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Reconstruction of the Contused Cat Spinal Cord by the Delayed Nerve Graft Technique and Cultured Peripheral Non-Neuronal Cells*

J. R. Wrathall[1], D. D. Rigamonti[1,3], M. R. Braford[1], and C. C. Kao[1,2]

[1] Department of Anatomy, Georgetown University and
[2] Division of Neurosurgery, Veterans Administration Medical Center, Washington, DC, USA

Summary. Previously, surgical reconstruction of the transected dog spinal cord by the delayed nerve graft technique has been shown to result in reinnervation of the nerve graft by axons. In the present study, we compared the results of surgical reconstruction of the severely contused cat spinal cord by the delayed nerve graft technique alone to those after reconstruction with a similar nerve graft plus cultured peripheral non-neuronal cells implanted between the grafted nerve and the spinal cord stumps. The spinal cord-nerve graft junction was examined by light and electron microscopy. The cultured cells were prelabelled with tritiated thymidine and their location after implantation determined by autoradiography. By 3 days after spinal cord reconstruction, the pelabelled cells were present at the junction and had migrated into the nerve graft and also into the spinal cord stumps where they were observed near axons. By 7 days, physical connections were observed bridging the junction between the spinal cord and nerve graft and axons ensheathed by Schwann cells had already penetrated at least 1 mm into the nerve graft. Wound healing took at least a week longer in animals repaired with a nerve graft alone. At one year or later after reconstructive surgery, in both groups of animals, the grafted nerve was reinnervated with myelinated and unmyelinated axons. Thus, the severely contused cat spinal cord could be reconstructed with the delayed nerve graft technique alone but the use of the cultured cells appeared to enhance wound healing and decrease the time required for axon elongation into the nerve graft.

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3 Present address: Letterman Army Institute of Research, San Francisco, CA, USA

Offprint requests to: Dr. Jean R. Wrathall, Dept. of Anatomy, Georgetown University, 3900 Reservoir Rd. N.W., Washington, DC 20007, USA

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Introduction

The successful regeneration of peripheral nerves is believed to be facilitated by peripheral non-neuronal cells that provide guidance and neurotrophic support for the regenerating axons [13, 25]. The absence of cells with such a supportive capacity in the mature mammalian CNS may contribute to the lack of effective CNS regeneration. Evidence bearing on this hypothesis has come from experiments in which peripheral non-neuronal cells in the form of segments of nerve have been surgical implanted into the gap of a transected spinal cord [1, 5, 8, 14, 18, 19, 26–28]. Early experiments [1, 5, 8, 28] were generally unsuccessful due to dislocation of the grafted nerve and/or the formation of scar tissue and cavities between the nerve graft and the spinal cord stumps. However, with improvement in microneurosurgical techniques [20] and the institution of a delay of about 1 week to allow the post-injury spinal cord cavitation phenomenon [15–18] to subside before implanting the nerve graft, good surgical reconstruction of the transected dog spinal cord has been achieved [18, 19]. At 1 month or later times after the surgical reconstruction, regenerated axons were observed within the nerve graft [19]. Junctional nodes of Ranvier in which axons were ensheathed by oligodendrocytes on one side and Schwann cells on the other, were observed at both the rostral and caudal junctions of the spinal cord with the nerve graft. Thus, at least some of the axons within the graft were believed to be of spinal cord origin. Additional evidence has recently been reported [26] for the transected rat spinal cord

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reconstructed with a modification of the same nerve graft technique. Three to 4 months after the reconstructive surgery, injection of horseradish peroxidase into the rostral or caudal junction of the spinal cord with the nerve graft revealed labelled spinal cord neurons on the opposite side of the nerve graft.

In both the dog and the rat, the number of axons from the spinal cord that elongated into the graft appeared to represent only a small proportion of the axons in a cross section of normal spinal cord. One possible way of improving the results might be to provide more rapid and intimate contact between the peripheral nonneuronal cells and axon terminals within the spinal cord stumps. In the transected dog spinal cord model, immediately after surgical reconstruction with a delayed nerve graft, microscopic spaces existed between the graft and the spinal cord stumps [19]. One week after reconstruction, the outgrowth of non-neuronal cells from the graft was observed that subsequently led to a physical union between the spinal cord and the nerve graft and allowed the elongation of axons into the nerve graft. If such spaces could be filled at the time of reconstructive surgery with non-neuronal cells with the capacity of effecting a more rapid union and of reaching spinal cord axons at an earlier time, before their regenerative attempts slow down [25], a better result might be obtained. To test this hypothesis, we have used cultured, peripheral, non-neuronal cells as an adjunct to surgical reconstruction with a delayed nerve graft of the cat spinal cord subjected to a severe contusion injury. The effect of reconstruction with a delayed nerve graft alone and with a nerve graft plus cultured cells, has been compared by light and electron microscopy.

Methods

Twenty-two adult female cats (3–4 kg) were used in this study. Each was anesthetized (Ketamine, Nembutal) and a laminectomy performed at T9 through T11. The blades of a pair of hemostatic forceps (5 mm width) were placed extradurally around the spinal cord at T10 and closely approximated for 2 s. After the compression injury the wound was closed in layers. The left sciatic nerve was then exposed, severed at the level of the mid thigh, and the wound closed. The animal was maintained for one week then reanesthetized and the wounds reopened. The dura and pia-arachnoid were opened by longitudinal incisions. The necrotic, custard-like, material within the spinal cord at the injury site was removed by gentle suction. In each case, the injury had produced a complete gap in the spinal cord 5–7 mm in length. An autologous nerve graft was obtained from the distal, predegenerated segment of sciatic nerve. The epineurium was removed and 3–4 nerve fascicles were cut to fill the gap that had been produced in the spinal cord. For cats in Group I ($n = 9$), the nerve segments were arranged longitudinally to fill the spinal cord gap and held in place by the addition of autologous plasma to form a clot. The incisions in the pia-arachnoid and dura were sutured separately and the wounds closed. For cats in Group II ($n = 13$), a slurry of homologous cultured peripheral non-neuronal cells was applied to both stumps of the spinal cord before the insertion of the nerve segments and the wound was closed in the same manner as in the Group I animals. The cultured cells were from either cell line GSA, a Schwann-like cell line derived from dissociated adult cat dorsal root ganglia [31], or line SNT, a mixed Schwann-like and fibroblastic cell line derived from trypsin-dissociated adult cat sciatic nerve [30]. Cells were grown as monolayer cultures, as previously described [31]. Tritiated thymidine (0.33 μCi/ml, 20 Ci/mmole, NET-027X, New England Nuclear) was added to the culture medium 48–72 h prior to harvesting to prelabel the cells. For each cat, $2-5 \times 10^7$ viable cells were harvested by trypsinization, washed by centrifugation (600 g $\times$ 3 min) in Hank's balanced salt solution, and the resultant cell pellet applied to the spinal cord stumps with a microsurgical spatula.

Post-operatively, all cats were given 600,000 units Bicillin, I.M. Daily expression of urine and feces by the Credé method augmented natural evacation. Short-term cats were sacrificed by fixation perfusion at 1, 3, 7, and 14 days through the descending aorta with a modified Karnovsky's fixative preceded by a saline wash. About 5 inches of spinal column with the injury area at the center, was removed and kept in fresh fixative for 3–5 days. The spinal cord was then dissected free and fixation continued for at least 2 more days. Three cm of spinal cord with the repair site at the center was cut in half via a sagittal longitudinal incision. One half of the spinal cord was paraffin-embedded and longitudinal serial sections (15 μm) prepared for light microscopic studies. Alternate sections were stained with Hematoxylin and Eosin, Masson's trichrome, Lapham's stain, DeMeyer's variation of Hortega's silver stain or processed for autoradiography. Sections for autoradiography were dipped in Kodak NTB3 emulsion, stored in the dark at 5° for 1–3 weeks, then developed and stained with HE or cresyl violet.

Samples for electron microscopy were taken in a longitudinal plane from the junction (rostral or caudal) of the nerve graft and the spinal cord stumps in the area of the lateral white matter tracts. Each was a rectangle of tissue about $3 \times 1 \times 0.5$ mm with the junction in the center and at right angles to the longest dimension. Samples were post-fixed with OsO_4, stained en bloc with uranyl acetate and embedded in EPON-Araldite. One micron sections were examined and areas chosen, thin sectioned, stained with uranyl acetate and lead citrate and examined with a AE1–801 electron microscope. Blocks of tissue from cats perfused at 7 or 14 days post-operatively were also marked at a point 1 mm into the nerve graft as determined from 1 μm plastic sections. These blocks were then reoriented and thin sectioned to allow visualization of a cross section of the nerve graft 1 mm from the junction with the spinal cord. Long-term cats, 1 year or more after reconstructive surgery, were perfused with fixative as described above. Tissue constituting a cross section of the nerve graft at its midpoint was cut into quarters, embedded, examined as 1 μm plastic sections and areas selected for electron microscopy.

Results

One day post-operatively (p.o.), the junction between the nerve graft and spinal cord stumps appeared similar in Group I and Group II cats except for the presence of the implanted cultured cells in specimens from Group II. The nerve graft was well approximated to the spinal cord tissue (Fig. 1) in many areas but new vascular connections had not yet been established as shown by the presence of packed erythrocytes in the blood vessels in the nerve graft even after perfusion-fixation. The spinal cord tissue immediately adjacent to the nerve graft appeared abnormal and many regions were necrotic. The presence of red and white blood cells in the parenchyma was frequently noted. Autoradio-

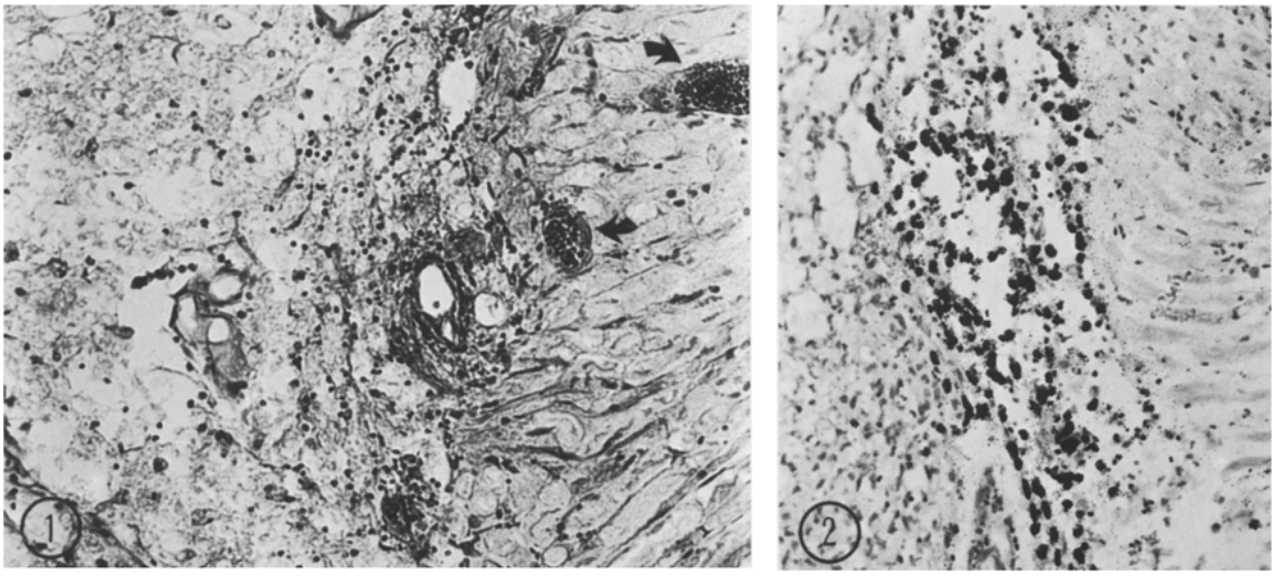


Fig. 1. A spinal cord-nerve graft junction from a Group I specimen, 1 day p.o. The nerve graft is well-approximated to the spinal cord tissue. Blood vessels within the nerve graft (*arrows*) are packed with erythrocytes. Red and white blood cells are also present within the junctional area and scattered in the degenerating spinal cord tissue. HE, ×540

Fig. 2. The junction between the nerve graft (*on the right*) and the spinal cord (*on the left*) in a Group II specimen at 1 day p.o. The nuclei of implanted cultured peripheral non-neuronal cells that were prelabelled with ^{3}H-thymidine are seen at the junction in the center of the field. Autoradiograph, HE, ×375

graphy (Fig. 2) demonstrated the prelabelled cultured cells present between the nerve graft and the spinal cord tissue in specimens from Group II.

At 3 days (Fig. 3) and 7 days p.o., the spinal cord-nerve graft junction of Group I animals (nerve graft alone) appeared similiar to that at day 1 except for evidence of somewhat increased necrosis in the spinal cord stumps, i.e. continuing cavitation. Silver-stained sections did not reveal evidence of axons reaching the nerve graft from the spinal cord. However, by day 7, functional vascular connections had apparently been established within the nerve graft as evidenced by the clearing of blood vessels by perfusion-fixation and the healthy appearance of nerve graft vessels as seen by electron microscopy (described below).

In Group II animals at 3 days p.o., the implanted cultured cells were seen as a zone of cells at the nerve graft-spinal cord junction. The proportion of cells that were heavily labelled with tritiated thymidine was decreased from day 1. Labelled cells could be detected up to 2 mm into the nerve graft (Fig. 4) and also appeared within the spinal cord stumps (Fig. 5). Examination of nearby sections stained with silver (Fig. 6), indicated that axons were present in the spinal cord in the region of these implanted cells and appeared to enter the spinal cord-nerve graft junction.

By 7 days p.o., the junctional region in Group II animals (Fig. 7) contained a thick cellular zone adjacent to the nerve graft and bridge-like strands of tissue connecting this zone to the more normal regions of the spinal cord stumps. Silver staining revealed axons traversing this region and apparently passing through the cellular zone to the nerve graft. Although autoradiography showed that most cells in this zone were unlabelled or very lightly labelled, the elongated form of the cells in this region resembled the labelled cells seen at 3 days p.o. Electron microscopy of longitudinal sections of the spinal cord-nerve graft junction showed axon terminal clubs (Fig. 8) and unmyelinated or occasionally myelinated axons (Fig. 9) in the junctional regions. The axons were ensheathed by cells with the characteristics of Schwann cells; generally a basal lamina was seen on the external surface of the ensheathing cells. Fine collagen fibrils were usually observed in the interstitial space. Axons ensheathed by Schwann cells were observed in the bridgelike structures adjacent to the nerve graft and could also be observed in the spinal cord stumps 1–2 mm from the junction.

By 7 days p.o. in Group II specimens, axons were seen to have penetrated at least 1 mm into the nerve graft and could be recognized within the Büngners' bands of the nerve graft in cross-sections studied by electron microscopy (Fig. 10). Careful study of cross sections of the nerve graft in Group I specimens failed to reveal any axons at 7 day, p.o., although by 14 days, some ensheathed axons were seen. Overall, wound-healing at the spinal cord nerve graft junctions in Group I specimens at 14 days p.o. appeared somewhat

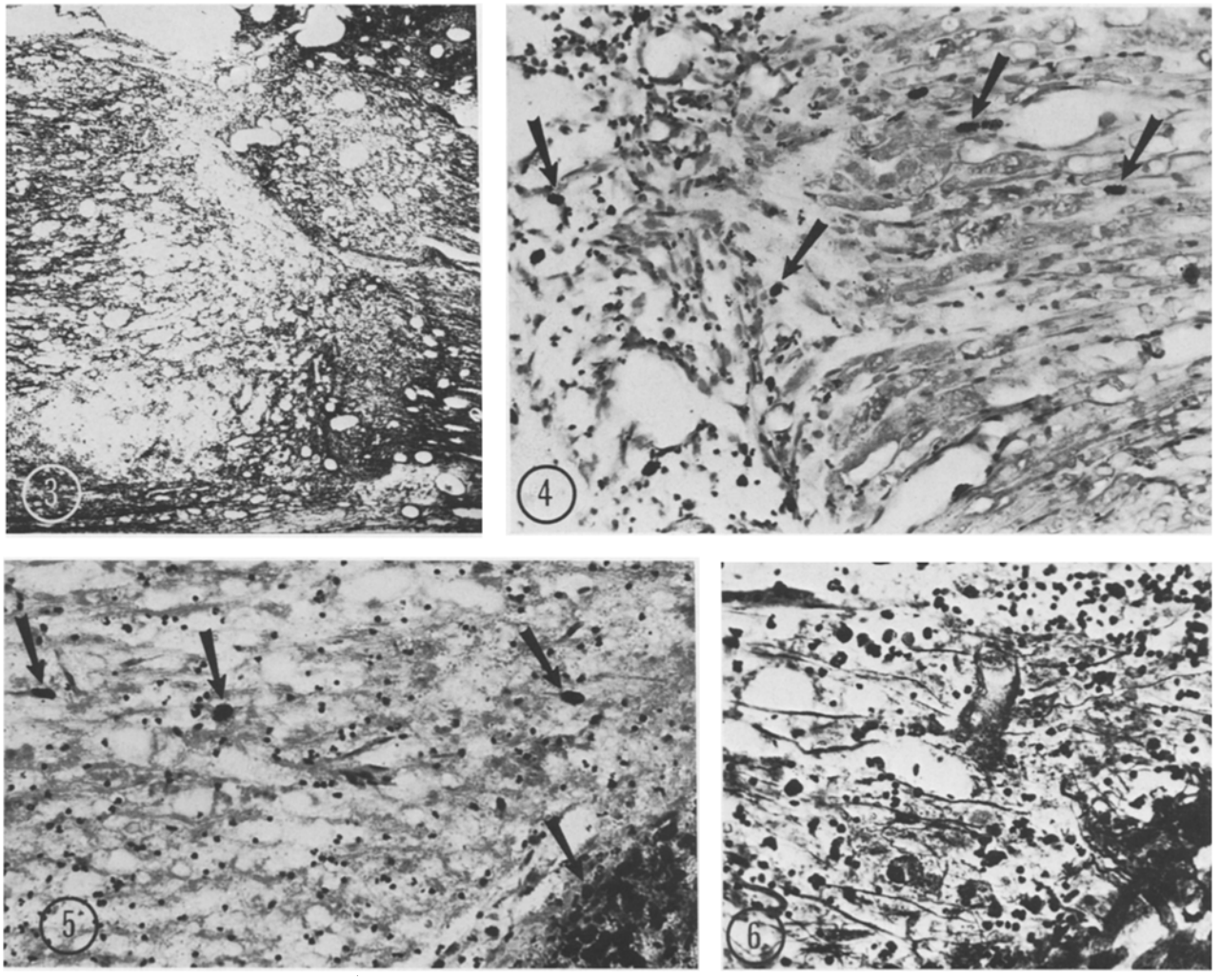


Fig. 3. A spinal cord-nerve graft junction from a Group I specimen at day 3 p.o. Two fascicles of the nerve graft are seen at the right of the field. The junction between the nerve graft and the more intact spinal cord tissue on the left consists of a rarified zone of degenerating spinal cord tissue. Axons have not yet penetrated this zone to reach the nerve graft. DeMeyer's silver stain, ×73

Fig. 4. A spinal cord-nerve graft junction from a Group II specimen at 3 days p.o. Labelled nuclei of the implanted cells (*arrows*) are seen among the thick zone of elongated cells in the junction and in the nerve graft (*on the right*), indicating the migration of the implanted cells into the nerve graft. Only the heavily labelled nuclei can be distinguished in the micrograph. Autoradiograph, HE, ×415

Fig. 5. Heavily labelled nuclei of implanted cells (*arrows*) are seen in the spinal cord tissue (*on the left*) and among the zone of cells at the junction (*lower right*) with the nerve graft. Group II specimen, 3 days p.o. Autoradiograph, HE, ×825

Fig. 6. The same area as seen in Fig. 5, in an adjacent section stained to reveal axons in the region of the spinal cord into which the implanted cells have migrated. Axons appear to approach and reach the spinal cord-nerve graft junction seen at the lower right of the field. Demeyer's silver stain, ×825

less advanced than in Group II specimens at 7 days p.o. Blood vessels seen within the graft at 7 or 14 days p.o., in both Group I and II specimens, appeared clear of erythrocytes, healthy and relatively normal (Figs. 11, 12). This is considered to indicate the establishment of vascular connections to the nerve graft by 7 days p.o.

At one year or later times after the reconstructive surgery, cross sections at the mid-point of the nerve graft of both Group I and II specimens revealed reinnervation (Fig. 13) of the nerve graft. Both myelinated and unmyelinated (Fig. 14) axons were seen. All of these were ensheathed by Schwann cells but some abnormalities were seen. For example, a small number of unusually large, unmyelinated axons were observed (Fig. 15). Axons were usually grouped into small fascicle-like structures often incompletely surrounded by processes of perineurial cells. Collagen fibrils filled much of the considerable interstitial space. The num-

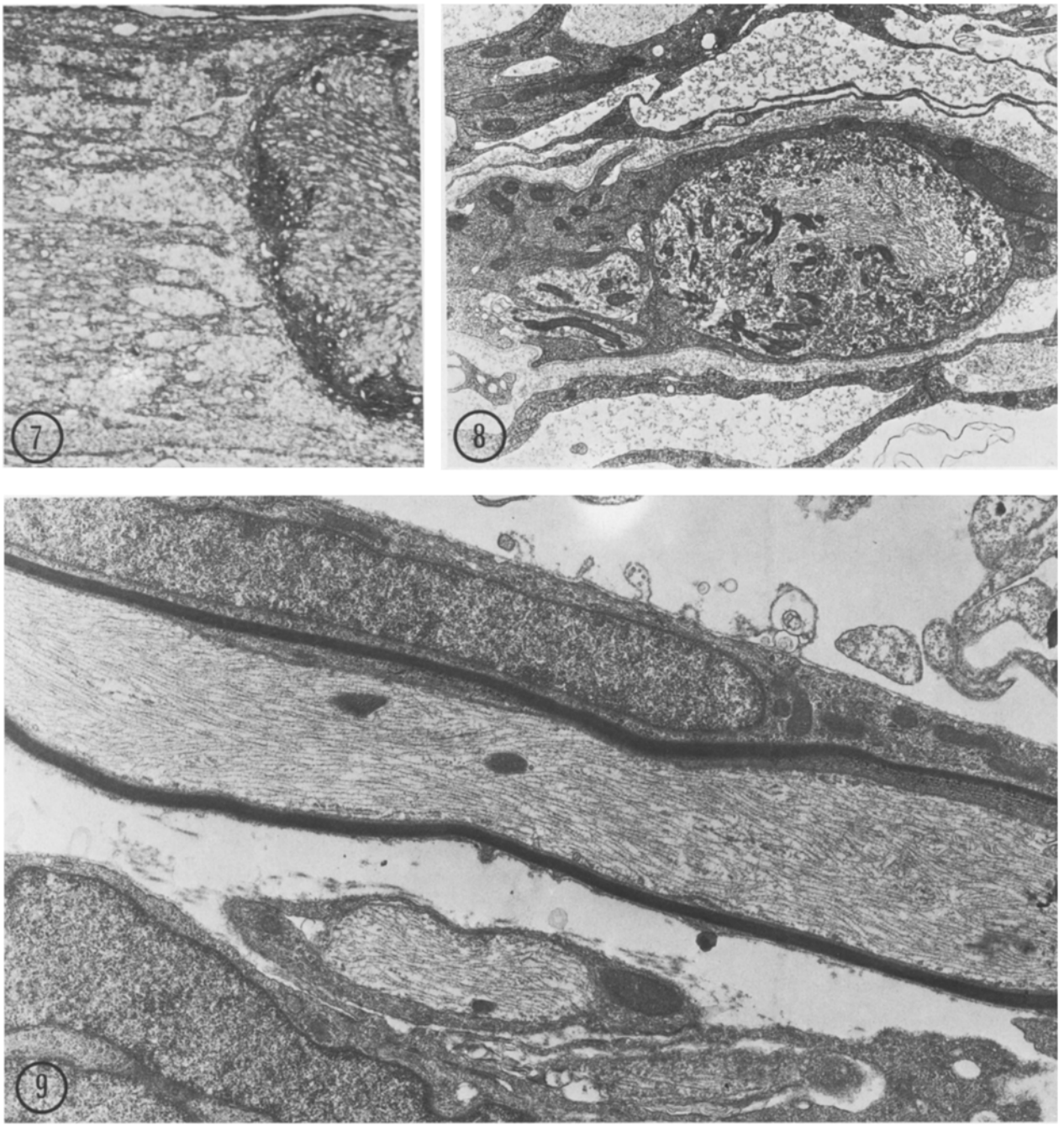


Fig. 7. A spinal cord-nerve graft junction from a Group II specimen at 7 days p.o. The nerve graft is on the right. There is a more darkly stained zone of cells at the junction and bridge-like structures appear to connect this zone to the spinal cord tissue on the left of the field. HE, ×65

Fig. 8. Electron micrograph of one of the bridge-like structures at the spinal cord-nerve graft junction from a Group II specimen at 7 days p.o. An axonal terminal club, filled with organelles and ensheathed by a Schwann cell is seen at the center of the field. A basal lamina and fine collagen fibrils are present external to the ensheathing cell. ×7,000

Fig. 9. Electron micrograph of axons in the spinal cord stump about 1 mm from the junction with the nerve graft. A thinly-myelinated axon traverses the field (*above*) and an unmyelinated axon is present in the lower center of the field. Both appear to be ensheathed by Schwann cells. Collagen fibrils are present in the interstitial space between the two axons. Group II specimen, 7 days p.o. ×11,700

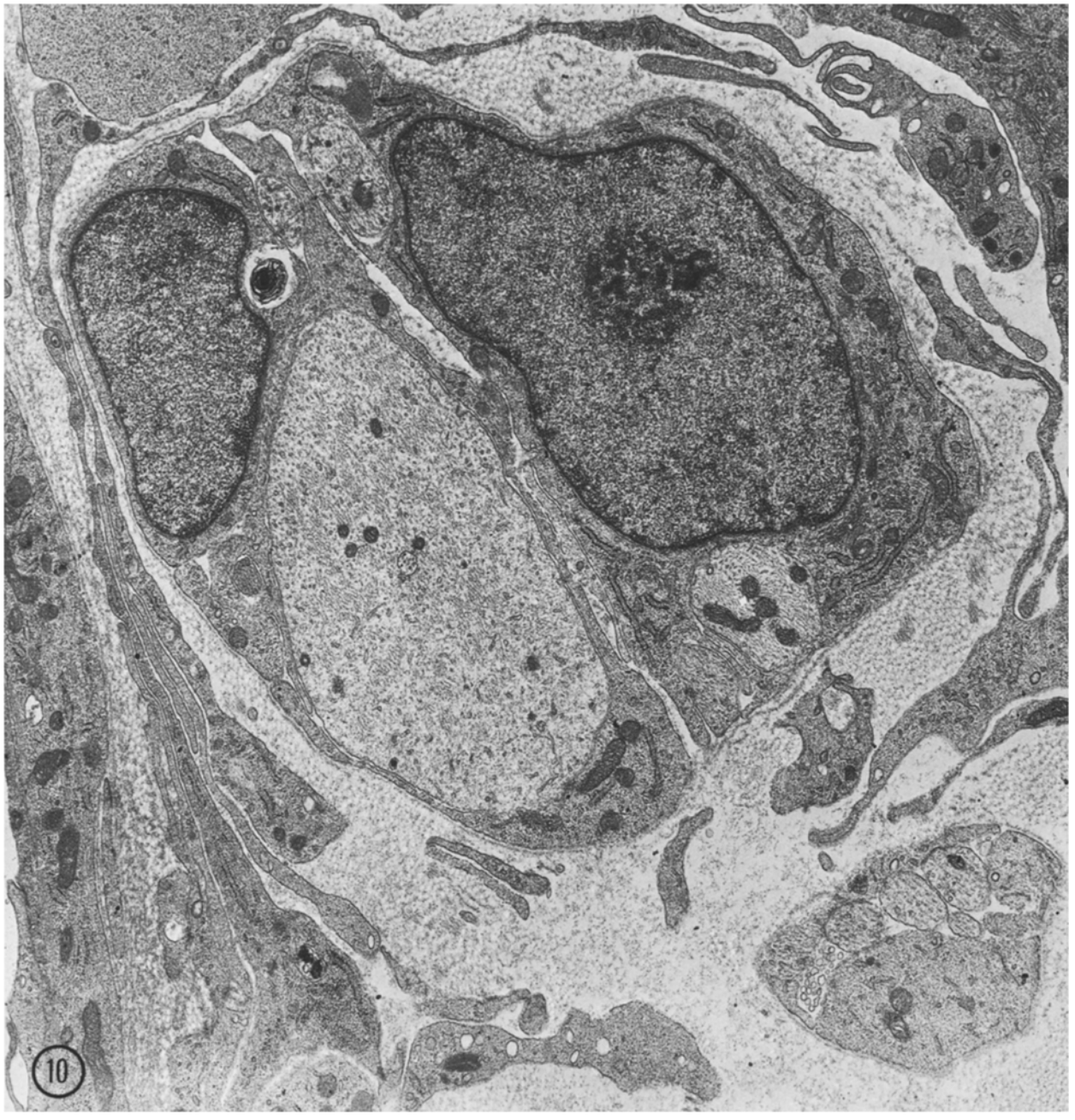


Fig. 10. Electron micrograph of a cross-section of the nerve graft 1 mm from the spinal cord-nerve graft junction from a Group II specimen at 7 days p. o. A Büngner's band containing ensheathed axons and surrounded by a basal lamina, fills the center of the field. Cross-sections of collagen fibrils are present in the interstitial space. ×11,500

bers of axons varied considerably in different regions of the cross-section from one specimen but no consistent pattern was found among different animals.

No differences attributable to the use of the two different cell lines (GSA, Schwann-like, or SNT, mixed type) were observed. Although the SNT cell line is believed to be a mixture of Schwann-like and fibroblastic cells, the amount of collagen in the junctional regions, as seen in paraffin sections stained with Masson's stain, was no greater than in Group II specimens in which GSA cells were used or in Group I specimens. Indeed, there appeared to be somewhat less collagen at the junctions in Group II specimens than in Group I specimens, as if the use of the cultured cells enhanced the effect of the nerve graft in preventing the formation of a collagenous scar. Further, although the

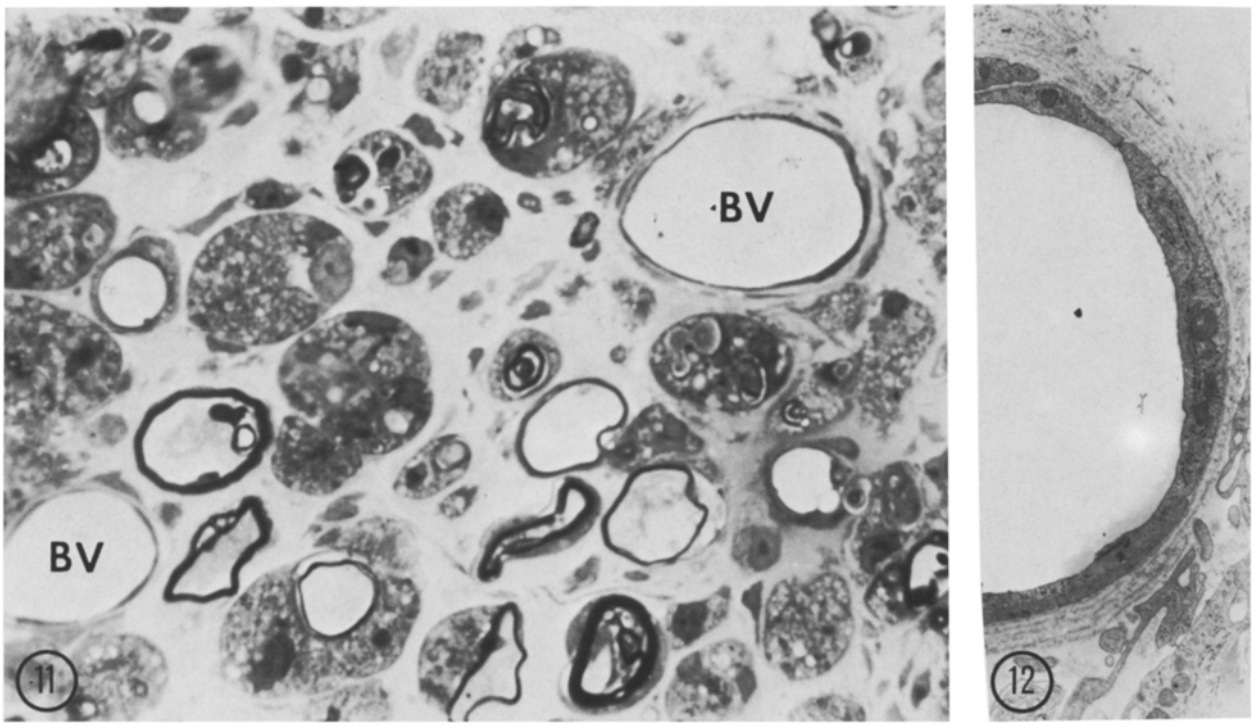


Fig. 11. Cross-section of the nerve graft 1 mm from the spinal cord-nerve graft junction from a Group I specimen at 7 days p.o. Blood vessels within the graft have been cleared by the perfusion fixation. Most cells within the graft are still filled with myelin debris. ×1,800

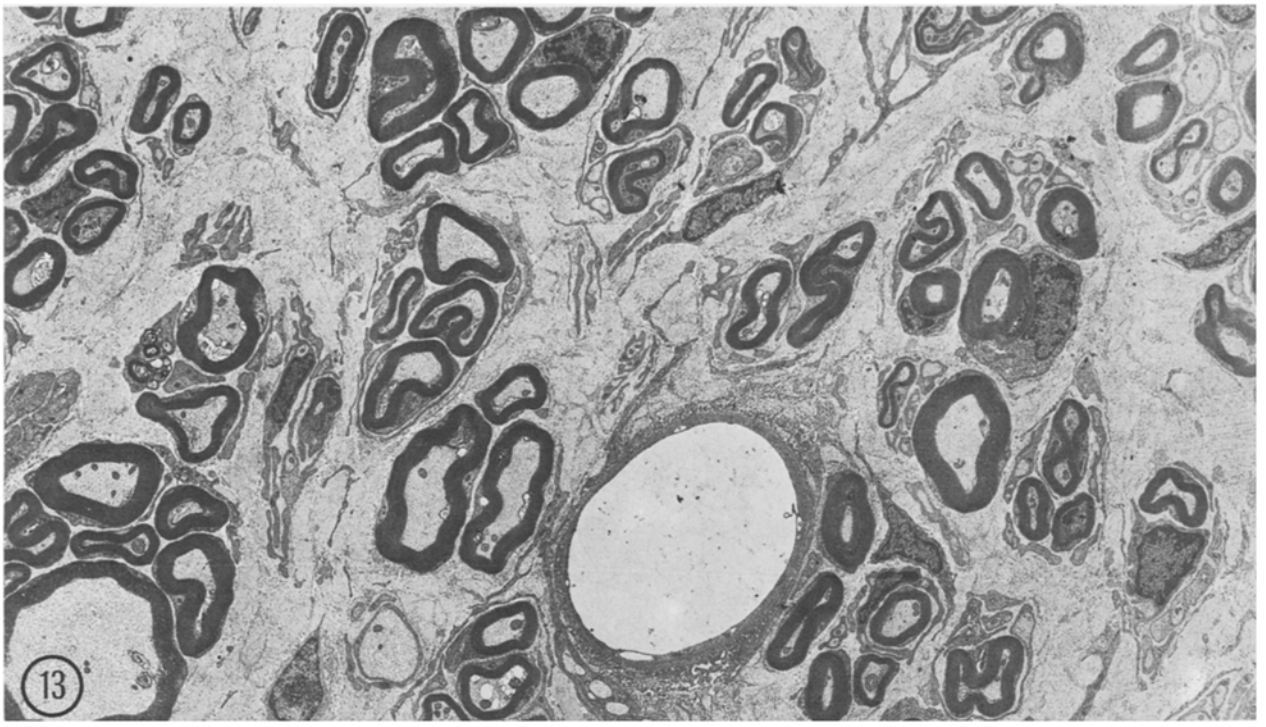
Fig. 12. Enlarged view of a portion of the wall of a blood vessel in the specimen shown in Fig. 11. The endothelial cell processes appear normal and connected by junctional complexes. ×11,500

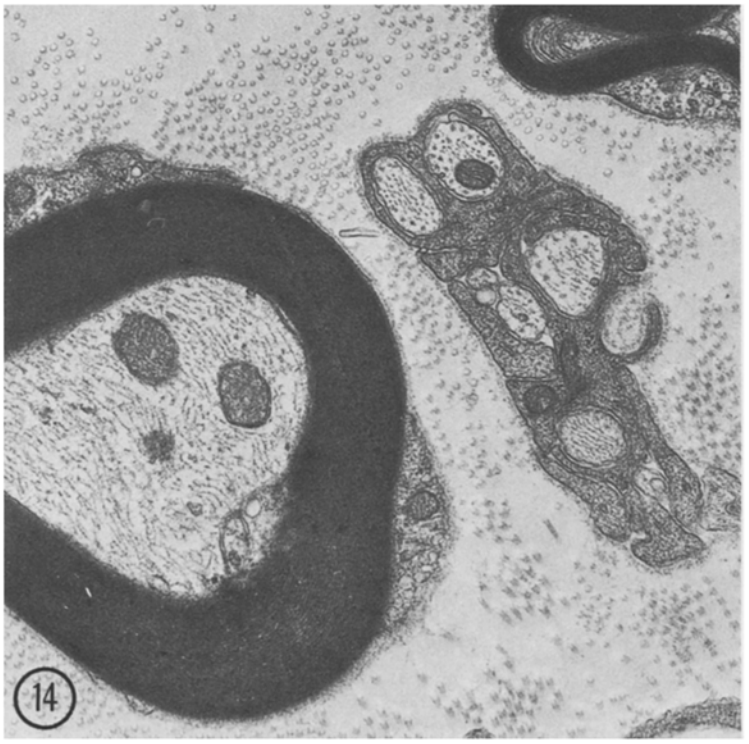
cultured cells were homologous rather than autologous, no increased inflammatory response distinguishable from that in Group I animals was noted at the time points studied.

Discussion

Cat spinal cords that were compressed between blades of hemostatic forceps developed a severe contusion injury. Post-injury spinal cord cavitation [15] produced a gap in the spinal cord of 5–7 mm by one week after injury, at which time the reconstructive surgery was performed. The results of repair with an autologous nerve graft (Group I cats) may be compared with a similar repair of the transected dog spinal cord, as previously reported [18, 19]. In the present study, in specimens studied from 1 day to 2 weeks post-operatively, the junction between the spinal cord stumps and the nerve graft appeared less satisfactory and wound-healing appeared slower than after reconstruction of the transected dog spinal cord. In large part, this appeared related to the original severe contusion injury that produced extensive hemorrhage and occasional secondary contusions distant from the site of injury. The spinal cord tissue adjacent to the nerve graft seen at 1 day to 2 weeks p.o. was not as healthy-appearing as the spinal cord tissue seen at the corresponding time points in the previous experiments [18]. Infiltration of the junctional area with red and white blood cells was noted in the present study and there was some indication of continuing cavitation after the repair. Nevertheless, in long-term animals, examined at 1 year or more p.o., reinnervation of the nerve graft was seen. Thus even in such a severe contusion injury, the delayed nerve-grafting technique allows axonal regeneration.

In Group II animals repaired with an autologous nerve graft and homologous cultured peripheral non-neuronal cells, wound-healing and the elongation of axons into the nerve graft occurred more quickly than with a nerve graft alone. Axons had penetrated into the nerve graft for a distance of at least 1 mm by one week after surgery, at least a week earlier than in Group I cats or in the transected and repaired dog spinal cords studied earlier [18, 19]. Although no signs of rejection of the cultured cells were observed in the short-term (up to 2 weeks, p.o.) animals, it is presumed that eventually these homologous cells were rejected in most recipient cats. However, at 1 year or later after repair, axons within the graft were observed as in animals repaired with an autologous nerve graft alone. Thus if the homologous cells were eventually rejected, their loss did





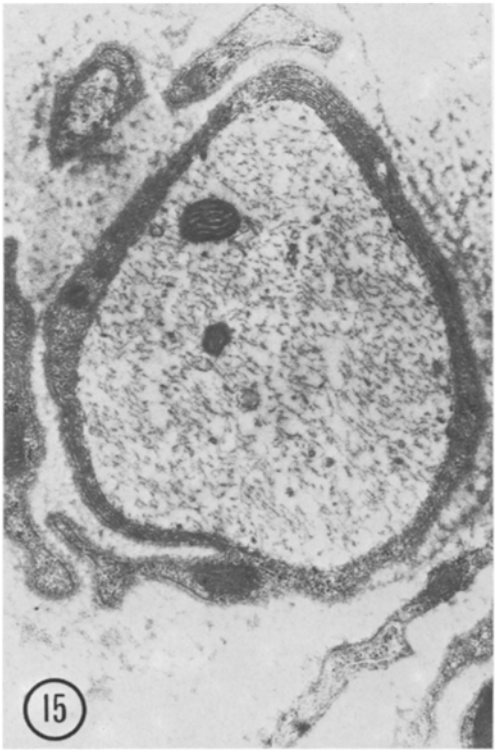


Fig. 13. Electron micrograph of a cross-section from the midpoint of the nerve graft from a long-term Group I cat at 15 months p.o. Many myelinated and unmyelinated axons have reinnervated the graft. ×2,500

Fig. 14. Enlarged view of a portion of the same specimen shown in Fig. 13. Small unmyelinated axons and a larger myelinated axon are seen with typical Schwann cell ensheathment. Collagen fibrils are present in the interstitial space. ×15,000

Fig. 15. The same specimen seen in Fig. 13 and 14. An unusually large unmyelinated axon, ensheathed by a Schwann cell, fills the center of the field. ×15,000

not at the same time cause the destruction of all the axons entering the graft.

The form of wound healing that was observed in the present study appeared to be similar to that reported previously after reconstruction of the transected dog spinal cord [18, 19]. It involved extension of PNS cells and PNS interstitial space into the spinal cord stumps. In an earlier study [19], the boundary between the spinal cord and the nerve graft at 1 month and later times after reconstruction with a delayed nerve graft was examined in detail. Finger-like extensions of peripheral interstitial space into the spinal cord were separated from the spinal cord tissue per se by a basal lamina and astrocytic processes. This very irregular and complex glia limitans was formed between 2 weeks and 1 month after the reconstuctive surgery. Axons bridging this boundary showed junctional nodes of Ranvier with oligodendrocyte-myelinated internodes on one side and Schwann cell-myelinated internodes on the other. In the present study, tissue from the short-term animals was examined at 1 day to 2 weeks p.o., before the expected formation of a glial boundary. None was observed in these specimens. However, axons observed in the region of the spinal cord-nerve graft junction were ensheathed by cells with the characteristics of Schwann cells, including a complete or partial basal lamina. Collagen fibrils were generally observed external to this basal lamina. Thus, axons appeared to reach the nerve graft via extensions of peripheral cells and peripheral interstitial space, as in the reconstructed dog spinal cord [19]. In Group II animals, these bridging structures had formed and axons had entered the nerve graft by 7 days p.o., whereas in Group I animals, repaired without the use of the cultured peripheral cells, these structures were first observed at 14 days p.o. The present observations are consistent with the implanted cultured cells actually forming part of these connecting structures but the interpretation of the exact role of the implanted cells is hampered by several factors.

The cultured cells, prelabelled in vitro with tritiated thymidine, could be followed in vivo for a period of timer after implantation. The degree of nuclear labelling decreased between day 1 and day 3 p.o., in a manner consistent with dilution of the label due to cell division. In addition, during the first 3 days in vivo, the cultured cells appeared to migrate both into the nerve graft and into the spinal cord stumps. In the latter area they could be detected in areas where axons could be seen in silver-stained sections and thus interaction of the cultured cells with spinal cord axons was possible by 3 days after implantation. However, as the nuclear label continued to diminish between 3 to 7 days p.o., few of the implanted cells could be identified by 1 week after surgery when axons ensheathed by Schwann cells were observed in the junctional regions. Thus, specific interactions between the implanted cells and spinal cord axons were not documented.

A reduction in the numbers of labelled cells in the junctional regions of Group II cats between day 1 and day 7 p.o. might also be expected if the homologous cells were being rejected in vivo. However, a similiar reduction in labelled cells has been observed in recent experiments (unpublished data) where cats were repaired using a nerve graft and autologous cultured cells or autologous cells alone. Further, when the prelabelled cells used in the present experiments were maintained in culture in medium free of label for 1 week, a marked decrease in percent labelled cells was seen (eg. 78% to 12%), presumably due to cell division alone.

Interpretation of the exact manner in which implantation of cultured cells enhanced axonal elongation into the graft is also hampered by the several sources of peripheral non-neuronal cells in the model employed. These sources include the cultured cells, the nerve graft, cells ensheathing peripheral axons associated with spinal cord blood vessels and cells that may have entered the spinal cord from the spinal nerve root entry zone.

Ensheathment and myelination of axons in the CNS by Schwann cells has been reported consequent to a number of naturally occurring or experimentally induced pathological conditions that result in CNS demyelination [2, 3, 9–12, 23, 33]. In these cases, it has been postulated [3, 12, 23] that the peripheral cells are derived from the nerve roots and/or blood vessels after damage to the glia limitans. However, little direct evidence is available as to their origin. In the case of a nerve graft implanted in the spinal cord [4, 19, 26] or brain [29], it is fairly clear that the peripheral non-neuronal cells so provided can ensheath and myelinate CNS axons. Further, cultured embryonic rat Schwann cells have been shown to myelinate CNS axons after they have been implanted into demyelinated areas of the spinal cord of immuno-suppressed mice [7]. However, as direct interaction between axons and implanted cultured cells was not detected in the present study due to the inability to trace the implanted cells for a sufficient period of time, it is possible that the enhancement of wound-healing we observed may be due, at least in part, to an indirect effect of the cultured cells. In this respect, the enhancement of neuron survival and regeneration in cultures in which peripheral non-neuronal cells are also present [6] and evidence of glial-axonal protein transfer [22] may be relevent. Neurotrophic support by non-neuronal cells has been long-postulated [25] but is still not clearly defined. However, such neurotrophic support may be involved in the enhancement of axonal elongation in spinal cords repaired with the use of the implanted cultured non-neuronal cells.

The model system employed in the current study was not designed to ascertain the specific effects of implanting the cultured cells. We are currently studying a "slit transection" model where post-injury spinal cord cavitation is significantly reduced by local hypothermia instituted prior to injury [32], and autologous cultured cells are implanted to completely fill the much smaller spinal cord gap. With this model, information on the interactions of the implanted cells with spinal cord axons may be more readily obtained.

The present results are highly encouraging. Current understanding of the considerable capacity of CNS neurons for axonal sprouting and synaptic plasticity has focused attention on the "pathway" problem as a key factor in preventing functional regeneration of CNS axons [21, 24]. Regenerative sprouts from CNS axons form but are normally unable to elongate and find a pathway to appropriate synaptic targets. Implanting cultured peripheral non-neuronal cells into the spinal cord appears to facilitate and speed the elongation of axons into the nerve graft which in turn provides a pathway [18, 19, 26] through which the axons may traverse the gap in the spinal cord. It is not yet known whether the implanted cells stimulate elongation of a particular class of axons in the spinal cord or have a general effect. However, increasing the speed of axonal elongation may be of critical importance. The regenerative attempts of CNS neurons begin to diminish at about a week after injury [25]. A glia limitans forms at the edge of the spinal cord stumps at 2–4 weeks after the injury [19]. It is unlikely that this barrier could be penetrated after it is formed. If axons can cross the injury site rapidly enough there is at least the possibility that useful functional reconnections might be formed.

In summary, the present results indicate that the use of cultured peripheral non-neuronal cells in the surgical repair of the severely injured spinal cord enhances wound-healing and speeds up the elongation of axons from the spinal cord stumps. Further studies are warranted to determine the mechanism of this effect and the degree to which spinal cord axon regeneration may actually occur.

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