# The Influence of Severity of Spinal Cord Ischemia in the Etiology of Delayed-onset Paraplegia

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**To clarify the cause of delayed-onset paraplegia, the authors evaluated the neurologic outcome after temporary (10 to 30 minutes) spinal cord ischemia in the awake rabbit. Loss of motor function occurred in less than 2 minutes in all animals. Restoration of flow within 16 minutes always resulted in full return of function, whereas with occlusion times of greater than 27 minutes all animals remained paralyzed. After temporary occlusion of 20 to 21 minutes, however, 71% of animals returned to normal neurologic function but developed delayed-onset paraplegia 14 to 48 hours later. This appears to be a reliable method for the creation of a model of delayed-onset paraplegia in the awake animal, and will facilitate more detailed studies of the pathophysiology of ischemia-induced paraplegia.**

PARAPLEGIA AFTER REPAIR of extensive thoracoabdominal aortic aneurysms has been reported with a frequency as high as 30% in some series.[1,2] The usual cause of acute spinal cord dysfunction after thoracic aortic occlusion is believed to be spinal cord ischemia from hypoperfusion during cross-clamping. Some patients undergoing thoracoabdominal aneurysm repair, however, awake with no neurologic deficit only to develop delayed-onset paraplegia 1 to 5 days later. The cause of this latter phenomenon is poorly understood, but it has been attributed to postoperative hypotension, embolization to the anterior spinal artery, occlusion of a reimplanted intercostal artery, or anterior spinal artery thrombosis. The precise pathophysiology of this phenomenon, however, has not been identified. The observation of such delayed neurologic deficits suggests the presence of additional mechanisms of spinal cord injury that might include cord edema in a confined space, cytotoxic action from leukocytes or microglia, vasoconstriction from arachidonic acid metabolites, and free-radical injury from metabolic byproducts of ischemia.[3-7]

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No reliable animal model of delayed-onset paraplegia has been documented, although previous studies have noted the worsening of neurologic function 24 to 48 hours after spinal cord ischemia in the rabbit model.[8-12] Furthermore because of the anesthetic agents used in the experiments reported, the interrelationships of time of onset and recovery of motor and sensory dysfunction has been inadequately delineated.

We undertook this study to develop a reproducible model for the study of delayed-onset paraplegia in the awake animal and to identify the temporal relationships of motor and sensory changes induced by spinal cord ischemia.

## Materials and Methods

Thirty New Zealand white rabbits weighing 4 to 6 kg were premedicated with atropine sulfate (0.005 mg/kg) administered subcutaneously and were anesthetized with intramuscular ketamine hydrochloride (40 mg/kg) and xylazine (3 mg/kg). Intermittent intravenous readministration of one-quarter doses of the anesthetic agents maintained an adequate level of anesthesia and prevented the need for endotracheal intubation and mechanical ventilation. The fur on the chest, abdomen, and back was clipped with electric shears and the skin prepared with Betadine® (Purdue Frederick Co., Norwalk, CT) solution. In the supine position, with the pelvis rotated 45° to the right, an oblique incision was made from the left costal margin directed toward the pubis; this facilitated retroperitoneal dissection and exposure of the infrarenal aorta. The Dunn vascular occlusion device (Solco Basle, Inc., Rockland, MA) was placed around the aorta, immediately inferior to the renal arteries (Fig. 1). The occluder tubing was tunneled, exteriorized, and sutured into a transcu-

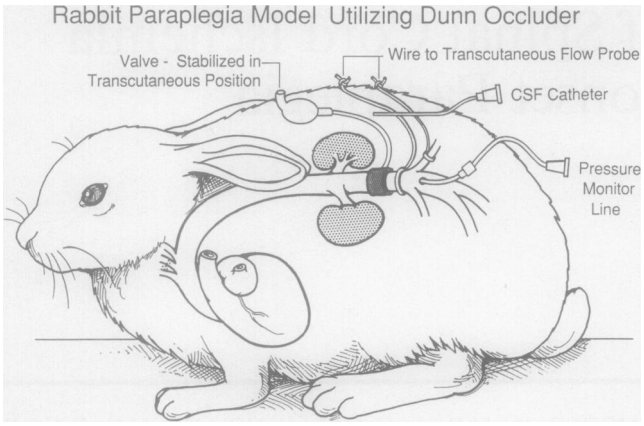


FIG. 1. Schematic diagram of experimental model. The aortic occluder device is positioned around the infrarenal aorta with the inflator valve stabilized in the transcutaneous position.

taneous position just to the left of the paraspinous muscles. The wound was closed in layers with absorbable fascial sutures and nonabsorbable skin sutures. A single dose of buprenorphine hydrochloride (0.1 mg/kg) was administered subcutaneously for postoperative pain control.

Confirmation of aortic occlusion during inflation of the occluder device and of subsequent restitution of distal aortic and lumbar arterial flow was obtained by the placement of electromagnetic and Doppler flow probes in the first five animals. An electromagnetic flow probe of appropriate size (2.5 to 3.5 mm) was secured 1 cm distal to the occluder device. A mini-Doppler flow probe was placed around an infrarenal lumbar artery. The leads from each of these devices were tunneled into a transcutaneous position and coupled to quantitative, directional flow monitors. This method of flow monitoring confirmed cessation of distal aortic and lumbar arterial flow during inflation of the occluder device and restitution of flow after release of the occluder in all five animals tested. Confirmation of return of distal flow after release of the occluder in the remaining animals was obtained by documenting return of femoral pulses.

Forty-eight hours after recovery from anesthesia, two leads of a neurosensory stimulation unit were attached by alligator clips to the skin of the hind limb, and the animal was stimulated every 5 seconds with the lowest voltage required to assess motor and sensory function. Aortic occlusion was then achieved by inflating the circum-aortic occluder with the rabbit in an awake and alert state. The exact times of loss of motor and sensory function after aortic occlusion (*i.e.*, onset of spinal cord ischemia) were recorded.

The aorta was occluded in 30 rabbits (48 trials) for a specified period (10 to 30 minutes), after which restitution of distal flow was permitted (Fig. 2). The neurosensory stimulation was repeated at 15-second intervals and the exact times of motor and sensory function recovery were recorded.

Some of the rabbits that underwent aortic occlusion for brief periods (10 to 16 minutes) experienced quite rapid neurologic recovery and were therefore selected for reocclusion after a period of rest of at least 7 days, resulting in a total of 48 trials in 30 rabbits.

The animals were killed after documentation of irreversible paraplegia or at the completion of the project. Killing was performed with a lethal intravenous dose of phenobarbital and potassium chloride. After death, representative animals from each group of clinical outcomes underwent detailed pathologic examination of the aorta, lumbar arteries, and spinal cord, the latter of which were fixed in formalin and examined microscopically after hematoxylin and eosin staining.

Animal handling, care, and disposal conformed to the guidelines set forth in the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication no. 86-23, revised 1985).

The data were subjected to standard statistical analyses using the nonpaired Student's t test, chi square, one-sided Fisher's exact test, and linear regression analysis.

## Results

### *Level of Ultimate Neurologic Function*

All trials of aortic occlusion resulted in spinal cord ischemia that led to total loss of motor and sensory function that persisted throughout the period of aortic occlusion (10 to 30 minutes). After restoration of blood flow, the clinical course of each animal was observed and could be categorized retrospectively into three groups. After brief periods of spinal cord ischemia (less than 17 minutes, group I, n = 11) the loss of hind limb neurologic function was reversible and resulted in permanent return of normal

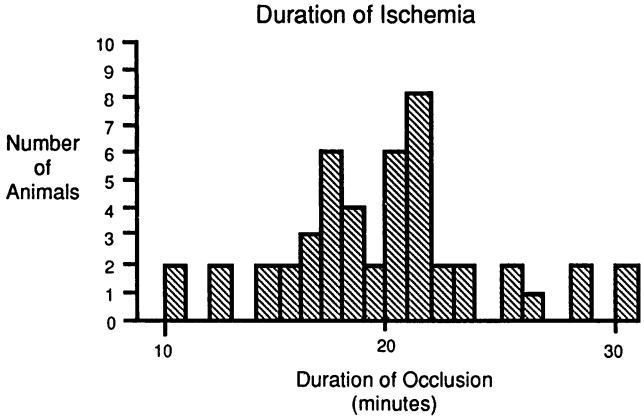


FIG. 2. Graph showing the number of trials for each specific time period of aortic occlusion.

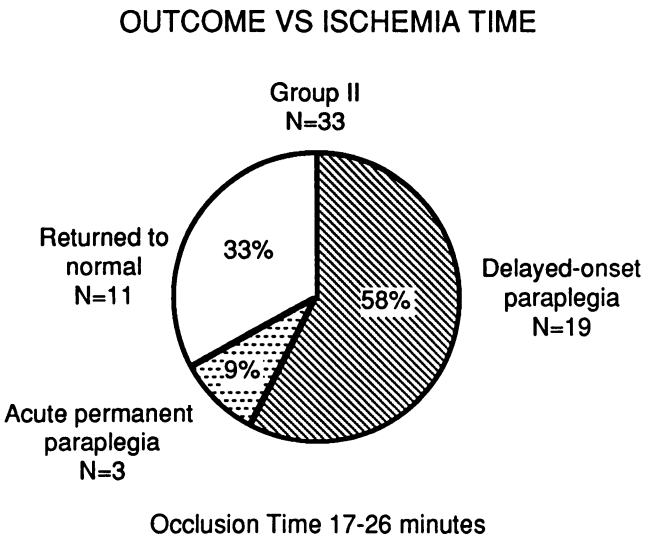


FIG. 3. Graph illustrating the neurologic outcome of the 33 animals that underwent occlusion times from 17 to 26 minutes. Fifty-eight per cent of these animals had return of normal neurologic function after occlusion but subsequently developed delayed-onset paraplegia.

neurologic function after reperfusion ($p < 0.001$). Loss of bladder and anal sphincter control was also noted, but likewise was reversible after brief periods of ischemia, corresponding to the recovery period of hind limb function.

After prolonged periods of spinal cord ischemia (more than 27 minutes, group III, n = 4) irreversible paraplegia was observed in all trials ($p < 0.05$).

Intermediate periods of spinal cord ischemia (17 to 26 minutes, group II, n = 33) resulted in three ultimate neurologic outcomes (Fig. 3). The first outcome was full return of neurologic function, as had been observed in all trials in group I. There were 11 of 33 trials (33.3%) that resulted in persistent, normal neurologic function after reperfusion. The second outcome was permanent, irreversible paraplegia noted in 3 of 33 trials (9%), as observed in all trials in group III. The third neurologic outcome observed in group II was delayed-onset paraplegia in 19 of 33 trials (57.6%). This subgroup of rabbits regained normal hind limb motor and sensory function, as well as normal bowel and bladder sphincter function after spinal cord reperfusion; however 14 to 48 hours (mean, 27 hours) after reperfusion, these rabbits developed delayed-onset, permanent paraplegia with loss of bowel and bladder sphincter control. Sensation of pain and coarse touch were retained in all animals experiencing delayed-onset paraplegia.

Linear regression analysis of these data confirm a direct relationship between the duration of spinal cord ischemia and ultimate neurologic outcome, correlation coefficient $r = 0.65$ ($p < 0.0001$). The incidence of delayed-onset paraplegia was highest after aortic occlusion periods of 20 and 21 minutes; therefore, this subgroup was subjected to independent analysis. After 20 to 21 minutes of ischemia, delayed-onset paraplegia occurred in 10 of 14 trials (71.4%), acute/permanent paraplegia in 2 of 14 trials (14.3%), and reversed/normal in 2 of 14 trials (14.3%) (Fig. 4). Linear regression analysis predicts an outcome of delayed-onset paraplegia after 20 to 21 minutes of spinal cord ischemia in over 70% of trials, with 95% confidence.

*Onset and Recovery of Neurologic Dysfunction*

The period between initiation of spinal cord ischemia and onset of motor and sensory dysfunction was analyzed. Because all 48 trials were identical with regard to this feature, pooled results from all three groups were analyzed. Loss of motor function occurred 10 to 120 seconds after aortic occlusion (mean, 55 seconds; n = 48). Loss of sensory function occurred 40 to 270 seconds after aortic occlusion (mean, 114 seconds; n = 32). Although there was variation in time until onset of deficits between the trials, motor loss preceded sensory loss in 100% of the trials. In an analysis of the means using an unpaired Student's t test, the time difference between onset of motor *versus* sensory loss is statistically significant ($p < 0.001$).

The time required for motor and sensory recovery in group II trials was analyzed in a similar fashion. The time required for full recovery of motor function ranged from 5 to 210 minutes (mean, 53 minutes; standard error, 11.4 minutes). The time required for recovery of sensory function ranged from 2 to 20 minutes (mean, 9 minutes; standard error, 1.7 minutes). Recovery of sensory function occurred before recovery of motor function in 100% of the trials, and the difference in the time interval required for recovery of sensory *versus* motor function is significantly different ($p < 0.001$) (Fig. 5).

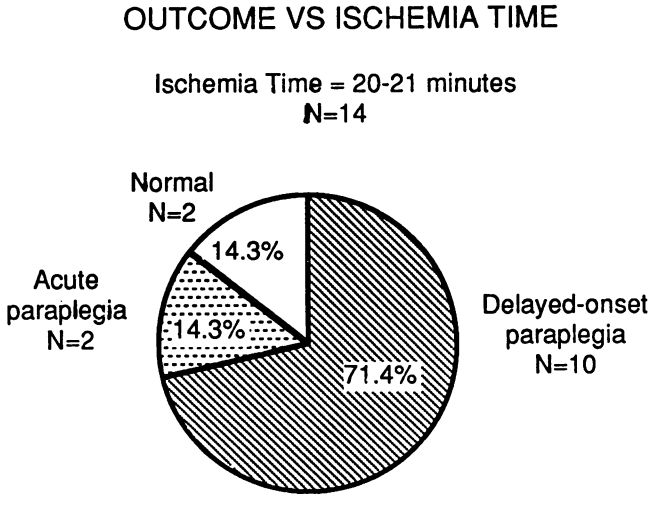


FIG. 4. Graph showing the incidence of delayed-onset paraplegia associated with a spinal cord ischemia time of 20 to 21 minutes.

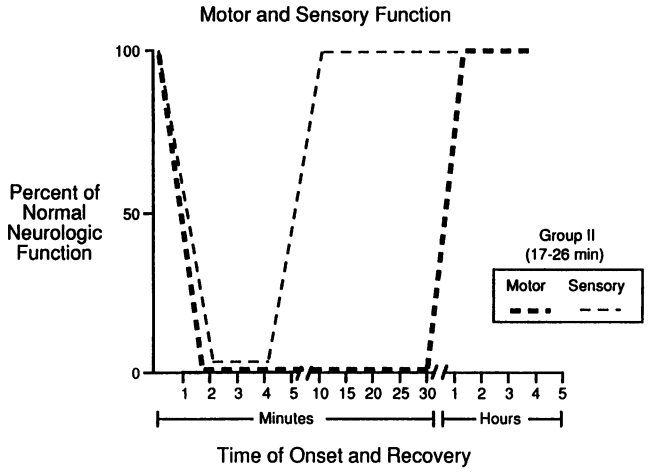


FIG. 5. Graph showing the relative time of onset and recovery of motor *versus* sensory function associated with spinal cord ischemia in the Group II animals.

An analysis of means was conducted between groups I and II with regard to recovery of motor function. The mean time required for full recovery of motor function in group I was 27 minutes (all animals reversed to normal), and in group II was 53 minutes (33% reversed to normal; 58% reversed to normal, then subsequently experienced delayed-onset paraplegia; and 9% experienced acute, permanent paraplegia.) (Fig. 3). The difference between 27 and 53 minutes is significant ($p < 0.01$). Therefore the time required for return of full motor function after temporary spinal cord ischemia is predictive of resultant neurologic outcome.

### *Pathologic Findings*

Pathologic examination of the animals after death was undertaken specifically to determine if paraplegia or delayed-onset paraplegia was related to thrombosis (acute or late) of the aorta or the lumbar arteries. In no animal was there any evidence of thrombosis of these vessels nor was there any evidence of embolization.

Histologic examination of the spinal cord permitted comparison of normal cord with the spinal cord of rabbits experiencing each of the three potential neurologic outcomes. Specimens of rabbits experiencing sustained recovery of neurologic function showed normal numbers of ganglion cells with normal nuclei and cytoplasm. Evaluation of spinal cord specimens from rabbits experiencing delayed-onset paraplegia showed decreased staining of ganglion cells. Many of the ganglion cells identified had hyperchromatic nuclei and mild to moderate vacuolization of cytoplasm. Diffuse destruction of gray matter with moderate to severe vacuolization and essentially no normal ganglion cells was observed in the spinal cord of rabbits experiencing acute, permanent paraplegia.

## Discussion

In the human, as well as in most animal models, prolonged clamping of the thoracic aorta can result in paraplegia. Because of the tremendous variability in the adequacy of collaterals to the spinal cord, however, the predictability of neurologic outcome after aortic cross-clamping is poor; this also makes prediction of the severity of neurologic injury difficult. Therefore a reliable model of defined ischemic spinal cord injury would be very helpful in studying the pathophysiology of both acute and delayed-onset paraplegia.

Other problems complicating attempts to study the variables associated with paraplegia are the marked hemodynamic and other physiologic changes associated with thoracic aortic cross-clamping. Because the rabbit has a segmented arterial supply to the spinal cord, occlusion of the infrarenal aorta will result in spinal cord ischemia, but is associated with minimal hemodynamic variation. Furthermore allowing the rabbit to recover after implantation of the occluder device permits evaluation of the responses to spinal cord ischemia in the awake model so that both sensory and motor neurologic changes can be assessed.

It is interesting to note that in these experiments, motor function was always lost before sensory loss, and sensory return always occurred before return of motor function. This may reflect an increased sensitivity to ischemia of anterior horn cells and motor tracts when compared with dorsal columns. We made no attempt to determine if blood flow during the time of ischemia differed from one area to another within a given segment of ischemic cord, although we did document such differential blood flows in the dog model.[13]

When one compares the time required for return of motor function after unclamping between those animals that developed delayed-onset paraplegia and those that recovered and maintained normal neurologic function, the difference is significant ($p < 0.01$). The mean time until motor recovery in those animals that developed delayed-onset paraplegia (59 minutes) was two times greater than the motor recovery time of those that had permanent recovery (25 minutes).

Sensory recovery did occur in all animals, even in those that sustained acute permanent paraplegia, but return of sensory function occurred later in those that developed acute paraplegia (20 minutes) than in those that experienced permanent recovery (8.5 minutes). This observation did not reach statistical significance because of the small sample size. It would appear, however, that the clinical usefulness of these findings is negligible.

This study clearly documented that paraplegia in the rabbit could be caused by spinal cord ischemia after as little as 10 seconds, but was reliably reversible after as

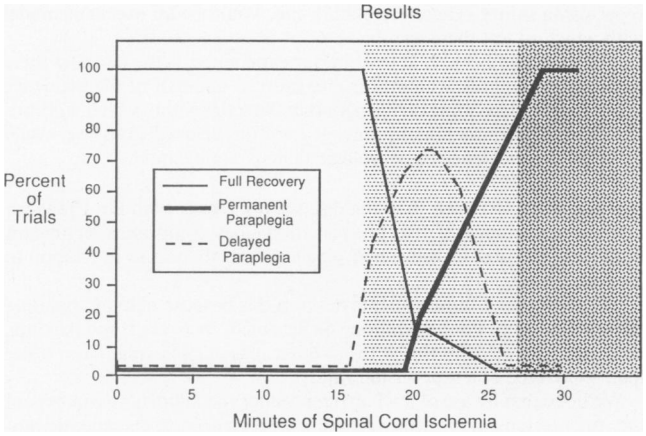


FIG. 6. Schematic graph illustrating the relationship of ultimate neurologic outcome in relation to duration of spinal cord ischemia.

much as 16 minutes of ischemia. It also showed that prolonged spinal cord ischemia in the rabbit, in other words, more than 27 minutes, will reliably produce acute, permanent paraplegia.

An intermediate level of spinal cord ischemia in the rabbit, in other words, 20 to 21 minutes, will usually result in full return of neurologic function after restoration of blood flow to the spinal cord, and the rabbit will be able to hop normally and will show no functional change; however, this is often (71%) followed by the onset of recurrent and progressive paraplegia within 14 to 48 hours (Fig. 6). The observation of delayed-onset paraplegia in this model is important because it documents that this phenomenon is primarily related to the duration or severity of the initial ischemic event and is not likely due to thrombosis or embolization of spinal arteries, nor to postoperative hypotension, as has been occasionally suggested in cases of delayed-onset paraplegia in humans. It is clear that, at least in the rabbit model, delayed-onset paraplegia can occur without antecedent thrombosis or hemodynamic dysfunction.

The precise cause of delayed-onset paraplegia remains unresolved. One must postulate, however, that cellular and metabolic dysfunction initiated by ischemia, as well as delayed hyperperfusion with resultant cord edema in a closed space, may all have a role in causing delayed-onset paraplegia.[14]

This paper documents a reliable method for the creation of delayed-onset paraplegia in the awake animal. This model will facilitate more detailed studies of the pathophysiology of ischemia-induced paraplegia and will allow the evaluation of those therapeutic modalities that may be beneficial in reducing the incidence of this dreaded surgical complication.

## Acknowledgments

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## References

1. Crawford ES, Crawford JL, Hazim, et al. Thoracoabdominal aortic aneurysms: preoperative and intraoperative factors determining immediate and long-term results of operation in 605 patients. J Vasc Surg 1986; 3:389–404.
2. Crawford ES, Svensson LG, Hess KR, et al. A prospective study of cerebrospinal fluid drainage to prevent paraplegia after high risk surgery on the thoracoabdominal aorta. J Vasc Surg 1991; 13: 36–46.
3. Hollier LH. Protecting the brain and spinal cord. J Vasc Surg 1987; 5(3):524–528.
4. Chen ST, Hsu CY, Hogan EL, et al. Thromboxane, prostacyclin, and leukotrienes in cerebral ischemia. Neurology 1986; 36:466–470.
5. North RJ. Concept of activated macrophage. J Immun 1978; 121: 806–809.
6. Giulian D. Ameboid microglia as effectors of inflammation in the central nervous system. J Neurosci Res 1987; 18:155–171.
7. Norris DA, Weston WL, Sams WM. The effect of immunosuppression and anti-inflammatory drugs upon monocyte function in vitro. J Lab Clin Med 1977; 90:569–580.
8. Zivin JA, DeGirolami U. Spinal cord infarction: a highly reproducible stroke model. Stroke 1980; 11:200–202.
9. Zivin JA, DeGirolami U, Hurwitz EL. Spectrum of neurological deficits in experimental CNS ischemia. A quantitative study. Arch Neurol 1982; 39:408–412.
10. Cheng MK, Robertson C, Grossman RG, et al. Neurological outcome correlated with spinal evoked potentials in a spinal cord ischemia model. J Neurosurg 1984; 60:786–795.
11. Robertson CS, Foltz R, Grossman RG, et al. Protection against experimental ischemic spinal cord injury. J Neurosurg 1986; 64: 633–642.
12. Jacobs TP, Shohami E, Baze W, et al. Deteriorating stroke model: histopathology, edema, and eicosanoid changes following spinal cord ischemia on rabbits. Stroke 1982; 18:741–750.
13. Bower TC, Murray MJ, Gloviczki P, et al. Effects of thoracic aortic occlusion and cerebrospinal fluid drainage on regional spinal cord blood flow in dogs: correlation with neurologic outcome. J Vasc Surg 1989; 9(1):135–144.
14. Hollier LH, Marino RJ. Thoracoabdominal aortic aneurysms. *In* Vascular Surgery: A Comprehensive Review, 3rd Edition. Philadelphia: WB Saunders, 1990, pp 295–303.

### DISCUSSION

DR. H. EDWARD GARRETT (Memphis, Tennessee): Dr. Crawford has led the way in the management of this devastating lesion and Dr. Hollier has continued to seek explanations for the dreaded complication of paraplegia.

Dr. Hollier now presents an experimental model to study further ischemia of the spinal cord and perhaps lead to preventive therapy.

We are all greatly indebted to these members of the Southern Surgical Association for their efforts and their contribution.

DR. E. STANLEY CRAWFORD (Houston, Texas): This model that Dr. Hollier and his group have developed provides an excellent opportunity to try many of the options available to determine if the incidence of paraplegia can be reduced. One of the most truly disappointing char-