

A Method for Shortening of the Rat Spine and its Neurologic Consequences

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SUMMARY

Large laboratory animals are the usual choice for complex surgical procedures on the spine and spinal cord, such as shortening of the spine. It would, however, be advantageous to be able to use a small inexpensive mammal like the rat. We describe a procedure which allows thoracic spondylectomy (T8-T9) to be performed in the rat with a satisfactory survival rate (69%). Functional consequences of the procedure on animals with uninjured spinal cord were monitored over a period of six months, at which time histologic examination was performed. There was a good correlation between operative trauma, consisting of mechanical injury to the cord assessed from the surgical notes, and the duration of postoperative spinal shock. Animals for which the cord appeared laminated or deformed at the time of sacrifice tended to show incomplete functional recovery. Cord cavitation developed in most surviving animals (78%) but did not have a measurable adverse effect on functional outcome. In the present study, the cord was not intentionally injured; however, availability of this procedure may facilitate the future development of methods to implement recovery of function following spinal cord injury.

KEY WORDS

rat, spondylectomy, spinal cord injury, thoracic spine

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INTRODUCTION

A variety of methods have been used in attempts to repair the transected spinal cord. These include methods to reduce scar formation /see 16 for review/ and, more recently, attempts to bridge the gap with fragments of transplanted fetal brain or spinal cord tissue /27/. A general obstacle in these studies has been the large gap caused by retraction of the stumps when the spinal cord is completely transected. This retraction not only greatly increases the size of the gap which must be bridged by growing neurites, but also increases the size of the resultant scar. Retraction therefore complicates attempts to repair the spinal cord which depend on scar reduction, methods to alter the substrate for regrowth, or transplantation of tissue. In all tissues, survival of a free graft is highly dependent upon local conditions and the presence of a large scar is likely to result in impaired graft survival.

In general, for neuronal tissue, it would seem that the type of junction most conducive to repair is a direct apposition between two surfaces of non-traumatized tissue, with no intervening area of scar or tissue reaction. In other words, the ideal tissue junction for studying nervous tissue "repair" is a junction of a given central nervous tissue with itself. However, this type of interface has almost never been analyzed because, for anatomical reasons, it is very difficult to obtain such an apposition without inflicting trauma on tissue which surrounds the injury. For the spinal cord, one prerequisite to obtaining a direct cord/cord junction is shortening of the spinal column. With the exception of treatment for spinal tumors, all reported experimental and clinical spondylectomies had had the same goal, abutting the cord stumps

/2,13,21,23,28,31/ or shortening the gap between the stumps prior to neural grafting /4/.

Removing one or more vertebrae can be done without excessive difficulty both in man and in laboratory animals. Stabilization of the column, however, is considered difficult, and usually requires special equipment. Experimental vertebrectomy has been described in dogs /21,31/, rabbits /23/ and cats /4/. The possibility of using rats would be advantageous because care of paraplegic rats is easier than that of larger mammals.

Here we describe a method for experimental shortening of the rat spine by thoracic spondylectomy. This technique requires minimal equipment and allows an operator to perform, unaided, thoracic spondylectomies in the rat. We analyzed behavioral, anatomic, and light microscopic changes, and attempted to correlate histologic findings with the type of physical damage sustained by the cord and with behavior. Many animals (69%) survived the procedure, and conservation of function in most of the surviving rats demonstrated that this procedure was only moderately harmful to the nervous system.

Abstracts of part of this work have been previously published /11,12/.

MATERIAL AND METHODS

Preliminary studies

The most appropriate site and technique for extensive laminectomies was sought in separate groups of animals.

The location of the animals' center of gravity, i.e., the site of maximal stress on the column during movements, was determined on 10 rats in the following manner: several threads were tied to the skin of euthanized animals before deep-freezing them in a crouched or stretched posture. The thread from which the stiffened animals hung in balance indicated the location of the center of gravity; this was found to be on a plane through L1. When extensive laminectomies were performed close to the center of gravity, i.e., below T11, they were sometimes followed by spontaneous fracture of the column, indicating excessive mechanical stress.

Conversely, when laminectomies were performed at or above T5, the animal could not lift its head to drink or feed in the postoperative period. We therefore selected the mid-thoracic area.

In additional groups of animals, the exposed cord was transected, and the width of the gap produced by retraction of the cord stumps was measured. We tested several methods suggested by other authors for bringing the stumps back together. Placing stitches through the cord /16,18/, suturing pia or dura /2,13,20/, modifying the flexion-extension of the spine /6,7/, and pulling the spinal cord by the roots /32/ were not effective. It was concluded that removal of a length of bone equal to 1 or 1.5 vertebral segments would be necessary and sufficient to eliminate the gap produced by transection.

Operative procedure

Thirteen Sprague-Dawley female rats weighing approximately 350 g at the beginning of the experiment were used. The goal was to remove the rostral 3/4 of the 9th thoracic vertebra and the caudal 3/4 of the 8th, and to approximate the adjacent stumps of the spine without damaging the cord. "Rods" for stabilization of the spine were made from the shaft of 25 gauge needles (Fig. 1).



Fig. 1: A "rod" for spinal stabilization: a 25 gauge, 38 mm long needle was shortened to 28 mm and its tip was bent with fine pliers.

Anesthesia was produced with pentobarbital and chloral hydrate i.p., gentamycin i.m. was administered, and the skin was surgically prepared. The animals were laid ventrally and a long, median incision was made over the thoracic spinous

processes. The exact site of surgery was determined using the landmark of the fifth thoracic vertebra, i.e., a large vein described previously /10/. On both sides, the caudal aspect of the transverse process of T6 was exposed. The 9th rib was freed from the pleura and cut at its posterior costal angle. The 8th and 10th ribs were dissected from the pleura but not cut. Pleura and mediastinal organs were dissected from the vertebral bodies. The 8th and 9th intercostal nerves were cut at their origin in order to avoid unwanted traction on the cord. The 8th and 9th vertebral vessels were avulsed. The medial stump of the right 9th rib was removed.

Four stitches were then prepared. A 3/0 and a 0 polypropylene monofilament (Ethicon) were placed around the column, just caudal to the 10th ribs. A 3/0 polypropylene monofilament was placed around the column, just rostral to the 8th ribs. A 3/0 silk "approximating" stitch was placed as shown in Fig. 2. The laminae of T9 and the caudal

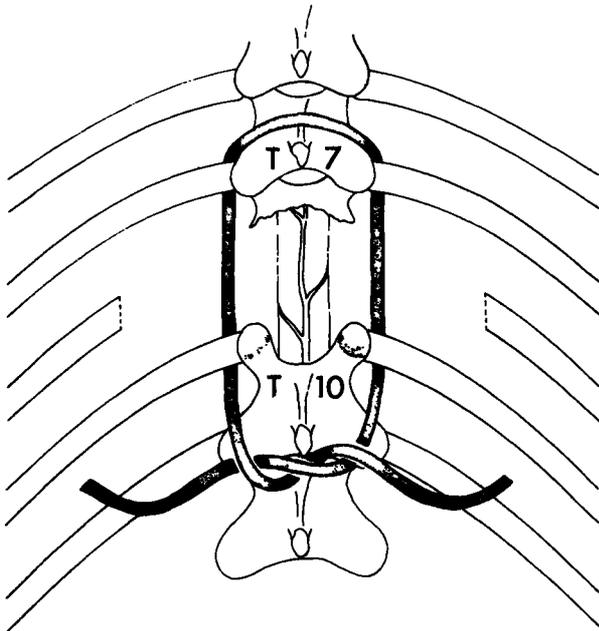


Fig. 2: Dorsal view of the spine showing the approximating stitch. Tying the knot brings the stumps of the spinal column into contact. During this movement, the hooked part of each rod abuts against the 5th transverse process (see Fig. 4), and the shaft of the rod slides towards the tail; the alignment of the spine remains unchanged.

half of the laminae of T8 were removed, and the right side of the vertebral bodies was clipped away. The animal was then tilted on its left side. Two transverse trenches were opened in the vertebral bodies, one caudal and the other rostral to the 9th ribs. These trenches were extended beyond the median line, thus occupying approximately 2/3 of the width of the spinal column.

The sharp-ended hook constituting one extremity of the rod was placed ventral to, and around, the right transverse process of T6. This automatically positioned it between the process and the rib lying ventrally to it (Fig. 3). The shaft of the rod was placed in the trench between the process and the corresponding right rib of T6, T7, T9 and T10. One of the stitches prepared caudally was then tied over the straight end of the rod. A small retractor kept apart the 10th and 8th ribs, preventing premature closure of the bone gap during subsequent steps.

The rostral trench was extended on the left side until the spine was completely cut. The caudal trench was similarly extended until the fragment of spine was loose, and could be pulled out together with the stump of the 8th rib attached to it. Because of the combined action of the right rod and of the small retractor, bone displacement was not possible and the rat could be tilted or moved around without injury to the denuded cord.

The hooked extremity of the second rod was

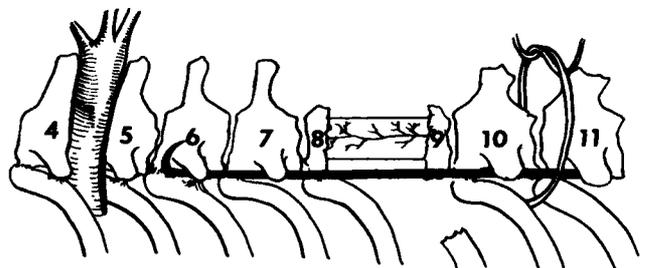


Fig. 3: Schematic representation of the placing of a rod. Precise location is determined by the landmark vessel in the interspinous space T4-T5 (see /10/). The rod is hooked around the transverse process of T6, and laid along the column. Tying the knot then presses it firmly against the column.

placed around the left transverse process of T6 and the shaft of the rod was laid along the left side of the column, between processes and ribs. The second caudal stitch was tied over the straight end of the rod, providing rigidity to the spine. The cranial stitch was then tied. By tightly pressing the rods against the cranial part of the column, this third stitch put the stumps of the spine in exact alignment. Longitudinal movements, however, were still possible after removal of the small retractor. Tying the approximating stitch reduced the space between what was left of the 8th and 9th vertebrae, and brought these two bone stumps into contact. At the end of this step, the cord was almost invisible, hidden in the reconstituted bone canal. Muscles and skin layers were then closed. The complete procedure lasted approximately 1.5 hours.

Animals were caged separately on soft bedding for one month and then housed under ordinary conditions. Animals were maintained and observed for a period of six months. No special postoperative care, such as regular bladder expression, was found necessary. Fig. 4 shows the rods *in situ*, approximately four months after the procedure.

Behavioral rating

Rats were rated by a "blind" observer, daily for the first 3 weeks, then once a week. The behavioral rating was derived from the scoring system described by Tarlov /29/, with addition of one category at each extremity of the scale. This scale was based on pilot observations made on approximately 240 animals, comparing the observations made by three raters.

Ratings:

"0" or *Complete physiologic spinal disconnection*. Spasticity and disinhibition of the hind quarters indicated release of the lower part of the cord. Spinal activity, however, was easy to distinguish from voluntary movements in that it was uncoordinated and erratic. More importantly, spinal activity was usually secondary to internal or external stimuli and disappeared with cessation of the excitation. The rat moved around by dragging its inert hind limbs. At rest, the animal lay on its abdomen with hind limbs extended, or on one flank.

"1" or *Complete paraplegia, without signs of*

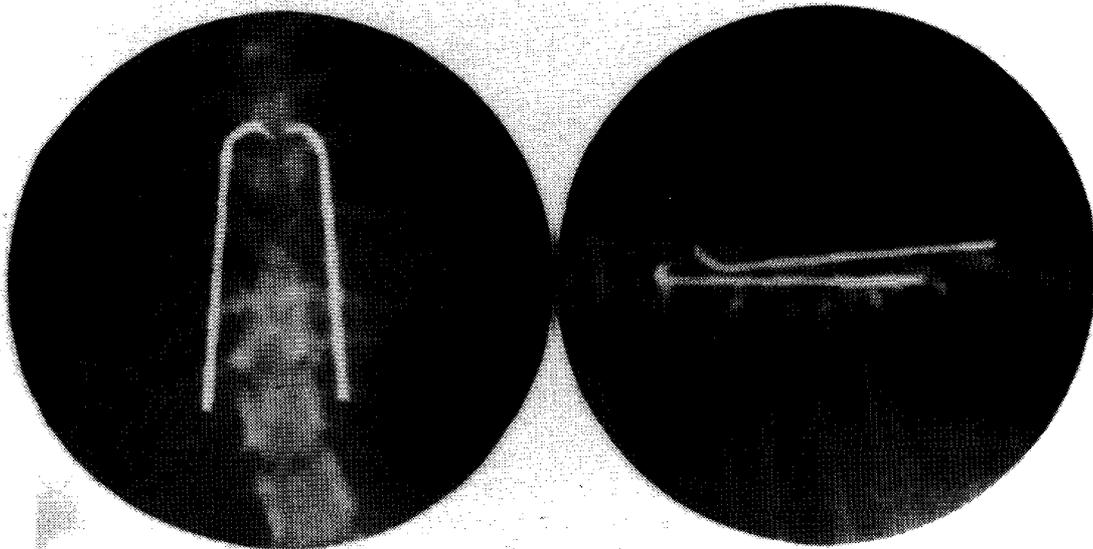


Fig. 4: X-ray of rods *in situ*, four months after the procedure.

spinal release. Strong stimuli, such as pinching the tail, did not elicit spastic activity in the hind quarters. Absence of spasticity was a sign of favorable prognosis, and subsequent improvement usually followed.

"2" or Incomplete paraplegia. At rest, the hind limbs were positioned under the body of the animal, in a normal crouching posture. When moving, the limbs were dragged behind, but exhibited strong coordinated pedaling movements. The rat responded to pressure or pricks applied to its hind quarters.

"3" or Notable deficit. The hind limbs could support the weight of the hind quarters at rest and during walking, but the gait was wobbly and slow. The animal could not turn around quickly without vacillating or tumbling. The animal could sit up with the help of its front limbs leaning against a wall, but could not then extend its hind limbs.

"4" or Slight deficit. The rat walked well and could stand up with the help of its front limbs leaning against a wall.

"5" or Absence of deficit. The criterion for this score was that the animals were able to stand unaided on extended hind limbs.

Radiographic examination of unrestrained rats

Two to four months after the procedure, the animals were placed in a high narrow dark cardboard box transparent to X-rays. An opening at the top of one side of the box formed a small window close to the ceiling. A transverse radiograph was taken when the rats stood up to look through the window. Fig. 4 is an enlarged view showing the rods *in situ*.

Anatomic assessment and histology

After six months, the animals were deeply anesthetized, and the thoracic spine was removed. The specimen was immersed in Bouin's fluid for one hour, rinsed for 1/2 hour in running water and placed in 10% buffered formalin for at least ten days. All laminae were then removed. Photographs were taken of the cord *in situ* and of the spinal canal after removal of the cord. The rods were removed and bone fusion was assessed by forcibly manipulating the spine under the operating microscope. The cord was embedded in paraffin,

cut longitudinally at 6 micrometer intervals, and sections were stained with hematoxylin & eosin (H&E), Bielschowsky, glial fibrillary acidic protein (GFAP), and luxol fast blue. About 120 sections of each cord were examined.

RESULTS

General

Four rats died between the 2nd and 5th postoperative days; death was attributed to anesthetic and hemorrhagic shock. The remaining nine animals survived until the end of the experiment, i.e. for six months. Improvement from the postoperative deficit was observed between the 2nd and the 25th day. Functional and histologic results are summarized in Table 1.

Behavior

In eight of the nine animals, recovery of function was quick and satisfactory. After one or two weeks, these rats were difficult to distinguish from naive animals. Spontaneous activity was normal. The animals could run and jump, and could stand up on extended hind limbs. Five of these eight animals essentially recovered completely, with final ratings of "5", indicating no detectable residual deficit (Table 1). The residual deficit in the other three rats, with final ratings of "4", consisted primarily of a lack of balance when standing up on hind limbs (Table 1).

The final result was mediocre in one rat (No. 33; cf. Table 1). The hind limbs of this animal could support the hind quarters' weight at rest and during walking, but the gait was wobbly and slow. The rat could not turn around quickly without vacillating or tumbling. The animal could sit up with the help of its front limbs leaning against a wall but could not stand up. Bladder and intestine function were normal. Spontaneous activity of the rats is shown in Fig. 5.

Types of trauma to the cord

During the surgical procedure, the cord potentially could have been submitted to insult or injury in three forms: mechanical trauma of brief duration, lasting compression, and vascular

TABLE 1
Anatomic and Behavioral Results

Animal Number	Peroperative Trauma	Long-Term Compression	Vascular Disturbances	Ratings on Postoper. Day 1(a)	Day of 1st Signs of Recovery	Date of Stabilization	Final Rating (a)	External Appearance of Cord at 6 Mos.	Cavitation (b)
660-24	Yes	None	--	1	12	1 mo.	4	Normal	Syringomyel.
660-28	Yes	Moderate	--	1	11	1 mo.	4	Slightly deformed	Hydromyelia.
660-29	Yes	--	--	1	Died on day 3	---	-	---	---
660-30	No	--	--	2	Died on day 3	---	-	---	---
660-31	No	None	--	2	3	18 d.	5	Normal	Hydro-syr.
660-32	Yes	None	--	1	8	12 d.	5	Normal	Hydro-syr.
660-33	Yes	Severe	--	1	25	2 mo.	3	Severely laminated	Syringomyel.
660-34	No	--	--	0	Died on day 5	---	-	---	---
660-35	No	--	--	3	Died on day 2	---	-	---	---
660-36	No	None	--	3	1	8 d.	5	Normal	Syringomyel.
660-37	Yes	Severe	--	1	20	1.5 mo.	4	Laminated	Hydro-syr.
660-38	No	None	--	2	2	15 d.	5	Normal	None
660-39	No	Moderate	--	1	10	25 d.	5	Normal	None

(a) Behavioral rating: 0 = spinal animal; 1 = complete paraplegia; 2 = incomplete paraplegia; 3 = notable deficit; 4 = slight deficit; 5 = absence of deficit

(b) Hydromyelia was characterized by dilation of the central canal and syringomyelia by cavitation in gray or white matter; both coexisted in hydro-syringomyelia.

disturbances. No blood-flow alterations were observed on the superficial vessels of the cord. Occurrences of the first two forms of insult are shown in Table 1. (i) Operative trauma, consisting of inadvertently touching the cord with an instrument, was assessed during the surgery. This usually provoked a strong motor response, and was quickly followed by a local change in color. Within one or two minutes, the surface of the cord turned pink or light purple. Minimal trauma of this type occurred for six of the 13 rats. These data are shown in Table 1, column 2. (ii) Compression was classified as none, moderate, or severe according to the appearance of the cords after sacrifice. These observations are shown in Table 1, column 3.

Radiography

Transverse radiographic views of unrestrained rats are shown in Fig. 6. The procedure caused little modification in the configuration and dynamics of the spine. There was good tolerance of the rods.

Gross anatomy of spine and cord

There were no signs of infection. In all specimens, the dura adhered to the bone at the site of surgery. It was not possible, however, to determine by gross examination whether or not the cord itself was also adherent. No macroscopic abnormalities attributable to vascular or hydrodynamic abnormalities were observed. Bone fusion was found to be complete in all specimens. In most cases, the spinal canal had been correctly reconstituted. In six animals, the exact site of spondylectomy was difficult to determine; no external abnormalities were seen on the cord of these animals (Fig. 7a). In one rat, there was a moderate bone protrusion at the site of fusion and a corresponding impression was visible on the surface of the cord (Fig. 7b). In the two remaining rats, the remnant of T9 was protruding into the canal, and the cord was severely laminated (Fig. 7c).

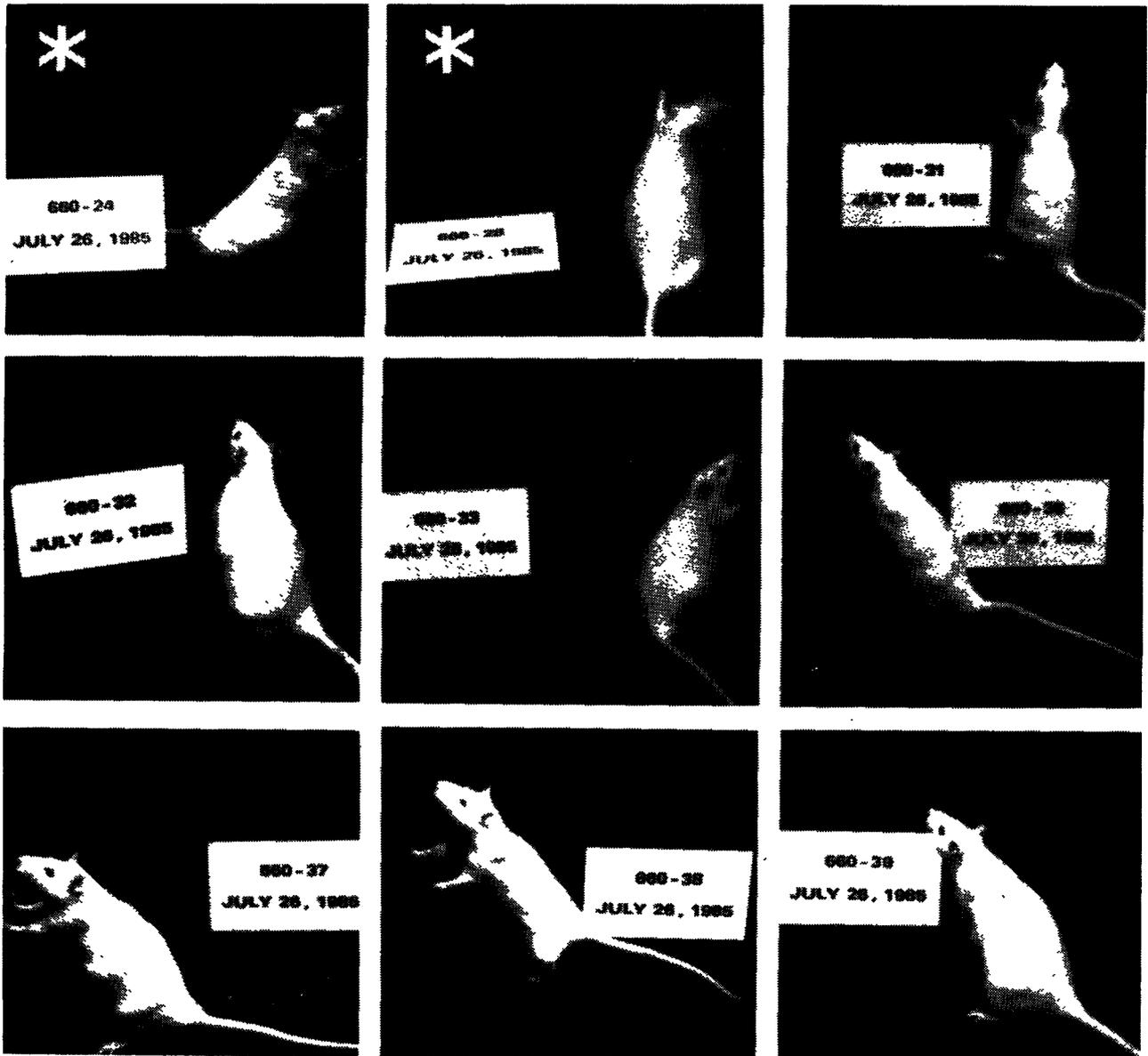


Fig. 5: Spontaneous standing activity of the animals, six months after the procedure. The asterisks indicate animals that needed to lean on the wall to steady themselves when standing (rated 4 on the behavioral scale).

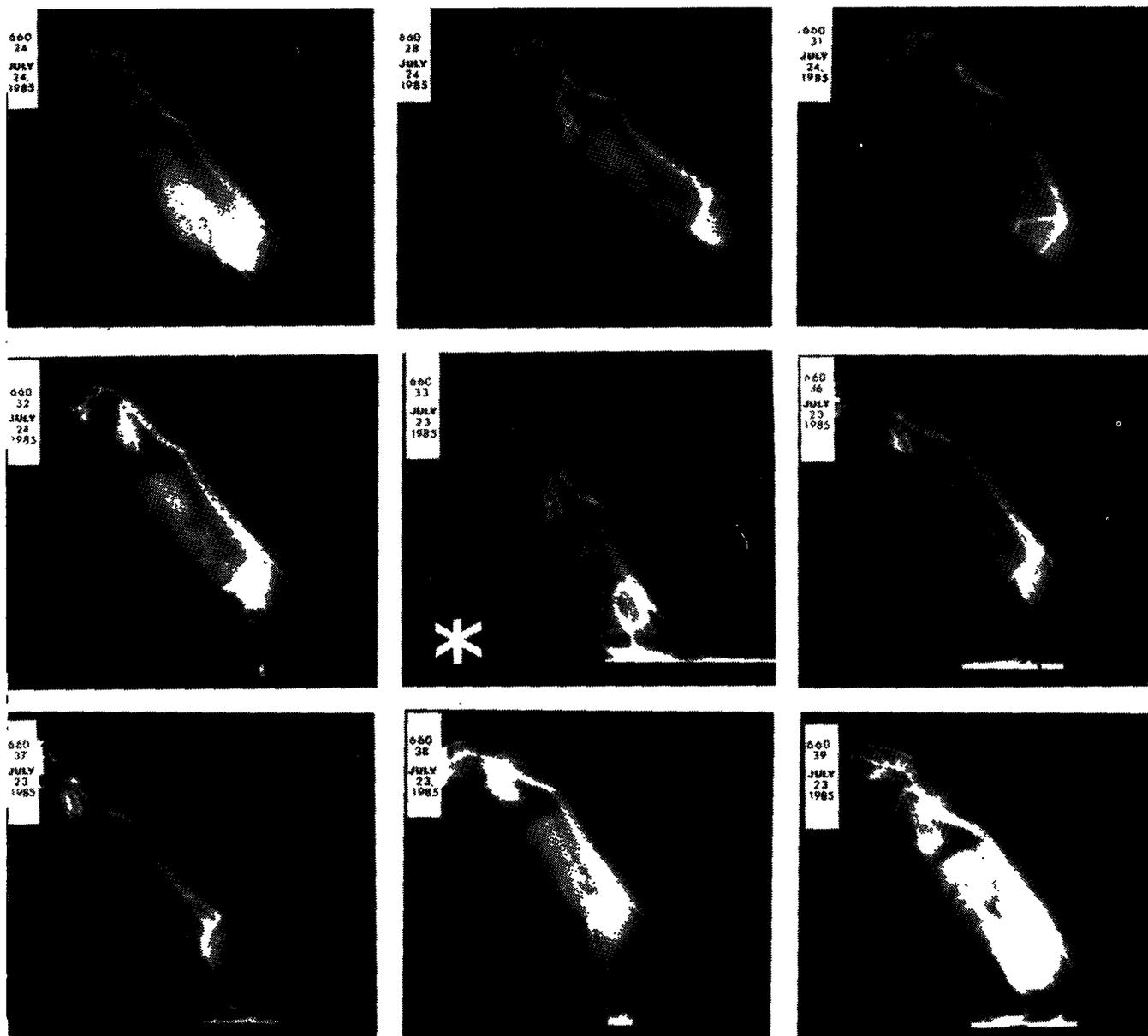


Fig. 6: Radiographic transverse view of unrestrained rats two to four months after the procedure. The asterisk indicates an animal that could walk and sit up but could not stand (rated 3 on the behavioral scale).

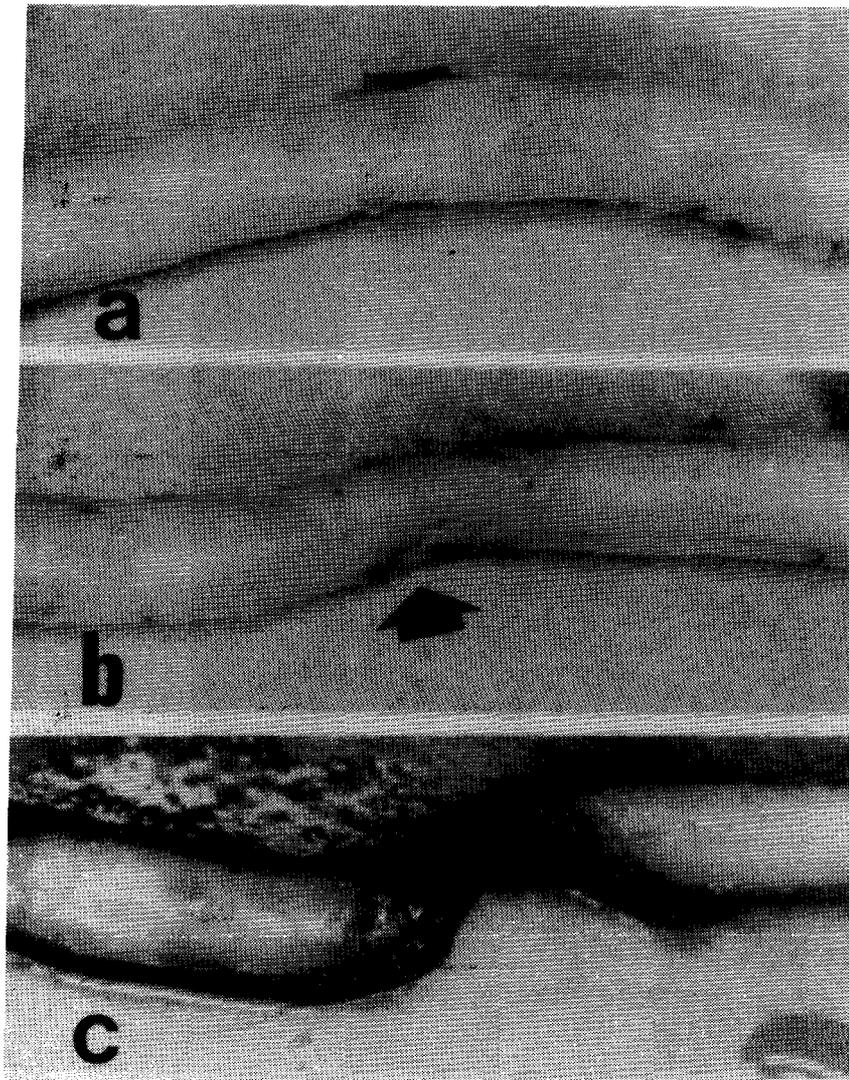


Fig. 7: External appearance of the cords. a) normal appearance (6 rats); b) slight localized compression (arrow) (1 rat); c) severe compression (2 rats). Compare this cord with the good functional level of the animal (center of Fig. 6 and 7).

Histology

Light microscopy of the cord showed excellent conservation of the neural structures in seven of the nine animals. In most of these cords, however, it was possible to find limited areas of neuronal death and fiber degeneration, probably corresponding to zones slightly traumatized during removal of the

bone (Fig. 8). These areas were characterized by spongiform changes and localized demyelination; reactive astrocytes, lipid-laden macrophages and chronic inflammatory cells were present; some neurons had degenerative changes. Meningeal sheaths were normal, as is usual with strictly extradural procedures /30/. Nerve roots and blood

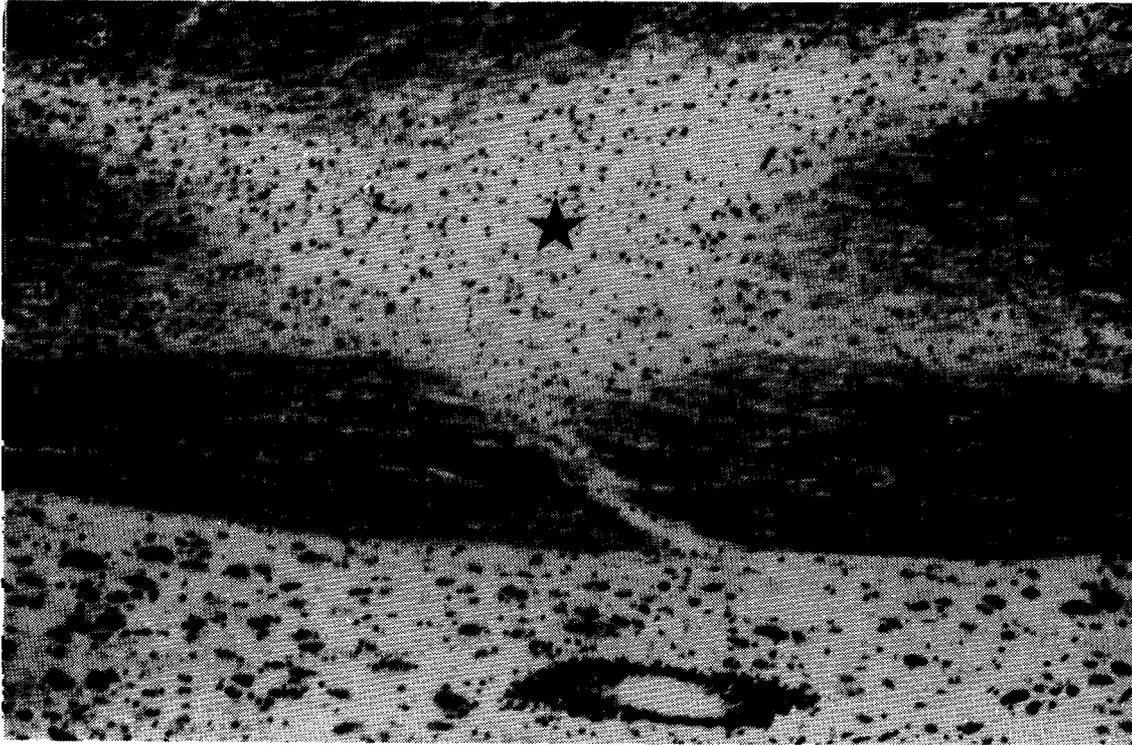


Fig. 8: Histologic appearance of the cords with moderate damage. A limited area of degeneration (star) is visible in the middle of normal fibers. The central canal (bottom) is not dilated (Bielschowsky stain).

vessels were normal. Cavitation was present in five of these seven animals. In one rat, cavitation was of the hydromyelic type, i.e. the central canal was dilated at some distance from the site of surgery (Fig. 9a). In two rats, cavitation was of the syringomyelic type, i.e. was located in the gray matter, without apparent connection to the central canal (Fig. 9b). In two animals, both abnormalities coexisted (Fig. 9c).

In the two rats with a laminated cord, gray matter had completely disappeared in the area of compression and no neurons could be seen. Cavitation was extensive: normal constituents of gray matter were replaced by a large cyst, which seemed to connect to the subarachnoidal space. Ribbons of normal-looking myelinated fibers

wandered from one extremity of the abnormal area to the other (Fig. 10). The percentage of surviving fibers thus bridging the gap was estimated to be 10% in one animal and 20% in the other.

Relationships between damage, histology and behavior

Operative trauma was correlated with the time interval to the first signs of functional recovery but not with the final degree of recovery of function. Operative trauma was not related to the occurrence of cavitation.

Cords that had not been traumatized had no microscopic parenchymal abnormalities; recovery

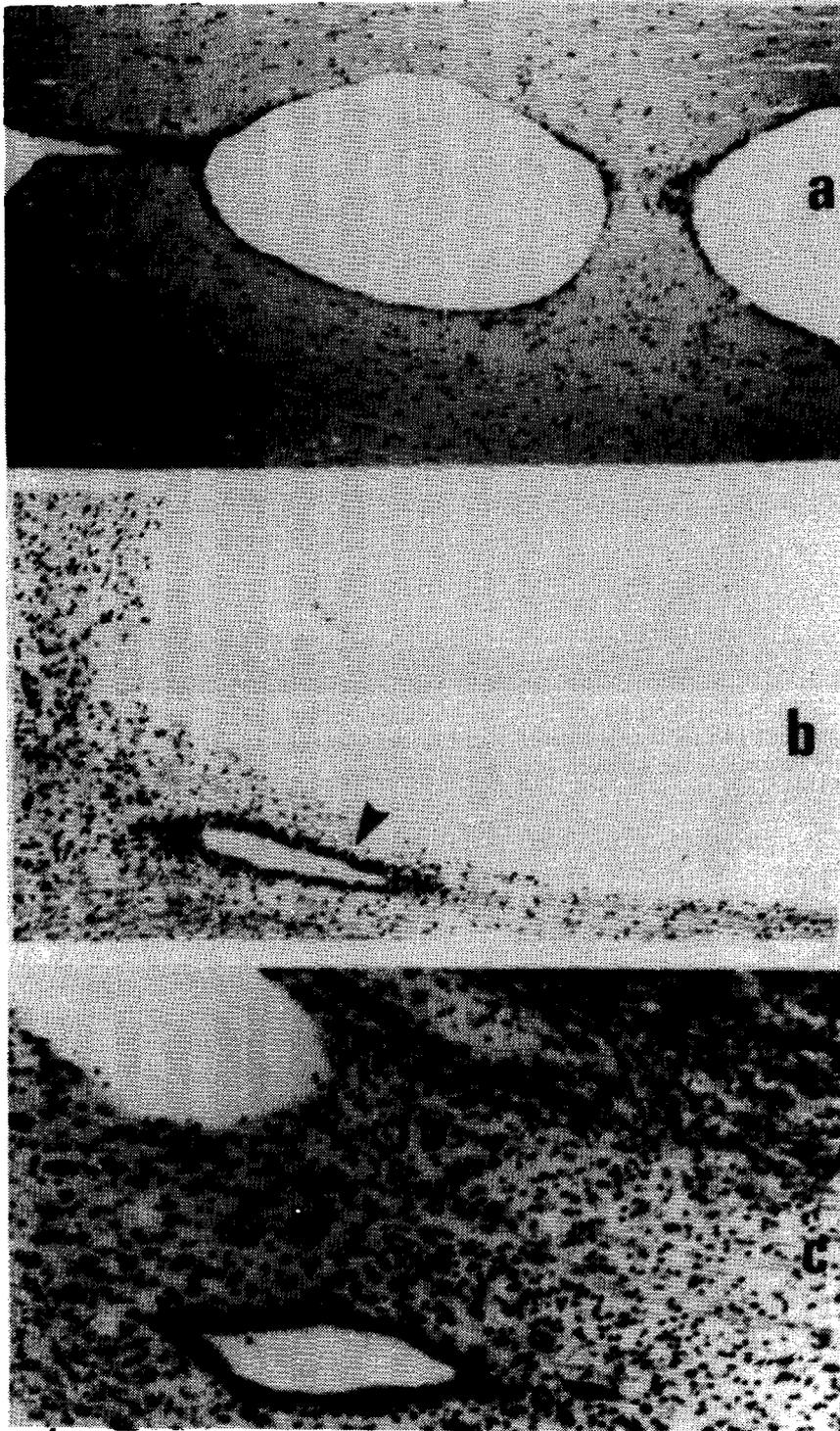


Fig. 9: Cavitation in the cords with no or moderate parenchymal traumatic damage. Cavitations were of the a) hydromyelic type: the central canal was dilated proximally and/or distally to the site of spondylectomy; b) syringomyelic type: the cavitation occurred in the gray matter. No connection with the normal central canal (arrow) could be found in these cases; c) hydro-syringomyelic type: both abnormalities coexisted (hematoxylin & eosin stain).



Fig. 10: Histologic appearance of a severely laminated cord. The area of compression was essentially occupied by a multilocular cyst, apparently ruptured in the subarachnoid space. Strands of normal fibers bridged the gap (hematoxylin & eosin stain).

was rapid and ultimate behavior was normal in these animals. Cavitation, however, was present in two of the four non-traumatized cases.

Cords that had been traumatized or compressed (Table 1, column 2) had moderate parenchymal damage. Recovery was slightly delayed, but was ultimately satisfactory. One of these five rats had no cord cavitation.

Finally, severe compression of the cord had evident histologic and behavioral consequences in 2 rats. Recovery was quite slow in the severely compressed cords but finally reached surprisingly good levels, of "3" and "4", even though only a fraction of the structure remained anatomically intact.

DISCUSSION

Methods

The procedure that was developed did not require the help of an assistant. Of the 13 rats initially operated, nine survived the operation and five of these showed essentially complete recovery. Thus, it is possible to perform thoracic spondylectomy in the rat without measurable permanent deficits from the procedure. Whether technical improvements would result in improved rates of survival or full recovery is not known.

Thoracic spondylectomy is more difficult to perform than lumbar spondylectomy because of the adhesion of pleura and mediastinal organs to ribs and column. Nevertheless, the thoracic level was selected for several reasons: i) normal mechanical stress was found to be less on the thoracic column than on the lumbar spine. Consequently, the chances of secondary displacements were fewer

after thoracic spondylectomy; ii) the ribs provided a good buttress for stabilization of the spine; iii) after complete cord lesion at the lower thoracic or lumbar level, hyperexcitability of the released lower portion can be strong enough to provoke spinal walking, especially when the bladder is distended or infected, or during defecation. This well-documented spinal activity /1,8,25,26/ differs from normal activity in that it is uncoordinated, erratic and usually disappears completely with cessation of the stimulus. It was thought preferable not to introduce this possibility of error into the behavioral assessment. Since spinal walking was not observed during pilot experiments in any of fifty rats with a mid-thoracic cord injury, the thoracic level was considered to be a more reliable location for spinal cord injury in terms of behavioral assessment.

Discussion of results

It is generally considered that "spinal shock", i.e. transient deficits of uncertain cause observed after trauma to the cord, is brief in lower species /5,9/. This was not confirmed by the present study: the duration of spinal shock was quite variable and seemed clearly related to operative trauma. Some of the animals that did not show signs of recovery for as long as 8-10 days showed essentially full functional recovery. However, the three animals with the longest durations of spinal shock, i.e., intervals to the first signs of recovery of 12, 20 and 25 days, did not show complete recovery. Therefore, in animals with long durations of spinal shock, the ultimate degree of functional recovery was incomplete. As reported by others /9,14,26/, a small number of intact fibers was sufficient to insure a surprisingly good level of function.

Cavitation was observed in seven of nine cords. This was an unexpectedly high incidence, since cavitation was not mentioned in previous studies of spine shortening (for example /13,23,31/). The development of cavitation in the cord could not be related to a single cause, and had no evident effect on the recovery of motor function. This is in agreement with clinical observations on syringomyelia and hydromyelia /3,17,22,24/.

In this preliminary study, the cord was not intentionally injured. The method presented here,

however, was designed to obtain close apposition of cord stumps after injury, and thus study the various factors that might influence the outcome of spinal cord injuries. Recent reports have analyzed some of the exchanges that occur between host and graft /27/, and especially the growth of blood vessels from the host to the transplant /19/. The method described here should make it possible to study the interface between two well-vascularized tissues and to determine the role of factors such as surface adhesion, trapping or ingrowth of foreign elements, edema, hemorrhage and vessel growth in CNS reactions to injury, wound healing, and methods of repair or treatment of CNS trauma.

ACKNOWLEDGEMENTS

We thank Susan Darlington and Courtney Davis for their excellent technical assistance.

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