ends of the proximal specimens were used for CE staining and immunohistochemical staining for neurofilament protein (Fig. 1). As a result, approximately serial sections were provided for the 3 stainings.

Staining Procedures

Immunohistochemical Staining for Neurofilament Protein. To identify all types of axons, a monoclonal antibody against neurofilament protein (160 kd; Novocastra Laboratories, Ltd, Newcastle-upon-Tyne, UK) was used. The axons positive for neurofilament protein corresponded to the total number of the regenerating myelinated axons in our light microscopic study.

Frozen sections (10 μm) of unfixed specimens were incubated for 20 minutes in 1.5% horse serum in phosphate-buffered saline as a blocking solution and subsequently with antineurofilament protein antibody for 60 minutes. The sections were then incubated with biotinylated anti-mouse immunoglobulin G (rat-absorbed; Vector Laboratories, Burlingame, CA) in phosphate-buffered saline including 1.5% horse serum for 30 minutes. After rinsing, the sections were incubated with avidin-biotin complex (Vector Laboratories). Development was performed with a 3, 3′-diaminobenzidine kit (Vector Laboratories) for 3 minutes. Following a brief rinse in distilled water and dehydration, the sections were mounted.

Histochemical Staining for Carbonic Anhydrase and Acetylcholinesterase Activities. Staining for CA activity was conducted according to a modification[17] of the method reported by Hansson.[14] In brief, every 30 seconds the slides were briefly dipped into an incubation medium (pH 6.3) containing 3.5 mmol/L $CoSO_4$, 11.7 mmol/L KH_2PO_4, 53 mmol/L H_2SO_4, and 157 mmol/L $NaHCO_3$. This process was repeated 30 times. The sections were then immersed for 30 seconds in 0.5% $(NH_4)_2S$ for visualization. Cholinesterase staining was performed according to the method of Karnovsky and Roots.[21] A modification of Karnovsky's method[23] was used in this study. The incubation medium was 0.1 mol/L NaOH-maleate buffer containing 0.1 mol/L Na-citrate, 30 mmol/L $CuSO_4$, 5 mmol/L $K_3[Fe(III)(CN)_6]$, and 1.8 mmol/L acetylthioline iodide. Iso-OMPA (10^{-4} mol/L; Sigma, MO) was used as an inhibitor of nonspecific CE activity. The sections were incubated for 3 hours at 37°C. Finally, the CA- and CE-stained sections were rinsed, dehydrated, and mounted as described above.

Morphometric Assessment

The stained sections were morphometrically evaluated with an image analyzer (Luzex-FS; Nireco Co, Tokyo, Japan). Digital images of the stained sections were binarized and the number of positively stained axons was measured. The data of the 3 experimental groups (death at 2 weeks, 4 weeks, and 12 weeks) were compared with those of the 18 controls.

Statistical Analysis

The data of the experimental groups and the control group were analyzed with 1-way ANOVA and a Bonferroni adjustment for multiple comparison. The differences were considered significant at $p < .05$.

Results

Animal Model

All nerve repairs healed without ruptures and no postoperative infections were observed. Six of 7 rats in the 2-week group, 5 of 7 rats in the 4-week group, and all 7 of the rats in the 12-week group completed the study; 3 of the 21 rats were excluded because of inappropriate handling and storage of the frozen specimens, resulting in the failure of CE staining.

Neurofilament Immunostaining for Total Axons

The sequential changes in the numbers of axons positive for neurofilament protein (160 kd) are shown in Figures 2 and 3. The number on the control side was significantly larger than that on the regeneration side at 2 weeks ($p < .05$). The number of total regenerating axons became almost equal to that of the control at 4 weeks, and thereafter increased in number as time progressed. The number at 12 weeks was significantly greater than at 2 weeks ($p < .01$).

Carbonic Anhydrase Staining for Sensory Axons

At 2 weeks after the nerve repair, CA-positive regenerating axons were already apparent, amounting to 600 ± 98 (mean $\pm$ SD) in number. Thereafter, the mean of the number increased as time progressed, but there were no significant differences between the experimental groups of 2 weeks, 4 weeks, and 12 weeks (Figs. 4,5).

Cholinesterase Staining for Motor Axons

Cholinesterase-stained cross-sections at 2 weeks after nerve repair showed only a few CE-positive axons. Even at 4 weeks, in contrast to the results of CA staining, the recovery rate (= no. of regenerating axons on operated side ÷ no. of regenerating axons on control side) was still small (9%). At 4 weeks,

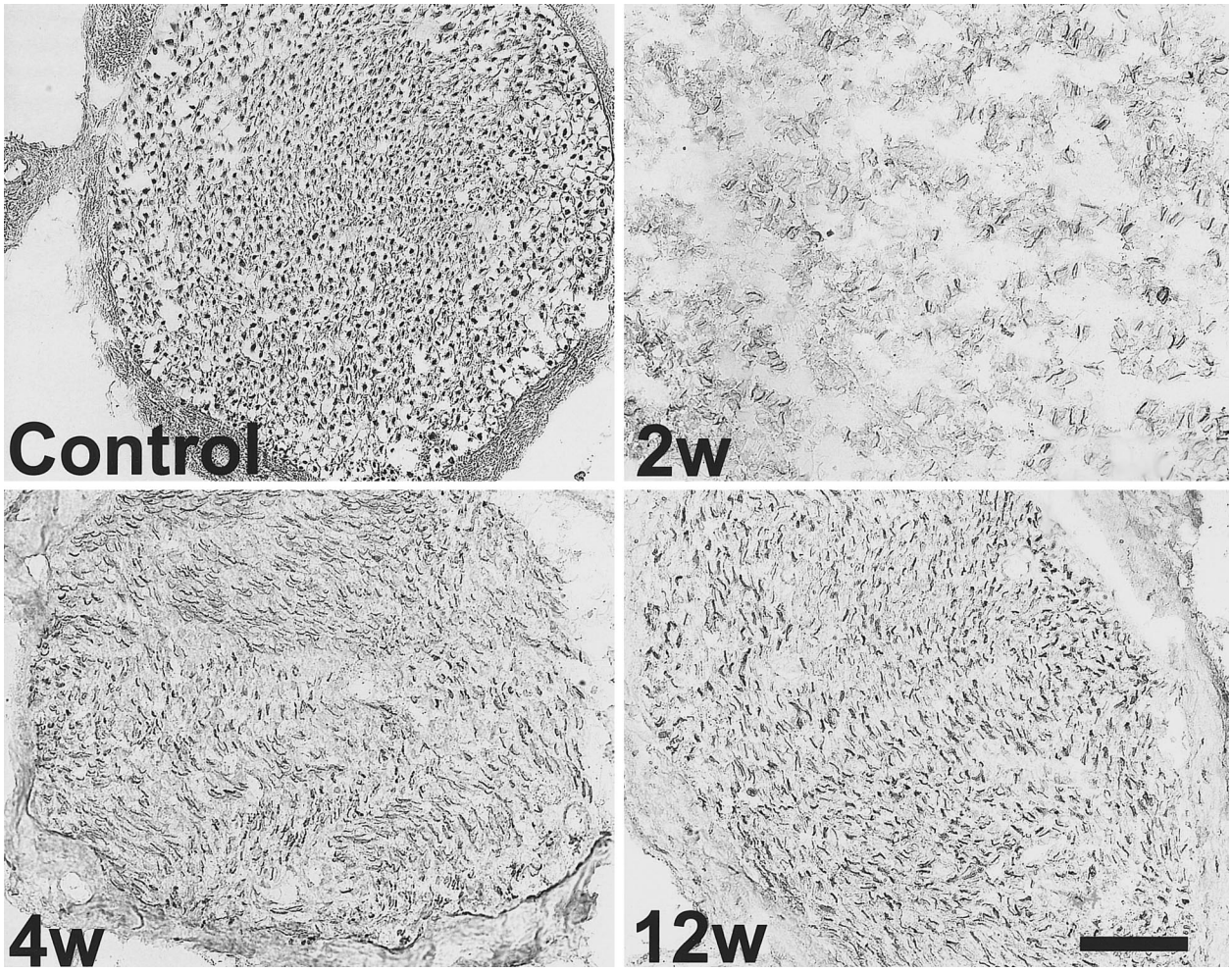

Figure 2. Cross-sections of the common peroneal nerve immunostained for neurofilament protein (160 kd). To identify both regenerating motor and sensory myelinated axons, frozen sections of unfixed specimens from the 4 groups (control group, 2-week group [2w], 4-week group [4w], and 12-week group [12w]) were immunostained for neurofilament protein. Bar = 100 μm.

there was a striking increase in the number of CE-positive axons, reaching 302 ± 50. The recovery rate at 12 weeks was 1.2 (Figs. 5,6).

Discussion

Experimental Method

Previous studies have yielded various results regarding the differences in regeneration between muscle nerves and sensory nerves. Shawe,[1] using Bodian's silver stain, reported that the fibers of muscle nerve in rabbit give rise to more branches than those of sensory nerve after crushing. Jenq and Coggeshall[3] found that regenerating nerve fibers in the sural nerve (purely sensory nerve) of rat was smaller in number than those in the innervating nerve of the

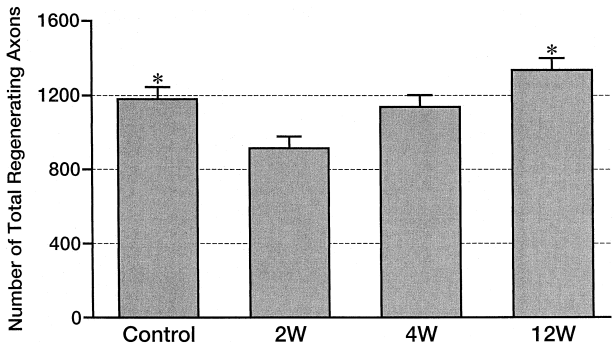

Figure 3. The sequential changes in the number (mean ± SE) of total regenerating axons were determined by counting axons positive for neurofilament protein in each group: control group (n = 18), 2-week group (n = 6), 4-week group (n = 5), and 12-week group (n = 7). * $p < .01$ versus 2-week group.

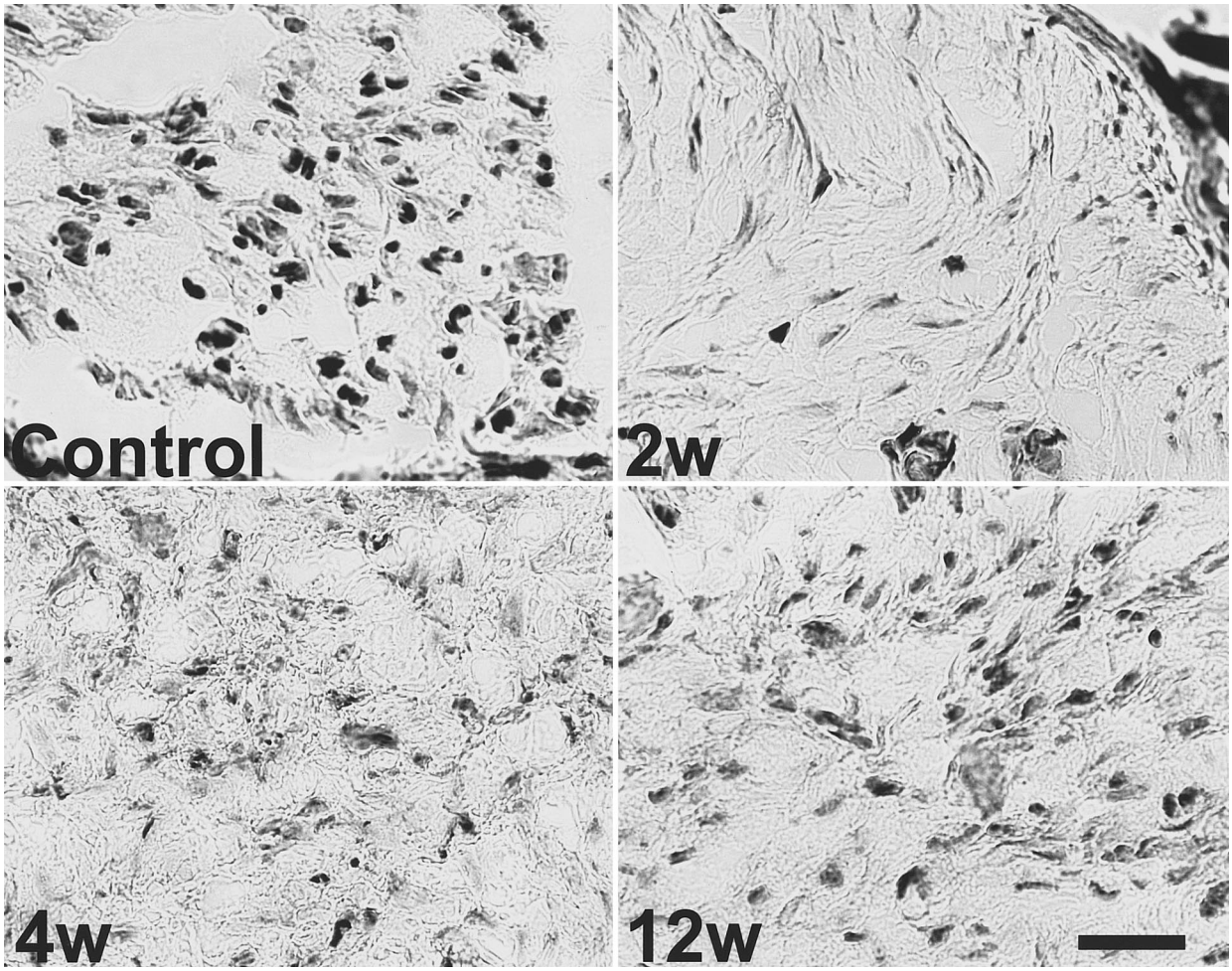

Figure 4. Cross-sections of the common peroneal nerve stained for CA. Carbonic anhydrase-positive regenerating axons (presumably sensory axons) showing scattered black spots were already apparent to a great degree at 2 weeks after the nerve repair in the 4 groups: control group, 2-week group (2w), 4-week group (4w), and 12-week group (12w). Bar = 25 μm.

gastrocnemius muscle (muscle nerve) and those in the tibial nerve (mixed nerve) after crushing at a point proximal to the bifurcation of the sciatic nerve. In addition, the regeneration of the saphenous nerve (purely sensory nerve) was slower than that of the tibial nerve after crushing in C57BL/Ola mice, the axons of which are very slow to degenerate, with many axons still present 3 weeks after axotomy.[24] Thus, sensory nerves seemed to regenerate slower than muscle nerves in such previous reports using a nerve crushing method.[1,3,24]

On the other hand, the results of transection of peripheral nerves varied according to the method of nerve repair. Jenq and Coggeshall[3] reapproximated both nerve stumps in a silicone tube immediately after transecting the sciatic nerve and measured the number of regenerating myelinated fibers in its trib-

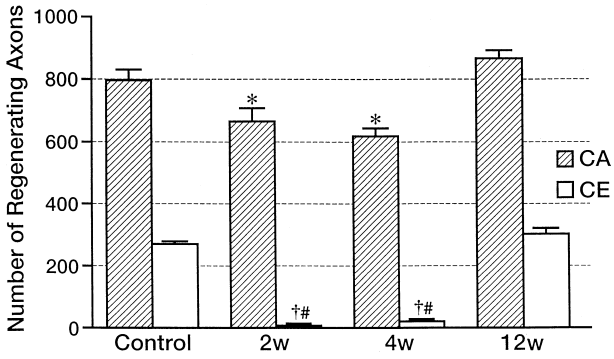

Figure 5. The sequential changes in the number (mean ± SE) of regenerating sensory axons (solid bars) and regenerating motor axons (open bars). Carbonic anhydrase-positive axons and CE-positive axons are shown for each group: control group (n = 18), 2-week group (2w, n = 6), 4-week group (4w, n = 5), and 12-week group (12w, n = 7). # $p < .01$ versus control, † $p < .01$ versus 12 weeks, * $p < .05$ versus control.

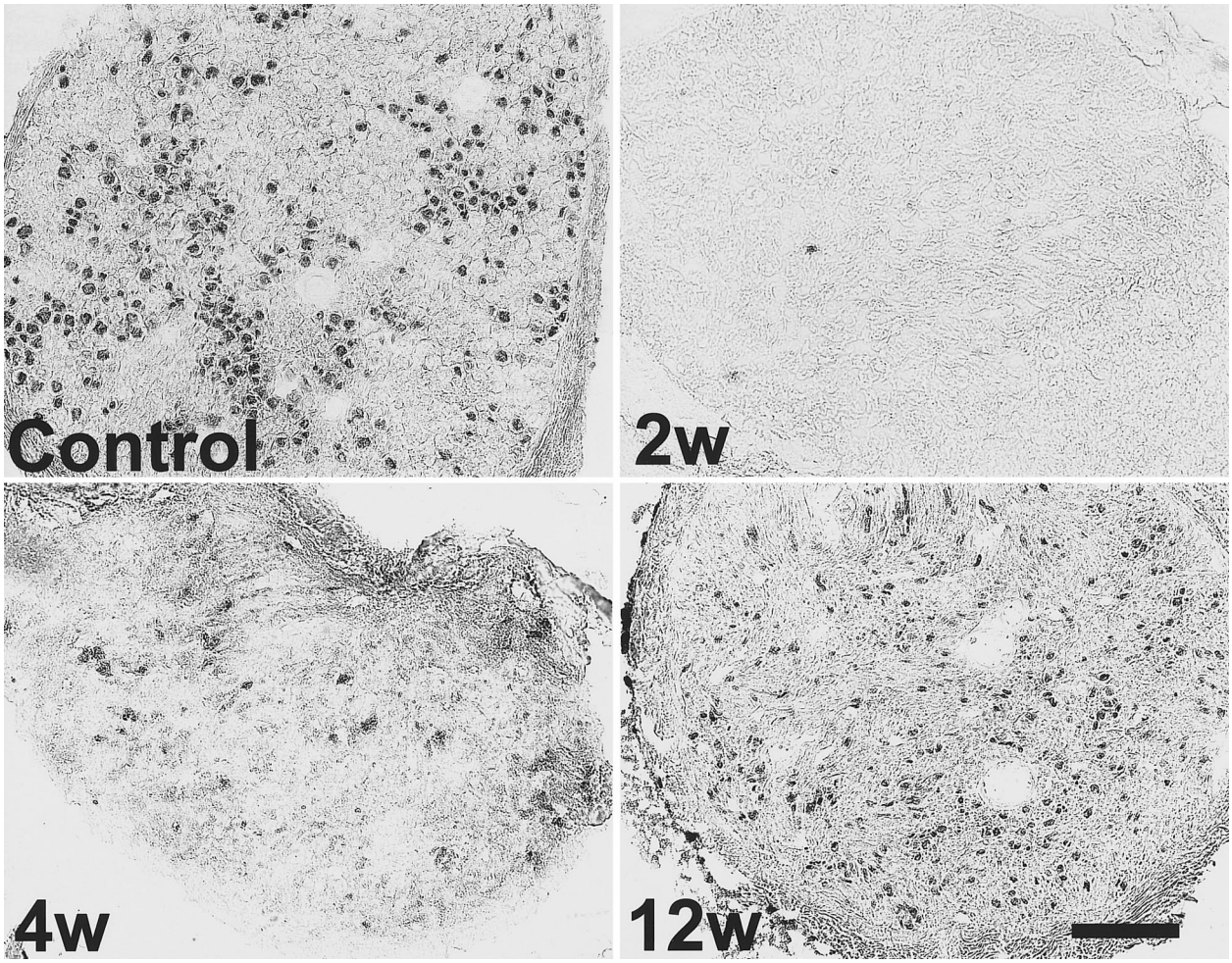

Figure 6. Cross-sections of the common peroneal nerve stained for CE in the 4 groups: control group, 2-week group (2w), 4-week group (4w), and 12-week group (12w). At 2 weeks after the nerve repair, there were only a small number of CE-positive axons. A striking increase of CE-positive regenerating axons (presumably motor axons) is evident, however, at 12 weeks after nerve repair. Bar = 100 μm.

utary nerves using an electron microscope. At 8 weeks, the recovery rate of the myelinated fibers of the sural nerve was almost the same as that of the innervating nerve of the gastrocnemius muscle, while the regeneration of sensory nerves repaired with a 4- to 8-mm gap was lower than that of muscle nerves.[5]

The results mentioned above, however, did not directly indicate whether regenerating myelinated sensory or motor axons grow faster after nerve repair, because a muscle nerve contains a certain amount of sensory axons (for proprioception)[8] and the degree of wallerian degeneration is different between purely sensory and muscle nerves.[25–27] It has been suggested that the degree of wallerian degeneration, the axonal environment, and the fiber composition of individual nerves play important roles in nerve regeneration.[24–26] Thus, to compare the regeneration of motor axons to that of sensory axons, using a single mixed nerve in which both motor and sensory axons regenerate in the same condition appears to be ideal.

Generally, axon counts and the regeneration rate may not necessarily equate with the normal function recovery. The accuracy of axon regeneration for the original pathway and the rate of reinnervating their target end organs[28–30] are additional problems for nerve regeneration studies, including ours. Brushart et al[28,29] suggested the "preferential motor reinnervation" and "pruning hypothesis" for these complex issues of misdirectedly projected axon collaterals of motoneuron to explain the contributions of pathway

and end organ for nerve reinnervation. Electromyography of the first dorsal interosseous muscle and the abductor digiti minimi muscle after complete ulnar and median nerve transection and repair showed a reinnervation rate of approximately 30% to 40%.[31] In general, clinical nerve repairs resulted in poorer outcome than experimental studies in rodents.[29]

Sensitivity and Specificity of Cholinesterase and Carbonic Anhydrase Stainings

We identified approximately 60% of the myelinated sensory fibers and 50% of the myelinated motor fibers in the controls at the microscopic level, compared with the results of an ultrastructural study, which reported that 600 myelinated motor fibers and 1,300 myelinated sensory fibers exist in the common peroneal nerve in Wistar rats.[32] The reduced number of fibers identified in this study is due not only to the limitations of a microscopic study using nonfixed sections but also to the specificity of CA and CE staining. In previous results of CE staining, the demonstration of CE activity varied from 70% to 90% in ventral roots.[6,8,22] On the other hand, the demonstration of CA activity was reported to be 60% in dorsal roots,[6] although it was 100% in the infraorbital nerve[18] and in thick sensory fibers.[8,17] Proprioceptive axons in a muscle nerve are highly CA positive[8] and CE negative.[17] The demonstration of CE and CA activities depends on the method of fixation of the samples and on the conditions of the staining procedure such as the temperature or pH of the incubation medium and the incubation time.[13,22,23] In this study, we used nonfixed sections for CE staining and iso-OMPA as an inhibitor for nonspecific CE to avoid detecting butyrylcholinesterase, which is abundantly present in unmyelinated sensory axons.[12,20] A characteristic of butyrylcholinesterase staining of unmyelinated fibers is a scattered spot or fleck form between the myelinated fibers, which may result in miscounting the axons.

Sequential Changes in the Numbers of Motor and Sensory Axons During Regeneration

Riley et al[6,7] stated that sensory axons regenerated earlier and to a greater degree than motor axons in the developing neuroma. Insufficient attention, however, was given to histoquantitative and sequential comparisons. Using horseradish peroxidase retrograde labeling, quantifying motor and sensory nerve regeneration has been done in several ways.[2,33–36] Da Silva and colleagues[33,34] reported that the motor neurons regenerated better than sensory neurons. On the other hand, Madorsky et al[35] and Baily et al[36] suggested that the sensory neurons regenerated consistently better than motor neurons using nerve repair with a 10-mm and 18-mm gap, respectively.

In this study, although CE and CA stainings do not enable visualization of all motor and sensory axons as described above, the results demonstrated the distinct sequential changes in numbers between regenerating motor and sensory axons. The number of total regenerating axons positive for neurofilament protein became almost equal to that of the control at 4 weeks, thereafter increased in number as time progressed, and exceeded that of the control at 12 weeks. The recovery rate at 12 weeks was 1.1. These results are almost in agreement with some previous reports.[1,10,11,37]

At 2 weeks the number of CA-positive axons corresponded to over two thirds of the total number of axons; it was already 80% of that of CA-positive axons at 12 weeks. On the other hand, CE-positive axons were only occasionally seen at 2 weeks. The number of CE-positive axons was only 24 ± 6 at 4 weeks, yet subsequently there was a striking increase of CE-positive axons, reaching to 302 ± 50 at 12 weeks.

Although CE and CA stainings do not enable complete visualization of motor and sensory axons, it is suggested that the myelinated sensory axons regenerate faster and are greater in number in the early stage of nerve regeneration, while the regeneration of the myelinated motor axons is prominent in the subsequent stage. Thus, our null hypothesis that both motor and sensory myelinated axons regenerate in the same manner is presumed to be false. It may be supported by the clinical finding that a mixed nerve regenerates slower than a pure sensory nerve.[38]

The authors thank Yuka Kuwahara and Takako Kato for their technical assistance and Dr Kazuki Ueda for his advice concerning statistics.

References

1. Shawe GDH. On the number of branches formed by regenerating nerve-fibres. Br J Surg 1955;42:474–488.
2. Forman DS, Barenberg RA. Regeneration of motor axons in rat sciatic nerve studied by labeling with axonally transported radioactive proteins. Brain Res 1978;156:213–225.
3. Jenq C-B, Coggeshall RE. Effect of sciatic nerve regeneration on axonal populations in tributary nerves. Brain Res 1984;295:91–100.
4. Jenq C-B, Coggeshall RE. Number of regenerating axons

in parent and tributary peripheral nerves in the rat. Brain Res 1985;326:27–40.
5. Jenq C-B, Coggeshall RE. Long-term patterns of axon regeneration in sciatic nerve and its tributaries. Brain Res 1985;345:34–44.
6. Riley DA, Sanger JR, Matloub HS, Yousif NJ, Bain JLW, Moore GH. Identifying motor and sensory myelinated axons in rabbit peripheral nerves by histochemical staining for carbonic anhydrase and cholinesterase activities. Brain Res 1988;453:79–88.
7. Sanger JR, Riley DA, Matloub HS, Yousif NJ, Bain JL, Moore GH. Effects of axotomy on the cholinesterase and carbonic anhydrase activities of axons in the proximal and distal stumps of rabbit sciatic nerves: a temporal study. Plast Reconstr Surg 1991;87:726–738.
8. Szabolcs MJ, Kopp M, Schaden GE. Carbonic anhydrase activity in the peripheral nervous system of rat: the enzyme as a marker for muscle afferents. Brain Res 1989;492:129–138.
9. Gutmann E, Sanders FK. Recovery of fibre numbers and diameters in the regeneration of peripheral nerves. J Physiol (Lond) 1943;101:489–518.
10. Giannini C, Lais AC, Dyck PJ. Number, size, and class of peripheral nerve fibers regenerating after crush, multiple crush, and graft. Brain Res 1989;500:131–138.
11. Choi S-J, Harii K, Lee M-J, Furuya F, Ueda K. Electrophysiological, morphological, and morphometric effects of aging on nerve regeneration in rats. Scand J Plast Reconstr Hand Surg 1995;29:133–140.
12. Yunshao H, Shizhen Z. Acetylcholinesterase: a histochemical identification of motor and sensory fascicles in human peripheral nerve and its use during operation. Plast Reconstr Surg 1988;82:125–130.
13. Kanaya F, Ogden L, Breidenbach WC, Tsai T-M, Scheker L. Sensory and motor fiber differentiation with Karnovsky staining. J Hand Surg 1991;16A:851–858.
14. Hansson HPJ. Histochemical demonstration of carbonic anhydrase activity. Histochemie 1967;11:112–128.
15. Carson KA, Terzis JK. Carbonic anhydrase histochemistry: a potential diagnostic method for peripheral nerve repair. Clin Plast Surg 1985;12:227–232.
16. Oswald T, Riley DA. Peripheral nerve carbonic anhydrase activity and choronic acetazolamide treatment of rats. Brain Res 1987;406:379–384.
17. Szabolcs MJ, Windisch A, Koller R, Pensch M. Axon typing of rat muscle nerves using a double staining procedure for cholinesterase and carbonic anhydrase. J Histochem Cytochem 1991;39:1617–1625.
18. Wilke RA, Riley DA, Sanger JR. Histochemical discrimination of fibers in regenerating rat infraorbital nerve. Microsurgery 1992;13:39–44.
19. Probhakar E, Lawson SN. The different electrophysical properties of rat primary afferent neurons with carbonic anhydrase activity. J Physiol (Lond) 1995;482:609–622.
20. Liu X-L, Zhu J-K. The histochemical study of rat sciatic nerve cholinesterase in degeneration and regeneration. J Reconstr Microsurg 1990;6:43–47.
21. Karnovsky MJ, Roots LA. A "direct-coloring" thiocholine method for cholinesterases. J Histochem Cytochem 1964; 12:219–225.
22. Gruber H, Zenker W. Acetylcholinesterase: histochemical differentiation between motor and sensory nerve fibres. Brain Res 1973;51:207–214.
23. Yoshimura K, Hatanaka CM, Harii K. Identification of peripheral nerve fibers in rats and humans with cholinesterase staining. J Jpn Plast Reconstr Surg 1994;14:131–139.
24. Chen S, Bisby MA. Impaired motor axon regeneration in the C57BL/Ola mouse. J Comp Neurol 1993;333:449–454.
25. Brown MC, Perry VH, Hunt SP, Lapper SR. Further studies on motor and sensory nerve regeneration in mice with delayed Wallerian degeneration. Eur J Neurosci 1994;6: 420–428.
26. Brown MC, Lunn ER, Perry VH. Consequences of slow Wallerian degeneration for regenerating motor and sensory axons. J Neurobiol 1992;23:521–536.
27. Gilliatt RW, Hjorth RJ. Nerve conduction during Wallerian degeneration in the baboon. J Neurol Neurosurg Psychiatry 1972;35:335–341.
28. Brushart TM. Motor axons preferentially reinnervate motor pathways. J Neurosci 1993;13:2730–2738.
29. Brushart TM, Gerber J, Kessens P, Chen TG, Royall RM. Contributions of pathway and neuron to preferential motor reinnervation. J Neurosci 1998;18:8674–8681.
30. Madison RD, Archbald SJ, Brushart TM. Reinnervation accuracy of the rat femoral nerve by motor and sensory neuron. J Neurosci 1996;16:5698–5703.
31. Thomas CK, Stein RB, Gordon T, Lee RG, Elleker MG. Patterns of reinnervation and motor unit recruitment in human hand muscles after complete ulnar and median nerve section and resuture. J Neurol Neurosurg Psychiatry 1987;50:259–268.
32. Schmalbruch H. Fiber composition of the rat sciatic nerve. Anat Res 1986;215:71–81.
33. Da Silva CF, Madison RD, Dikkes P, Chiu TH, Sidman RL. An in vivo model to quantify motor and sensory peripheral nerve regeneration using bioresorbable nerve guide tubes. Brain Res 1985;342:307–315.
34. Madison RD, Da Silva CF, Dikkes P. Entubulation repair with protein additives increases the maximum nerve gap distance successfully bridged with tubular prostheses. Brain Res 1988;447:325–334.
35. Madorsky SJ, Swett JE, Crumley RL. Motor versus sensory neuron regeneration through collagen tubules. Plast Reconstr Surg 1998;102:430–436.
36. Baily SB, Eichler ME, Villadiego A, Rich KM. The influence of fibronectin and laminin during Schwann cell migration and peripheral nerve regeneration through silicone chambers. J Neurocytol 1993;22:176–184.
37. Tohyama K, Osawa T, Tajima K. Nerve allot graft. In: Shimizu Y, Ide C, Kawamura M, et al, eds. Nerve regeneration and functional reconstruction. Niigata, Japan: Nishimura, 1997:244–246.
38. Snell RS. Clinical neuroanatomy for medical students. Niigata, Japan: Nishimura, 1992:103–106.