Peripheral Nerve Implantation in Experimental Paraplegia*

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At present, the concept of "abortive regeneration" in the central nervous system of mammals is no longer tenable. Many investigators have demonstrated that elements of the mammalian central nervous system are capable of regeneration, which process can be enhanced by treating the lesion with trypsin, roentgen-ray radiation, or by the administration of systemic pyrogens. As a rule, regeneration of traumatized central axons in the spinal cord proceeds under optimal conditions, viz., clean surgical lesion, vascular integrity, hemostasis, early approximation of severed ends and excellent physical condition of the animal. It is apparent that such an ideal milieu for neurofibrillary neoformation is seldom if ever present in traumatic paraplegia as encountered in clinical practice. Therefore, it is not surprising that re-establishment of functional continuity between the completely transected segments of the spinal cord by means of regenerating central neurofibrils has never been unequivocally demonstrated in man.

The use of centrally connected implants of peripheral nerve into the distal segment of the transected spinal cord offers an inviting alternative for establishing neuronal continuity between the proximal and distal stumps of the severed spinal cord. In the present experiment intercostal nerves or sympathetic-nerve trunks were used as implants and their effect on the functional performance of paraplegic dogs was studied.

Methods and Materials

Thirty-nine adult mongrel dogs of both sexes were used. The animals were separated into three major groups.

Group I—Controls.

a) Sham controls 2 dogs
b) Controls 4 dogs

Group II—Sympathetic nerve-trunk implantation.

a) One-stage 11 dogs
b) One-stage treated with ethanolamine- 10 dogs
   c) Two-stage 2 dogs

Group III—Single and multiple intercostal-nerve implants.

a) Single 1 dog
b) Bilateral 7 dogs
c) Triple 2 dogs

Laminectomy was performed extending from T10 to T12. In sham controls, the operation was terminated at this stage, while in controls the dura mater was incised longitudinally and a 1-cm. segment of spinal cord was removed. The dural edges were approximated but were not sutured.

For implantation of a sympathetic nerve laminectomy with excision of a cord segment was followed by subperiosteal resection of ribs T11 and T12. Epipleural dissection of the sympathetic trunk was carried out caudad for 7 to 10 cm. The rami communicantes were cut and the trunk was divided; the free end of the centrally connected sympathetic nerve was next drawn through the longissimus dorsi muscle into the site of the laminectomy and implanted in the distal stump of the transected spinal cord by means of a 6 O silk guide suture (Fig. 1). In the two-stage operation (Group II c) the sympathetic trunk was inserted into the intact spinal cord. Two months later the segment of spinal cord was excised proximal to the site of implantation. Ten dogs of the sympathetic-
implant group were treated postoperatively with subcutaneous injections of ethanolamine-1,2 for 30 consecutive days, dosage being 20 mg./kg. of body weight.

The intercostal nerves to be implanted were dissected 1 segment above the level of the spinal cord transection. A 5–7 cm. portion was freed and divided, and the end of the central segment was inserted in the distal stump of the spinal cord as described previously (Fig. 1).

The dogs were observed from 100 to 285 days. Weekly neurological examinations were carried out to assess the reflex activity and locomotor function of the animals and observations were recorded on individual charts of performance.

In 6 dogs of Group II and 2 dogs of Group III the nerve implant was sectioned subsequently from 103 to 208 days after operation. Electrophysiological studies were performed on 13 treated dogs as well as on normal animals. These consisted of recording spontaneous electrical activity and induced action potentials resulting from stimulation of the implanted nerve.

At the completion of the experiment the dogs were sacrificed by means of a lethal dose of pentobarbital sodium and were perfused using a pulsatile perfusion-fixation technique. Serial sections of the spinal cords were stained with Bodian's protargol method and a variety of other stains.

**Observations**

1) **Function.** The following grading was employed for the evaluation of reflex and locomotor activity:

**a) Locomotor function.**

- Hind legs of the animal unable to support the weight of the body when the animal is placed in the standing position.
- Hind legs can support superincumbent weight of body when animal is placed in the standing position.
- Animal able to stand unassisted.
- Animal able to stand unassisted; walks short distances. Front and hind limbs not co-ordinated.
- Co-ordinated walking ability.
- Normal locomotion.

**b) Reflex function; spasticity.**

- Failure to elicit reflex; markedly diminished tonus.
- Normal reflex; normal tonus.
- Increased reflex activity; medium spasticity.
- Hyperreflexia; hyperspasticity.

**Group I (Controls).** In sham control dogs no neurological deficit was present. In other control animals, following spinal shock, depressed reflex activity was observed for a period varying from 1 to 6 weeks during which time these dogs also exhibited the Schiff-Sherrington phenomenon. Subsequently there developed a pronounced hyperreflexia and spasticity: foot drop, exaggerated segmental flexion and brisk tendon reflexes were seen in all animals. There was a marked variation in locomotor recovery of the control animals. The lowest level of functional attainment was represented by dogs that were unable to support their paralyzed body segment when placed in the standing position. These animals moved about by dragging their paralyzed limbs which were either extended completely or hyperab ducted. Spasticity in the hind limbs was considerable and often arthrodesis of the hyperextended limbs developed despite physiotherapy. In contrast, 2 of the control dogs were observed to stand up unassisted and to take a few steps. These dogs initiated locomotion by straining forward and by flexion of the neck. The paralyzed portion of the body was next visibly raised off the ground mainly
by the pull of the longissimus dorsi muscles. Reflex stepping of the hind limbs propelled the body forward whenever the hind paws were in contact with the ground. A variety of other adaptive movements was also observed.

The bladders of the paraplegic dogs had to be expressed at least once every 24 hours. After a varying interval of time reflex control of the bladder returned in 34 of the dogs. However, even these dogs had to be checked for residual urine every day. The animals had their paralyzed limbs put through a full range of motion by passive exercise as part of the daily care. No pressure ulcers were observed.

Group II (Sympathetic-Nerve Implant). Spinal shock and hyporeflexia lasted for 3 to 24 days and were then replaced by hyperreflexia and spasticity. In 4 animals these decreased within 1 month to levels slightly above normal. Three of these 4 subsequently demonstrated almost normal walking for distances up to 20 feet. Standing was achieved by simultaneous extension of the hind limbs using only a minimum amount of ancillary splinting by the rostral portion of the body. The activity of the front and hind limbs was well synchronized.

In 8 dogs differences in muscle tone between the two hind limbs were noted. The leg showing less tone and weaker quadriceps reflex was on the side of sympathetic-chain interruption.

As in controls, it was noted that animals maintained their standing position more readily during excretory activity.

No variation from the above findings was observed in animals treated with ethanola- mine-1,2 (Group II b). The two-stage operation (Group II c) had no demonstrable advantage over the standard procedure.

Group III (Intercostal-Nerve Implant). Reflex and locomotor activity of this group was essentially the same as of dogs with sympathetic-trunk implants. Only 1 dog (III c) showed good recovery of walking. This animal was injected at the site of nerve implant with 2 per cent procaine hydrochloride, 145 days after operation. (No cerebrospinal fluid was aspirated prior to injection.) Complete spastic paraplegia followed this maneuver (Fig. 2). The dog was unable to support its weight in the standing position; reflexes were accentuated in the caudal portion of the body. Two hours after the injection, the dog again demonstrated almost normal walking ability.

2) Nerve-Implant Section. Eight animals were reoperated upon; the nerve implant was defined clearly and interrupted in 5 dogs. Following the procedure, 4 of them showed deterioration in locomotor ability and an increase in spasticity. One dog reverted from a functional grading of 4+ to 1+. Sufficient time was allowed for recovery from operation, but no improvement in function was noted subsequently.
Table 1 illustrates the neurological status of representative animals.

3) Electrophysiology. In 13 animals electrical stimulation of the implanted sympathetic chain was performed paraspinally and epipleurally, approximately 7–10 cm. rostral to the site of implant in the spinal cord. Recording electrodes were inserted into the spinal cord at the site of implant and at various distances up to 3–4 cm. caudal. In 4 of 13 animals (2 intercostal-nerve implants, 2 sympathetic-trunk implants) stimulation of the implant induced compound action potentials in the distal segment of cord. Crushing of the nerve distal to the stimulating electrodes abolished the evoked response (Fig. 3).

Stimulus artifact was recorded in 8 preparations and was attributed to electrical “spread” since it was not abolished by crushing the nerve.

4) Histology. To date 7 spinal cords were sectioned serially; the nerve implant is present in the distal segment of the spinal cord in 4 of them. The terminal portion of the nerve implant shows an active growth cone in 3 of these cords (Dogs S1, S8, I3). Collateral and terminal neurofibrillar neoformation13 is evident in both sympathetic- and intercostal-nerve implants (Fig. 4). It is interesting that very little mesenchymal and glial reaction is seen in the area of the growth cone.

Discussion

The limb reflexes and functional activity of the paraplegic dog have been analyzed previously.11,16 Sherrington16 observed that standing, stepping and a type of forward locomotion resembling walking may be seen in spinal dogs; however, co-ordinated walking is never demonstrated by such animals.

In the present study, 4 dogs with nerve implants showed co-ordinated walking ability. The failure of the other animals to do so may be attributed variously to surgical technique, necrosis of the nerve implant, physical condition and clan of the animal.

That the paraplegia following injection of procaine was reversible and that deterioration and increased spasticity followed section of the nerve implant, indicate that nerve implantation produced the improved locomotor function seen in these dogs. The existence of growing neurofibrils at the terminal end of the nerve implant was demonstrated; it was also shown that stimulation of the nerve implant evoked action potentials in the distal segment of the cord. The relative absence of scar formation at the end of the nerve implant was less than anticipated without Piromen and roentgen-ray therapy.8

<table>
<thead>
<tr>
<th>Dog</th>
<th>Days Observed</th>
<th>Earliest Appearance of Function (Wks.)</th>
<th>Functional Grading</th>
<th>Spasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>137</td>
<td>1+ 2+ 3+ 4+</td>
<td></td>
<td>3+</td>
</tr>
<tr>
<td>Best Control, C2</td>
<td>216</td>
<td>4 6 14</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Best Group IIa, S4</td>
<td>221</td>
<td>5 8 16 16</td>
<td>4+</td>
<td>2– Complete paraplegia after implant section</td>
</tr>
<tr>
<td>Best Group IIb, E6</td>
<td>161</td>
<td>4 7 7 7</td>
<td>4+</td>
<td>2+</td>
</tr>
<tr>
<td>Best Group III, I3</td>
<td>193</td>
<td>3 4 4 4</td>
<td>4+</td>
<td>2+</td>
</tr>
</tbody>
</table>

Fig. 3. Dog 13. Oscillographic records from distal segment of spinal cord. (Left) Stimulation of intercostal-nerve implant followed by action potential, and (right) stimulation of same nerve implant after crushing it distal to the stimulating electrodes. Note absence of action potential.
There are two possible mechanisms by which the nerve implant may produce the observed improvement in walking ability. First, it is possible that neural continuity is established between the proximal and distal segments of spinal cord by regenerating fibrils creating functional synapses with motor elements. On the other hand, Freeman and Turbes theorized that in implants of intercostal nerve “the growing tip of axons exudes a material which either stimulates or inhibits the chemical mechanisms responsible for the reflex activity of the motor and sensory pools in the distal segment of the spinal cord.” Thus it is possible that biochemical changes associated with growth of the nerve implant influence the neuronal pool in the distal stump of the cord, affecting an equilibrium between the central excitatory and central inhibitory states. This may result in decreased spasticity and potentiate integration of reflexes in the caudal segment of the
spinal cord, leading to improved spinal walking.

That there were no significant differences noted in the functional behavior of animals treated by implantation of sympathetic chain and insertion of intercostal nerve, lends support to the latter hypothesis.

Others have observed a decrease in muscle tone in the sympathectomized extremity when compared with the normal side under anesthesia. A similar decrease in tonus was seen in the unanesthetized paraplegic animal on the side of the sympathetic-chain interruption in this study.

Summary

Centrally connected sympathetic-nerve trunks were implanted in the distal segment of the transected spinal cord in 23 dogs. Three of these subsequently showed coordinated walking ability. The previously reported procedure of intercostal-nerve implantation was also investigated.

Conclusions

1) After complete transection of the spinal cord and excision of a 1-cm. segment at T10, dogs may stand or walk but have no coordination of front and hind limbs.

2) The implantation of sympathetic trunks into the distal segment of the cord may produce improvement of spinal walking similar to that produced by intercostal-nerve implantation in the dog.

3) Similar alterations in reflex activity have been reported in a human paraplegic.

4) Both sympathetic-trunk and intercostal-nerve implants may grow in the distal stump of the spinal cord and are capable of nerve-impulse conduction to the stump.

5) No histological changes were seen at the site of the nerve implant that could be attributed to injection of ethanolamine.

6) Sympathetic denervation of the hind limb of a paraplegic dog with transection of the low thoracic spinal cord is followed by decreased muscle tone and diminished reflexes of that limb.

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References


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