A reproducible spinal cord injury model in the cat

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Allen's weight-drop method for producing experimental spinal cord injuries was improved by placing a curved stainless steel plate anterior to the spinal cord to provide a smooth, hard surface for the receipt of posterior cord impact. In addition, an electronic circuit was used to ensure that cord injury was produced by a single impact, thereby enhancing the reproducibility of the injury mechanism. Using a spinal cord injury model with these modifications, the author found that the recovery of hindlimb function and the histopathological appearance of the injured cord 6 weeks after upper lumbar injury were closely related to injury magnitude. The curve of functional recovery versus injury magnitude has a sharp transition centered at 10 gm \times 15 cm, and indicates that an injury of 10 gm \times 20 cm produces a "threshold" lesion suitable for the future evaluation of spinal cord treatment methods.

DEVELOPMENT of a reproducible model of spinal cord injury has been the object of much research since Allen first designed a technique for producing measurable experimental spinal cord trauma. He produced cord injuries by dropping a 30-gm weight from various heights onto the exposed dura of dog spinal cords. Other quantitative methods of injuring the spinal cord have been developed and include compressing the cord with epidural balloons, circumferential cuffs, weights, or aneurysm clips, and by spinal distraction. To be useful for the assessment of treatment methods, the injury technique should consistently produce a "threshold" lesion, that is, the minimum lesion that, without treatment, results in permanent paraplegia.

Allen's weight-drop technique, with various modifications, has been the most widely used method for producing experimental spinal cord injury. This technique has not found universal acceptance as a means of creating a reproducible lesion, however. Animals with apparently identical threshold injuries inflicted by Allen's technique do not all become permanently paraplegic, nor does the histological appearance of their chronically injured cords consistently relate to functional recovery. This communication describes a modification of Allen's method in which the recovery of hindlimb function and the histopathology of the spinal cord correlate well with the magnitude of the injury.

Materials and Methods

Thirty-three healthy adult male cats, each weighing 3.3 to 5.7 kg, were used for the development of this model. The experiments were carried out in two series. The first determined the approximate magnitude of the smallest injury that resulted in paraplegia of at least 6 weeks' duration. Eleven cats were used and the magnitudes of injuries ranged from zero to 10 gm \times 60 cm. The transition zone of the recovery versus injury magnitude curve was then studied with a second series of 22 cats in which injury magnitudes were varied between 10 gm \times 5 cm and 10 gm \times 35 cm.

Anesthesia

Animals were premedicated intramuscularly with xylazine (0.5 mg/kg) and atropine (0.02 mg/kg), anesthetized with ketamine (20 mg/kg intramuscularly), intubated, and ventilated with a Bird respirator and Bain circuit.* Anesthesia was maintained with 70% N\textsubscript{2}O, 30% O\textsubscript{2}, and 0.25% to 0.5% halothane. Fentanyl (2 \mu g/kg) and pancuronium (0.05 \mu g/kg) were given intravenously as required to ensure adequate anesthetic depth and muscle relaxation. Muscle relaxation was reversed at the end of the operation by an intravenous injection of neostigmine (0.1 mg/kg) and atropine (0.05 mg/kg).

*Bird respirator manufactured by the Bird Corp., Mark 3 Respirator Lane, Palm Springs, California. Bain circuit manufactured by Respirator Care, Inc., Toronto, Canada.
Once animals were anesthetized, arterial and venous cannulas were placed in the femoral vessels and, for animals in the second series, a Foley catheter (No. 8 French) was placed in the bladder through a small midline abdominal incision.

Monitoring

The arterial pressure waveform, mean arterial pressure, electrocardiogram, and heart rate were monitored with Hewlett-Packard monitors and displayed on an oscilloscope screen. The end-tidal CO$_2$ was monitored with a Beckman CO$_2$ analyzer and, together with blood gas analyses, was used to maintain the arterial pH at between 35 and 40 mm Hg. The animals' esophageal temperature was measured with a Yellow Springs 401 probe and held within the normal range with a heating blanket. The arterial waveform, mean arterial pressure, heart rate, end-tidal CO$_2$, and esophageal temperature were continuously recorded throughout the operative period on a six-channel Honeywell chart recorder.

Description of Injury Apparatus

The apparatus for producing the injury consists of an 80 cm-long brass tube (inner diameter 5.5 mm) supported by a micromanipulator, and vertically aligned with the aid of an attached bubble level. Warping of the tube is prevented by fastening it to a rigid piece of angled aluminum with precision-fabricated plastic gussets. A curved stainless steel plate, or anvil, fixed to the lower end of the brass tube, provides a solid support for the spinal cord and its meninges. A Teflon hammer (1.40 gm), or impounder, protrudes from the lower end of the tube and rests on the spinal cord (Figs. 1 and 2).

Spinal cord injuries are produced by dropping a 10-gm weight onto the hammer from a known height.

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† Hewlett-Packard 78200 series monitors manufactured by Hewlett-Packard Co., Rockville, Maryland.
‡ Beckman model LB-2 CO$_2$ analyzer manufactured by Beckman Instruments Inc., Fullerton, California.
§ Yellow Springs probe manufactured by Yellow Springs Instruments Co., Yellow Springs, Ohio.
¶ Honeywell MFE/1000 chart recorder manufactured by Honeywell Inc., Minneapolis, Minnesota.
Prior to being dropped, the weight is held by a pin inserted through holes drilled at 5-cm intervals along the length of the tube. The tube is vented through these holes, as well as by a groove in the Teflon hammer and by the space between the weight and the inner wall of the tube.

To ensure that the cord receives only a single blow, an electronic peak-detecting circuit monitors the inductance of a sensing coil at the lower end of the brass tube (Fig. 3). The center of the coil is positioned to coincide with the lowest point of travel of the falling weight. The inductance of the coil increases as the iron weight falls into it, and reaches a peak at the instant the weight reverses its direction of travel. This peak causes an electromagnet situated just above the sensing coil to be energized. Thus, after the first impact, the weight is pulled up by the magnet and held within the column, preventing further trauma to the cord.

Reproducibility of the impact was tested using an accelerometer.* The impact surface of the transducer head was covered with a 3-mm thick piece of Silastic to attenuate the deceleration to a value within the accelerometer’s measurement range. The 10-gm weight was dropped 10 times in random order from each 5-cm height between 5 and 60 cm and the root mean square (RMS) values of the decelerations were recorded. Mean values and standard deviations of the accelerometer readings were plotted as a function of drop height (Fig. 4). The coefficients of variation at each height ranged from 1.4% to 8.2%, which indicates that at each height, the deceleration (a measure of impact magnitude) is repeatable.

After each impact the weight became magnetized when it was caught by the pick-up magnet. To rule out the possibility that magnetic braking reduced impact magnitude, decelerations were measured using a magnetized weight and were compared with those obtained using a demagnetized weight. No differences between the two groups of readings were found.

Spinal Cord Injury Technique

With the animals under general anesthesia, the monitors were placed and a partial laminectomy was performed at either the L-1 or L-2 level. In addition to the spinous process, the central portion of the lamina and the middle third of the attached pedicles were removed as far anteriorly as the posterior surface of the vertebral body (Fig. 2). The superior and inferior facet joints were not disturbed and retained their attachments to the vertebral body via the remaining parts of the pedicles. The continuity of the vertebral column was, therefore, preserved. The lamina and pedicles were removed with Lempert rongeurs without disrupting the underlying ligamentum flavum and without touching the cord.

Following partial laminectomy, the ligament and the epidural fat were gently dissected away to expose the dura mater. The curved anvil was then carefully placed anterior to the cord between the dura and the vertebral body. The animal was fixed to the framework of the injury apparatus by two clamps attached to the spinous pedicles.

* Vibration meter Type 2511 manufactured by Bruel & Kjaer, Pointe Claire, Quebec, Canada, and accelerometer transducer Model 2215 manufactured by Endevco, Brampton, Ontario, Canada.
Spinal cord injury model

TABLE 1
Criteria for scoring motor recovery in cats

<table>
<thead>
<tr>
<th>Score</th>
<th>Motor Recovery</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>unable to walk</td>
</tr>
<tr>
<td>1</td>
<td>walks a few steps only</td>
</tr>
<tr>
<td>2</td>
<td>unlimited weak gait</td>
</tr>
<tr>
<td>3</td>
<td>walks with slight weakness</td>
</tr>
<tr>
<td>4</td>
<td>jumps up 3 feet</td>
</tr>
<tr>
<td>5</td>
<td>normal</td>
</tr>
</tbody>
</table>

processes adjacent to the laminectomy site. In this way, respiratory and cardiac movements of the vertebral column at the injury site were essentially eliminated. The vertically aligned brass tube with the 10-gm weight in place was then positioned over the curved anvil and carefully lowered so that the anvil support entered the hole in the anvil (Fig. 1). When fully engaged and the set screw tightened, the anvil rested firmly on the posterior surface of the vertebral body, providing a hard smooth immobile surface for cord impact (Fig. 2). After the injury, the apparatus was removed and the anvil was carefully rotated out from under the cord. The cord was inspected and its appearance noted. Finally, the dura was covered with a piece of Gelfoam to reduce scarring, and the incision closed in layers with interrupted Vicryl sutures.

To minimize experimental bias, the injury height was chosen at random just prior to producing the injury. In each series, the injury height was not revealed to the experimenter until all of the animals had been sacrificed.

Postinjury Management

Postoperatively, animals were kept in a heated cage until fully recovered from the anesthetic. They were then housed in groups of six in large animal pens. To prevent pressure sores, the floors of the pens were covered with fine wood shavings. Five cats developed small perineal ulcerations which in four cases healed in 2 weeks. Injury heights for these five animals ranged from 15 to 30 cm and none of them recovered any hindlimb motor function.

In the first series, bladders were emptied by daily catheterization or manual expression until spontaneous voiding occurred. Three cats required catheterization for the 6-week recovery period, while another three developed sphincter spasm to the extent that a suprapubic catheter had to be inserted. In the second series, a suprapubic catheter was inserted in each cat at the time of surgery and was removed when spontaneous voiding was established. All cats in this series were voiding spontaneously 3 weeks after injury, the onset being directly related to the degree of injury. Bladder care in the second series was less traumatic and more easily accomplished than in the first.

Intramuscular injections of penicillin (100,000 units) and streptomycin (0.125 mg) were given daily for 5 days, followed by daily oral administration of sulfisoxazole (Gantrisin, 125 mg). The Gantrisin was continued as long as a suprapubic catheter was in place or catheterization was required.

Four of the original 33 cats were removed from the study. Of these, three were sacrificed on the 9th, 13th, and 14th days after injury for problems related to catheterization. The fourth cat died of unknown causes on the 33rd day. These animals were injured with drop heights in the range of 20 to 40 cm. They had recovered no hindlimb function at the time of death.

Assessment of Recovery

Each animal was assessed daily for 6 weeks by an individual unaware of the magnitude of the injury. During that time, the 1st day that an animal was observed to elevate its hindlimbs and take a few steps was recorded. On the 42nd day after injury, hindlimb motor function of all animals was scored according to the criteria shown in Table 1.

Histological Study

Six weeks after injury, all animals were placed under general anesthetic and perfused through the left ventricle (inferior vena cava severed) with 500 to 700 cc of normal saline followed by 400 cc of 10% buffered formalin. The spinal cord from T-11 to L-4 was then removed and pinned to its full length in a bath of 10% buffered formalin. Blocks from the site of injury as well as from above and below the site were prepared for study by light microscopy and stained with hematoxylin and eosin and Luxol-fast blue.

Results

Immediately following injury, subdural hemorrhage at the impact site was clearly evident in all animals and appeared more extensive for the larger injury heights. No dural tears were seen after injury.

Mean values of basic physiological parameters immediately prior to injury are given in Table 2. Blood pressure changes peaked at 31 ± 8.7 seconds after injury and were quite variable in magnitude. There was no statistical relationship between the peak change in mean
TABLE 3
White matter preservation as a function of injury magnitude

<table>
<thead>
<tr>
<th>Injury Height (cm)</th>
<th>Normal-Staining White Matter (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
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<td>10</td>
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<tr>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

arterial pressure and the injury height (correlation coefficient: $r = 0.067$, not significant). Following the peak increase, arterial pressure gradually fell to slightly below preinjury levels approximately 30 minutes later. There were no hypotensive episodes during surgery.

Functional recovery, assessed 6 weeks after injury, is shown in Fig. 5. Control animals are represented at zero injury height. There is a sharp transition between permanent paraplegia and nearly normal recovery at an injury magnitude of 10 gm $\times$ 15 cm. No animal subjected to a larger injury walked even a few steps during the 6-week assessment period, whereas those with lesser injuries walked with only slight weakness or better.

The onset of functional recovery was also found to be directly related to injury magnitude. Uninjured control animals walked within 24 hours of surgery. Those injured by 5-cm drops walked on the 3rd or 4th day after injury. Animals injured by 10-cm drops first walked at between 8 and 16 days, while those injured by 15-cm drops either began to walk at between the 13th and 22nd postinjury day or did not walk at all.

Representative cord sections at the site of maximum injury are shown in Fig. 6. Destruction of the central gray matter was virtually complete in all injured animals, and white matter destruction increased toward the cord periphery with increasing injury magnitude. Cord Section A, from a control animal, was taken at the site of intended injury, that is, where the curved support plate was positioned. There is no damage evident in this section. Approximately one-half, one-third, and one-tenth of the visible cord tissue in Sections B, C, and D, respectively, are composed of normal-staining white matter. The small tissue rims seen in cord Sections E and F had no areas of myelin staining. For injury magnitudes in excess of 10 gm $\times$ 15 cm, the injury site was usually devoid of normal-staining cord tissue and often consisted of a large cystic cavity.

First-order estimates of white matter preservation, made without prior knowledge of injury height, are given in Table 3. The average area of normal-staining white matter at the site of maximum injury for each injury height was computed and expressed as a percentage of the area of white matter in control animals. No corrections were made for differences in cord size among animals.

These results indicate that with the modifications described in this study, the Allen weight-drop method is a suitable technique for creating reproducible experimental spinal cord injuries.

Discussion

Threshold Injury

The data in Fig. 5 suggest that a threshold injury is achieved by dropping a 10-gm weight 20 cm onto the exposed dura and spinal cord. A threshold injury in this model is defined as the smallest injury that consistently renders a cat unable to walk for at least 6 weeks. Other authors have found that an impact of 20 gm $\times$ 20 cm produces a threshold injury in the cat. The threshold injury magnitude in the cat reported by Dohrmann, et al., is not comparable to that reported in the present study as the weight and height were not specified separately. It has been shown that different injuries result from different height-weight combinations having the same product. Lewin, et al., reported injury magnitudes and functional recoveries similar to the results of this study.

Reduction of Variables

A curved supporting plate beneath the spinal cord reduces or eliminates a number of potential sources of variability in the production of spinal cord injuries. The anterior surface of the vertebral canal has small humps and hollows which may lead to uneven cord impact. The flat smooth surface of the supporting anvil elimi-
Fig. 6. Representative low-power photomicrographs of spinal cord sections taken from the site of injury. Section A is from a control animal. Sections B, C, D, E, and F are from animals injured by a 10-gm weight falling 5, 10, 15, 20, and 30 cm, respectively. H & E and Luxol-fast blue, × 10.
that a significant portion of the impact was expended in displacing the entire vertebral column when the latter was unsupported. In the present experiments, the diversion of impact energy to structures other than the cord was minimized by securing the anvil to the drop tube and allowing it to rest on the vertebral column, which in turn was clamped rigidly to the supporting framework.

Multiple impacts constitute another source of variation that has been eliminated from the current model. Cord compression due to the second impact has been reported by some to be 40% to 60% of that produced by the initial impact \(^{31,33,38}\) and by others to be insignificant.\(^{42}\) Clearly, elimination of this source of variation is important to the production of a reproducible injury. In agreement with the results of this study, Ducker, et al.,\(^{26}\) using a manual method to prevent multiple impacts, observed good correlation between cord destruction and behavioral recovery in monkeys after graded injuries.

### Assessment of Recovery

The scoring system used in this study is similar to that described by Goodkin, et al.,\(^{26}\) and Campbell, et al.\(^7\) It differs from other commonly used scoring methods\(^{20,48}\) in that assessments of sensation or non-walking voluntary movements are avoided. Sensory testing was found to be inconsistent in this and previous studies.\(^{10,19,20,34,35,49}\) Differentiation of reflex and voluntary movement was also found to be inconclusive in this study. For example, some animals in which the cords were found to be completely destroyed at the injury site made crawling movements with their hind legs and used their front legs as a fulcrum to elevate their hind quarters. It was difficult to distinguish this activity from voluntary movement. For the purposes of this model, unassisted elevation to the standing position and the completion of at least three steps serves as the minimum criterion for the existence of a functional connection along the injured spinal cord. It is likely that functional connections exist in many injured animals unable to walk, but the uncertainty in evaluating their function makes the above criterion suitable for the future study of treatment methods.

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### References

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