The mechanism of spinal cord cavitation following spinal cord transection

Part 3: Delayed grafting with and without spinal cord retransection

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Cavitation adjacent to transection of spinal cords can be successfully eliminated by a second operation 1 week after the initial spinal cord transection. The second operation consists of removal of the necrotic spinal cord tissue, thus producing a gap. Segments of autogenous sciatic nerve are inserted into the gap between the spinal cord stumps. If the spinal cord is injured by retrandsection at the second operation, cavitation again occurs in the spinal cord stumps resulting in separation of the nerve grafts from the spinal cord. The results of the present experiments support the concept that lysosomal spinal cord autotomy, which causes spinal cord cavitation, is a self-limiting process and that once the spinal cord has completed the autotomy, the process will not occur again unless the spinal cord is again traumatized.

KEY WORDS • spinal cord injury • spinal cord necrosis • spinal cord cavitation • nerve graft • spinal cord regeneration

THE pathological changes that occur in severely injured spinal cords, whether produced by transection, contusion, or compression, are identical. The damaged spinal cord tissue is eventually removed and is replaced by either a single cavity or a connective tissue scar surrounded by cavities. Mechanisms such as direct tissue damage, hemorrhage, and ischemic necrosis of the spinal cord have been proposed as the cause of posttraumatic spinal cord cavitation.

We have previously proposed a neurogenic theory of spinal cord cavitation. Using a transection model, we have observed that posttraumatic spinal cord cavitation begins as numerous microcysts, at a distance of 1 to 2 mm from the point of transection, within the non-contused, non-hemorrhagic spinal cord stumps, both rostral and caudal to transection. As the microcysts enlarge, coalesce, and rupture, 1 to 2 mm of morphologically unaltered spinal cord tissue between the microcysts and the transection site (the “preserved” segment) is completely detached (spinal cord autotomy) by the microcysts from the remaining viable spinal cord (the metamorphic segment). Electron microscopy shows in each microcyst an axonal terminal club containing numerous lysosomes together with other axoplasmic organelles. As the terminal clubs...
later rupture, the lysosomes once contained within the terminal clubs are released to the extracellular space of the microcyst. Autolysis of the 1- to 2-mm “preserved” segment of the spinal cord subsequently occurs. The microcysts enlarge to encompass the degenerating “preserved” segment and thereby form large cavities. The autolytic process is attributed to the effect of activated lysosomal enzymes from the ruptured terminal clubs and is called “lysosomal spinal cord autotomy.”

If spinal cord repair by the surgeon is to become a reality, one must first find a way to correct for the damage of lysosomal spinal cord autotomy. As it has been shown, a spinal cord can be sharply transected, and autogenous brain slices, nerve segments, or nodose ganglia can be transplanted into the gap and closely approximated to the spinal cord stumps.

Unfortunately, autotomy of the spinal cord stumps follows and, as a consequence of autotomy, the “preserved” segment of the spinal cord is lysed and replaced by cavities. Most of the grafts are, therefore, isolated from the spinal cord by cavities.

From our previous observation, lysosomal spinal cord autotomy appears to be a self-limiting process. This observation can be used to correct for the effect of spinal cord autotomy on spinal cord cavitation. Rather than immediate grafting at time of spinal cord transection, a transected spinal cord ought to be delayed for grafting until spinal cord autotomy is nearly complete, approximately 1 week after the initial transection. At the reopening, only the necrotic cord tissue (that is, the “preserved” segment) is removed and segments of autogenous sciatic nerve are grafted to the viable portion (the metamorphic segment) of the spinal cord.

This paper reports the results of such experiments. In the control group, the previously transected spinal cords are retransected at the time of reopening, and segments of autogenous sciatic nerve are grafted to the cut ends of the retransected spinal cords.

Materials and Methods

Fourteen adult female dogs, each weighing from 20 to 30 lbs, were divided into two groups. Seven dogs in Group I were made paraplegic by a complete subpial spinal cord transection at the T-10 cord level. The gap between the cord stumps was made approximately 5 mm. The dura and the wound were closed. One week after the initial transection, the laminectomy wound was reopened. The dura was opened along the previous longitudinal incision. Necrotic spinal cord tissue was removed subpially, thus creating a gap of approximately 10 mm between the spinal cord stumps. The spinal cord was then transected again at 5 mm from the ends of the spinal cord stumps, both proximally and distally. We used a microsurgical subpial technique described previously, and removed the tissue between the two new cut ends of the cords without tearing the pia-arachnoid membrane. The gap between the cord stumps created by the second operation measured about 2 cm. Segments of sciatic nerve were obtained via a left gluteal incision and the nerve was placed longitudinally to fill the gap and fixed in position by a plasma clot. The pia-arachnoid incision was closed with interrupted 6-0 black silk sutures. A watertight closure of the dura was again achieved with a continuous suture of 6-0 black silk. The wound was closed in layers.

In Group II, seven dogs underwent complete subpial spinal cord transection at the T-10 cord level, and were operated on again 1 week after the first operation. The dura was opened along the previous longitudinal dural incision and the previous pia-arachnoid incision was identified. The pia-arachnoid incision was opened and the necrotic semifluid custard-like material in the gap was carefully removed microsurgically by gentle suction with the least possible injury of the pia-arachnoid membrane and the spinal cord stumps. The gap so produced usually measured 1 cm.

Segments of sciatic nerve were obtained as in Group I and grafted to the gap; the grafts were fixed in position by a plasma clot. The pia-arachnoid and the dura were closed as in Group I animals. The wound was closed in layers.

One dog in each group was sacrificed at 1 hour, 4 hours, 1 day, 1 week, 1 month, 3 months, and 10 months after the second operation. The spinal cords were fixed in situ by a perfusion technique with buffered 10% formalin. Tissue blocks of about 7 cm, containing cord within the vertebral column,
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were removed and placed in buffered 10% formalin for 10 days, at which time the spinal cord was removed from the canal. Longitudinal sections of the cord were made approximately 10 μ thick. To evaluate the cellular and fibrous changes, stains such as hematoxylin and eosin, Nissl's stain, and Masson's trichrome were used. DeMyer's, Holmes', and Bodian's silver impregnations were used to demonstrate the axons, and Luxol fast blue and Lapham's methods were employed to stain the myelin.

**Results**

**Findings at Wound Reopening**

At surgery and under the dissecting microscope, the spinal cord wounds in both Group I and II animals appeared similar. There was only minimal adhesion of the dura to the spinal cord and the spinal cord appeared surprisingly clean. There was usually an outflow of clear cerebrospinal fluid. The arachnoid and the blood vessels appeared clean and the spinal cord was pulsatile. A thin fibrin-like coat was seen covering the pia-arachnoid incision made during previous surgery.

When the pia-arachnoid incision was re-opened, there was a gush of necrotic semi-fluid custard-like material. An obvious increase in the gap between the cord stumps (about 10 mm longitudinally) from that which was made at the initial transection (about 5 mm longitudinally) was revealed upon removal of the necrotic material.

**Histological Studies**

**Group I.** Histological study of the spinal cords from the seven Group I dogs, transected for the second time 1 week after the initial spinal cord transection, showed that extensive spinal cord autotomy did indeed occur repeatedly (Fig. 1).

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**Fig. 1.** A schematic drawing showing the effect of spinal cord autotomy, developing after spinal cord transection, in relation to methods of nerve grafting. **Left:** Group I, delayed nerve grafting with spinal cord retranssection. The previously transected spinal cord is retranssected 1 week after the initial transection and segments of autogenous sciatic nerve (b-c) are immediately transplanted into the gap, oriented end-to-end and in close approximation to the retransected spinal cord stumps (upper). Autotomy of the retransected spinal cord stumps again occurs, and is nearly completed at 1 week (lower). The new ends of spinal cord stumps (a and d) are thus located at a distance from the ends of the grafted nerves. **Right:** Group II, delayed nerve grafting without spinal cord retranssection. A previously transected spinal cord is shown 1 week after initial transection (upper). At this time, the spinal cord autotomy is nearly complete and the new ends of spinal cord stumps are clearly delineated (a and d). During the second operation 1 week after the initial spinal cord transection, the necrotic cord tissue between the spinal cord stumps is removed without damaging the spinal cord, and the grafted nerves are closely approximated to the new ends of spinal cord stumps (a and d). No spinal cord autotomy follows and the new ends of spinal cord stumps heal to the grafted nerves without cavitation (lower).

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The immediate appearance of the spinal cord stump, transected a second time, is shown in Fig. 2. The cut end of the spinal cord was sharply limited and was closely approximated to the cut end of the grafted nerve. Since the metamorphic segment, which occupied approximately 2 to 3 mm in area at the new ends of the spinal cord stumps, was removed at the second operation, the spinal cord stump contained no terminal clubs or segregated balls. Only a few dilated myelin sheaths and swollen axons were seen. There was no hemorrhage or contusion of the spinal cord from the operation.

Autotomy of the cord stump began at 1 day and the 1- to 2-mm of spinal cord tissue bordering on the cut end of the spinal cord was subsequently replaced by cavities (Fig. 3). However, closer examination of the cavitated cord-nerve junction showed the presence of a thin layer of tissue covering the cut end of the grafted nerve. As previously reported, this thin layer of tissue was due to proliferation of neurilemmal cells and fibroblasts from the cut end of the grafted nerve and was of considerable significance in the healing between the spinal cord and the grafted nerve.

Despite favorable cellular proliferation from the grafted nerve, spinal cords obtained after 1 month showed two cysts, one on each end of the spinal cord stumps, separating the nerve graft from the spinal cord (Fig. 4). The spinal cord stump was lined with either fibrous astroglial cells or, occasionally, a layer of ependymal cells.

The original axons and myelin sheaths of the grafted nerve underwent rapid Wallerian degeneration. At 1 week after transplantation, the original axons of the grafted nerve appeared as beaded fragments or droplets within dilated neurilemmal sheaths (Fig. 5). After 1 month, no trace of axons or myelin in the grafted nerve was demonstrable (Fig. 4). The original neurilemmal sheaths of the grafted nerve remained and were occupied by neurilemmal cells and formed the Büngner's bands.

After 10 months, the grafted nerve appeared much smaller in size, presumably due to shrinkage of the tissue. It then consisted mainly of collagenous fibers and only a few Büngner's bands with no demonstrable axons or myelin.

Group II. In this group, the spinal cord stumps were not disturbed. Only the necrotic
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Fig. 4. A longitudinal section of spinal cord from a Group I dog, 1 month after the second cordotomy and sciatic nerve autograft. The spinal cord was transected 1 week before the second cordotomy. Two cysts are clearly shown (a-b, c-d), one on each end of the spinal cord, separating the nerve graft (b-c) from the spinal cord. The section is taken from the right lateral column. The head of the animal is to the left of the picture. A dorsal root (dr) is located outside the pial membrane (pm). Axons and myelin sheaths of the grafted nerve have completely degenerated. Bodian's stain, × 5.

Semifluid custard-like material in the gap was removed. The gap between the spinal cord stumps was therefore shorter than in Group I (Fig. 1).

Since the second operation was performed 1 week after the initial spinal cord transection, autotomy of the spinal cord stumps was nearly complete and the new ends of the previously transected spinal cord stumps, the metamorphic segments, were clearly delineated. The metamorphic segments, undisturbed at the second operation, were grafted with segments of autogenous sciatic nerve.

Figure 6 shows the immediate result of grafting. The spinal cord stump, as compared with the comparable picture in Group I (Fig. 2), appears considerably more edematous; it is disorganized and many extremely dilated myelin sheaths are seen. The blood vessels were engorged. In addition, there were many terminal clubs and segregated balls, which is characteristic of the metamorphic segment.

At 1 week after the second operation, the spinal cord stump remained adherent to the

Fig. 5. The typical appearance of the grafted nerve 1 week after nerve grafting (same dog as in Fig. 3). The degenerating axons appear as beaded fragments or droplets within dilated neurilemmal sheaths. Bodian's stain, × 220.

Fig. 6. A longitudinal section of a spinal cord from a Group II dog, showing the cord-nerve junction (arrow a) 1 hour after a delayed sciatic nerve autograft. The spinal cord was initially transected 7 days previously. The picture is taken from the right lateral column with the head of the animal to the left of the picture. The spinal cord stump is a typical 1-week-old metamorphic segment in which dilated myelin sheaths, swollen axons, terminal clubs, and segregated balls are seen. Three engorged blood vessels are shown (bvs). Bodian's stain, × 55.

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Fig. 7. Appearance of a cord-nerve junction (arrow a) 1 week after delayed nerve grafting without retranssection of cord from a Group II dog, viewed from the right lateral column with the head of the animal to the left. With the Group II procedure, the spinal cord stump remained adherent to the grafted nerve in contrast to the gap formed after retranssection (Fig. 3). Left: The terminal clubs within the spinal cord stump are smaller than those shown in Fig. 6. Spinal cord edema is also much less conspicuous. Bodian's stain, X 55. Right: The cellular proliferation at the cord-nerve junction (arrow a) can be seen. These cells extend into the spinal cord stump in longitudinally oriented cell columns. The 1-week-old cord-nerve junction becomes obscure due to the proliferation of these cellular elements. H & E, × 55.

Fig. 8. Photomicrograph of sagittal section through the lateral column of a spinal cord 5 weeks after transection and 4 weeks after delayed sciatic nerve autograft without retranssection of the spinal cord (from a Group II dog). The head of the animal is to the left. Arrows a and d indicate the ends of spinal cord stumps. One segment of grafted nerve (N₁) is shown between arrows a and d. At arrow a, the cord-nerve junction is obscure because of invasion by regenerated fibers, whereas at arrow d, the cord-nerve junction is still identifiable. No cavitation is seen between the grafted nerve and the spinal cord stumps (compare with Fig. 4). Another segment of grafted nerve (N₂), cut obliquely, is seen ventral to the nerve segment described. A layer of extradural scar tissue (s) is present dorsal to the spinal cord. A dorsal root (dr) is seen beneath the dura, but separated from the spinal cord by a continuous pial membrane (pm). Bodian's stain, × 5.

At 1 month after the second operation, the grafted nerves remained well approximated to the spinal cord stumps with no cavities formed at the cord-nerve junction (Fig. 8). In many areas, it was impossible to draw an exact boundary between the spinal cord and the grafted nerve.

At 3 months and 10 months after the second operation, the grafted nerve remained adherent to the spinal cord stumps. The junc-
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Fig. 9. A sagittal section through a spinal cord in a Group II dog examined 10 months after the second operation. The section is through the lateral column with the head of the animal to the left. Upper: Arrows a and d indicate the ends of the spinal cord stumps. Two nerve segments (N₁) and (N₂) fill the gap between the spinal cord stumps. Nerve N₁ is cut tangentially at the left upper portion of the picture, but still clearly extends between the spinal cord stumps. Nerve N₂ is slightly rotated and its caudal end is not ideally approximated to the spinal cord stump. The junctional areas between the spinal cord stumps and both ends of Nerve N₁ are obscure due to bridging by many axons extending between the spinal cord stumps and the transplanted nerve segment. The axons within the nerve segment are parallel to the long axis of the spinal cord and do not appear to have received any contribution by axons from sources other than the spinal cord. A small cyst (cy) is seen in the caudal spinal cord stump. A ventral nerve root (vr) is also present. Bodian's stain, × 22. Lower: High magnification of grafted nerve (area A, above) showing presence of axons (arrows). As the original axons of the grafted nerve are known to degenerate, the presence of axons in the grafted nerve 10 months after nerve grafting indicates that the axons are derived by ingrowth and are, therefore, regenerated axons. Compare with Fig. 4. Bodian's stain, × 220.

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The idea of grafting segments of peripheral nerve into transected central tracts has probably existed since the dawn of modern experimental neurology. In the classic monograph of Ramón y Cajal, Tello, Leoz, and Arcaute were cited as transplanting segments of autogenous sciatic nerve into the transected gap of the optic nerve in the rabbit. It is interesting to see that Ramón y Cajal did encourage the idea, but disapproved their surgical skills stating that “they were unable to avoid, in nearly all the cases, the dislocation of the graft and the entrance into the scar of external disturbing factors, such as adipose and muscular tissue, suture threads, etc.”

In 1940, Sugar and Gerard published the first report of successful implantation of segments of homograft sciatic nerve into the gap of spinal cord transection in the rat. In their report the importance of surgical skill in achieving a success was emphasized. All of their best cases of spinal cord regeneration were involved with implanted nerves, which retained a favorable orientation. In cases with poor results, the presence of a connective tissue scar and the formation of cysts, one on each end of the spinal cord stump, was seen. Unfortunately, following Sugar and Gerard’s experiment, many discouraging reports were published in which the grafts were either missing or separated from the spinal cord stumps by fibrous scars and cavitation. These findings were ascribed to lack of spinal cord regeneration and were not treated as a problem of poor wound healing. Hence, no further effort was made to improve the surgical technique.

In 1970, Kao, et al., described a microsurgical technique, “subpial spinal cord transection,” which was designed to achieve effective implantation of the grafts. The spinal cord was severed without cutting across the pia-arachnoid tubing, so that the grafts were not only prevented from dislocation but also favorably oriented within the gap. The results showed that the formation of a connective tissue scar was prevented by grafting regardless of whether cultured brain tissue, nodose ganglion, or segments of sciatic nerve were used. However, it was also reported that immediate grafting at the time of spinal cord transection could not eliminate spinal cord cavitation. Even if a sharp, non-contused, non-hemorrhagic cut end of spinal cord was produced by microsurgical transection, lysosomal spinal cord autotomy eventually occurred and a segment of spinal cord tissue bordering at the cut end of the spinal cord (the “preserved” segment) was lysed and replaced by cavities which separated the grafts from the spinal cord.

Our present study demonstrates, for the first time, that there is a surgical technique to eliminate spinal cord cavitation. A second operation was performed at a suitable time after spinal cord transection. The wound was reopened and necrotic spinal cord tissue removed microsurgically. Segments of autogenous sciatic nerve were then grafted between the viable spinal cord stumps.

In many ways, this approach is similar to the time-honored principle in plastic surgery that skin autografts should be applied to a burn wound only after the necrotic eschars are removed. The preserved segment of the spinal cord formed after spinal cord transection is analogous to the necrotic eschar and the metamorphic segment so formed is analogous to the granulation tissues formed in a burn wound (Fig. 1).

Spinal cord autotomy thus represents only the initial phase of the entire wound-healing process in severely injured spinal cords. After the delayed nerve grafting, the remaining stages of the healing process continued without repeating the initial autotomy. To produce the best surgical result, the spinal cord stumps should not be injured at the second operation so that no further autotomy occurs. Exactly how many times an axon in the central nervous system can be injured and still be capable of repeating axonal autotomy is yet to be determined.

Successful elimination of spinal cord cavitation has produced, in Group II animals, not only approximation of the grafted nerves to the spinal cord stumps, but also bridging by many axons at the cord-nerve junction. Axons were also identified within the grafted nerves. As was reported previously, and again confirmed in the present experiments (Figs. 4 and 5), the original axons and myelin sheaths of the grafted nerve underwent rapid degeneration. Approximately at the end of the second week, both the original axons and
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the myelin sheaths were removed and Büngner's bands were formed. Axons, identified within the grafted nerves, more than 1 month after transplantation, are thus derived by ingrowth and are therefore regenerated axons.

Only three possible origins of the regenerated axons in the grafted nerve can be considered: 1) spinal cord axons, 2) peripheral dorsal root fibers, and 3) autonomic nerve fibers accompanying the blood vessels of the spinal cord. Myelination of the axons in the grafted nerve precludes their origin as regenerated autonomic nerve fibers. The absence of axons in the grafted nerves in Group I animals strongly opposes the possibility of fiber contribution from a nearby peripheral dorsal root, as the chance for their invasion was equal in Group I and Group II animals. In addition, the microsurgical technique has preserved and reapproximated the pia, and a barrier is present against peripheral invasion (Figs. 4 and 8).

Thus, the bridging by many axons between the spinal cord stumps and the grafted nerves at both ends, the undisturbed parallel course of axons within the grafted nerves (Fig. 9), all favor the conclusion that these regenerated axons in the grafted nerves are of spinal cord origin.

We are currently studying by electron microscopy the mechanism of regeneration of the spinal cord axons following delayed nerve grafting. Initial observations seem to indicate that some axons remained arrested within the spinal cord stumps. Still another group of spinal cord axons were apparently blocked by a basement membrane formed between the spinal cord stump and the grafted nerve. Despite the above observations, the presence of regenerated myelinated and unmyelinated axons was confirmed by electron microscopy. As the myelinated axon bridged the cord-nerve junction, it formed a junctional node of Ranvier. On the spinal cord side, the axon was myelinated by the oligodendrocyte, whereas on the nerve side, the same axon was clearly myelinated by neurilemmal cells.

Although a transection model is used in this study, based on previous reports, it may be predicted that a similar result may also be obtained in the severely contused or compressed spinal cord by delayed nerve grafting.

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