■ LABORATORY REPORT

Anesthesiology
1996; 85:1184-9

Intrathecal Catheterization in the Rat

Improved Technique for Morphologic Analysis of Drug-induced Injury

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Background: **The authors previously described an *in vivo* model suitable for investigation of functional impairment induced by intrathecally injected local anesthetic. However, meaningful histologic analysis could not be performed because catheterization, *per se*, induced morphologic changes in control animals. In the current experiments, the authors sought to identify an alternative, less reactive, catheterization technique for intrathecal drug administration.**

Methods: **Twenty-five rats received an intrathecal infusion of normal saline through a catheter composed of either 28-gauge polyurethane, 32-gauge polyimide, 32-gauge polyurethane, PE-10 polyethylene, or PE-10 polyethylene that had been stretched to twice its original length. Seven days after infusion, sensory function was assessed using the tail-flick test, and the spinal cord and nerve roots were prepared for neuropathologic evaluation.**

Results: **There was no significant difference in sensory function among groups. Animals in which 28-gauge polyurethane, 32-gauge polyimide, PE-10, and double-stretched PE-10 had been implanted had moderate to severe nerve injury in 11%, 14%, 23%, and 8% of fascicles, respectively, whereas none of the animals in which 32-gauge polyurethane was implanted had any evidence of moderate or severe damage.**

Conclusions: **Morphologic changes induced by intrathecal catheterization in the rat can be minimized by the use of 32-gauge polyurethane tubing. (Key words: Anesthetic techniques, spinal. Catheterization, intrathecal: rat. Complications, neurologic: cauda equina. Pathology, nerve injury: axonal degeneration.**

RECENT reports of major and minor neurologic sequlae occurring after spinal[1-7,‖,#] and epidural[8,9] anesthesia highlight substantial gaps in the current understanding of local anesthetic neurotoxicity. Study of neurotoxic damage to the spinal cord and nerve roots can be facilitated by the development of more effective experimental models. Modifying well-validated techniques for intrathecal catheterization, drug delivery, and sensory assessment in the rat, we previously described an *in vivo* model that closely parallels clinical injury.[10] However, intrathecal catheterization induced moderate to severe morphologic damage in control animals, precluding meaningful histologic analysis and suggesting cautious interpretation of functional data. In the current experiments, we sought to identify an alternative, less reactive, catheterization technique.

Materials and Methods

These studies were approved by the Committee on Animal Research of the University of California, San Francisco. All experiments were conducted on male Sprague-Dawley rats (200-300 g).

Catheter Material

A scalpel was used to cut segments of catheter composed of either 28-gauge polyurethane (28-PU; Micor, Allison Park, PA; n = 6), 32-gauge polyimide (32-PI; TFX Medical, Duluth, GA; n = 5), 32-gauge polyurethane (32-PU; Micor, Allison Park, PA; n = 6), PE-10 polyethylene (PE-10; Becton Dickinson, Sparks, MD; n = 3), or PE-

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Received from the Department of Anesthesia, University of California, San Francisco, California. Submitted for publication February 23, 1996. Accepted for publication June 28, 1996. Supported in part by the Committee on Research of the Academic Senate, University of California, San Francisco, and the National Institutes of Health (grant GM51887).

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‖ FDA Safety Alert: Cauda equina syndrome associated with the use of small-bore catheters in continuous spinal anesthesia, May 29, 1992.

Fenerty J, Sonner J, Sakura S, Drasner K: Transient radicular pain following spinal anesthesia: Review of the literature and report of a case involving 2% lidocaine. Int J Obstet Anesth 1996; 5:32-5.

10 tubing that had been stretched to double its original length (DS-PE-10; n = 5). The outer (OD) and inner diameters (ID) of the catheters, as determined by a measuring microscope (Mitutoyo TM-500, Tokyo, Japan), were as follows: (28-PU: 0.014″ OD × 0.007″ ID); (32-PI: 0.009″ OD × 0.0072″ ID); (32-PU: 0.0107″ OD × 0.005″ ID); (PE-10: 0.023″ OD × 0.0105″ ID); and (DS-PE: 0.0174″ OD × 0.0076″ ID). The manufacturing tolerance for the 32-PU catheter is 0.01 ± 0.001″.

Surgical Procedure

Rats were anesthetized by intraperitoneal injection of methohexital (40–60 mg/kg), and the catheter was introduced into the subarachnoid space using a previously described modification[10] of the method of Yaksh and Rudy**: catheters were passed through a slit in the atlanto-occipital membrane and advanced 11 cm, to lie with the tip caudal to the conus medullaris. (A 0.003″ stainless steel stylet was used to facilitate placement of 32-PI and 32-PU catheters.) Animals were allowed to recover for 24 h before the study began. Rats that exhibited any evidence of sensory or motor dysfunction were excluded from the study.

Functional Measurement

To assess sensory function, the tail-flick test was performed, as described previously.[10] To prevent tissue damage, the heat stimulus was terminated if no response occurred by 8 s (cut-off).

Experimental Protocol

Twenty-five rats received an intrathecal infusion of preservative-free normal saline (Abbott Laboratories, North Chicago, IL). The animals were placed in a horizontal acrylic restraint, and baseline tail-flick latency was assessed immediately before infusion. Infusions were administered *via* a mechanical infusion pump at a rate of 1 μl/min for 4 h (the longest period of infusion used in our previous studies). A segment of calibrated polyethylene tubing was inserted between the syringe and the intrathecal catheter, and the infusion was monitored by observing the movement of a small air bubble within the tubing. Animals were evaluated for alteration in tail-flick latency 7 days after infusion.

Tissue Preparation

Animals were killed by injection of an overdose of pentobarbital 7 days after infusion. They were perfused intracardially with a phosphate-buffered glutaraldehyde-paraformaldehyde fixative. The spinal cord and nerve roots were dissected out, immersed in the same glutaraldehyde solution used for perfusion fixation, and embedded in glycol methacrylate. The embedded tissue was sectioned at the conus medullaris, 6 mm rostral, and 6 and 12 mm caudal, using a Sorvall JB-4 microtome (1-μm transverse sections). The tissue stained with hematoxylin-eosin or was treated with 4% osmium tetroxide and stained with toluidine blue. Histopathologic evaluation was performed, using light microscopy, by a neuropathologist blinded to the intrathecal catheter implanted, the results of sensory function testing, and the solution infused (histologic sections from these experiments were interspersed with specimens obtained from other studies in which local anesthetic was administered).

Table 1. Nerve Injury Scoring System

Score	Category	Description
0	Normal	No edema; no injured nerve fibers
1	Mild	Edema; little or no nerve fiber degeneration or demyelination
2	Moderate	Less than 50% of nerve fibers with degeneration and demyelination
3	Severe	More than 50% of nerve fibers with degeneration and demyelination

Data Analysis

Functional Assessment. Tail-flick latencies at the proximal, mid, and distal portions of the tail were averaged to give a mean tail-flick latency. Mean baseline tail-flick latencies for the five groups were compared using one-way analysis of variance. To assess the effect of catheter type on sensory function, average tail-flick latencies were converted to percent maximal possible effect, calculated as {(tail-flick latency − baseline)/(cut-off − baseline)} × 100, and compared using one-way analysis of variance.

Histologic Analysis. Specimens obtained 12 mm caudal to the conus were used for quantitative comparison of catheter-induced damage. This region was chosen because cross sections obtained below the conus have a greater number of fascicles and because preliminary studies suggested that catheters produce more damage in this area. Each fascicle present in the cross-section was assigned an injury score of 0–3, where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe injury (table 1). The injury score for each cross-section

** Yaksh T, Rudy T: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 17:1031–6.

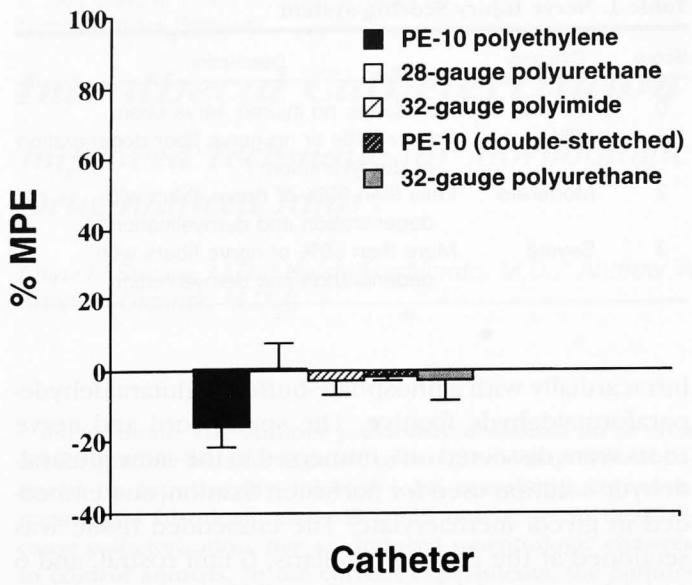

Fig. 1. Sensory function 7 days after a 4-h intrathecal infusion (1 μl/min) of normal saline through a catheter composed of either PE-10 polyethylene, 28-gauge polyurethane, 32-gauge polyimide, double-stretched PE-10, or 32-gauge polyurethane. Tail-flick latency values were calculated as the average of latencies for the proximal, mid, and distal portions of the tail, and are expressed as percent maximum possible effect, where percent maximal possible effect = {(tail-flick latency − baseline)/(cut-off − baseline)} × 100. Data reflect the mean ± SEM. There was no difference in percent maximal possible effect among the five catheters.

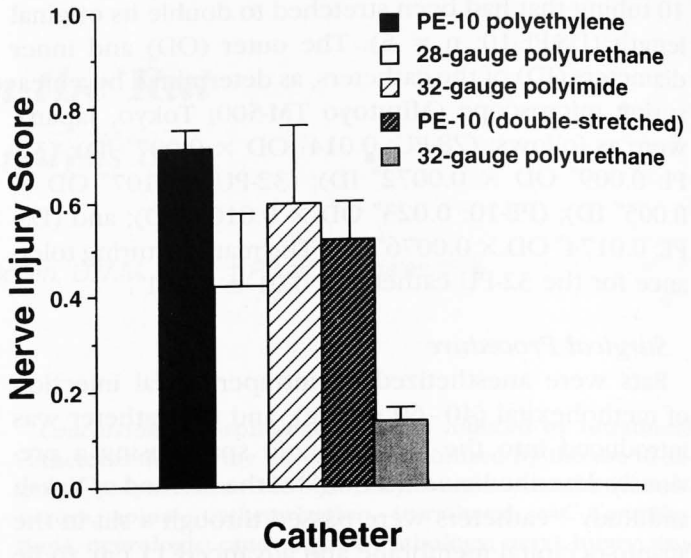

Fig. 2. Nerve injury score for sections obtained 12 mm caudal to the conus medullaris, 7 days after an intrathecal infusion of normal saline through a PE-10 polyethylene, 28-gauge polyurethane, 32-gauge polyimide, double-stretched PE-10, or 32-gauge polyurethane catheter. Nerve injury scores were based on all fascicles present in each cross section. Each fascicle was assigned an injury score of 0–3, where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe injury. The injury score for each cross-section was calculated as the average score of all fascicles in the section. Data reflect the mean ± SEM. Animals in which 32-PU catheters had been implanted had lower nerve injury scores than those with other catheters; this difference was statistically significant for all groups except those receiving 28-PU catheters.

was then calculated as the average score of all fascicles present in the cross section. These data were compared using the Kruskal-Wallis test and Dunn's test. For all comparisons, $P < 0.05$ was considered significant. Injury scores and percent maximal possible effect were compared using simple linear regression; a correlation coefficient was calculated using the least squares method.

Results

Neurologic Function

There was no significant difference in baseline tail-flick latencies for the five groups. When assessed on day 7, sensory function did not differ significantly from baseline or among groups (fig. 1).

Histopathologic Findings

Sections obtained from animals in which 28-PU, 32-PI, PE-10, DS-PE-10, and 32-PU catheters had been implanted demonstrated moderate to severe injury in 11%, 14%, 23%, 8%, and 0% of fascicles, respectively. Animals in which 32-PU catheters had been implanted had lower nerve injury scores than those with other catheters; this difference was statistically significant for all groups except those that received 28-PU catheters (fig. 2). Representative sections from animals implanted with 32-PI, DS-PE-10, and 32-PU catheters are presented in figures 3, 4, and 5, respectively. The correlation coefficient for percent maximal possible effect and nerve injury score was 0.078.

Discussion

In 1976, Yaksh and Rudy described a method to implant PE-10 tubing in the subarachnoid space of small animals.[**] As originally detailed in the rat, catheters were passed through the atlanto-occipital membrane and advanced 8.5 cm, to lie with the tip at the rostral portion of the lumbar enlargement. This simple, yet effective, technique permitted intrathecal injection in the intact,

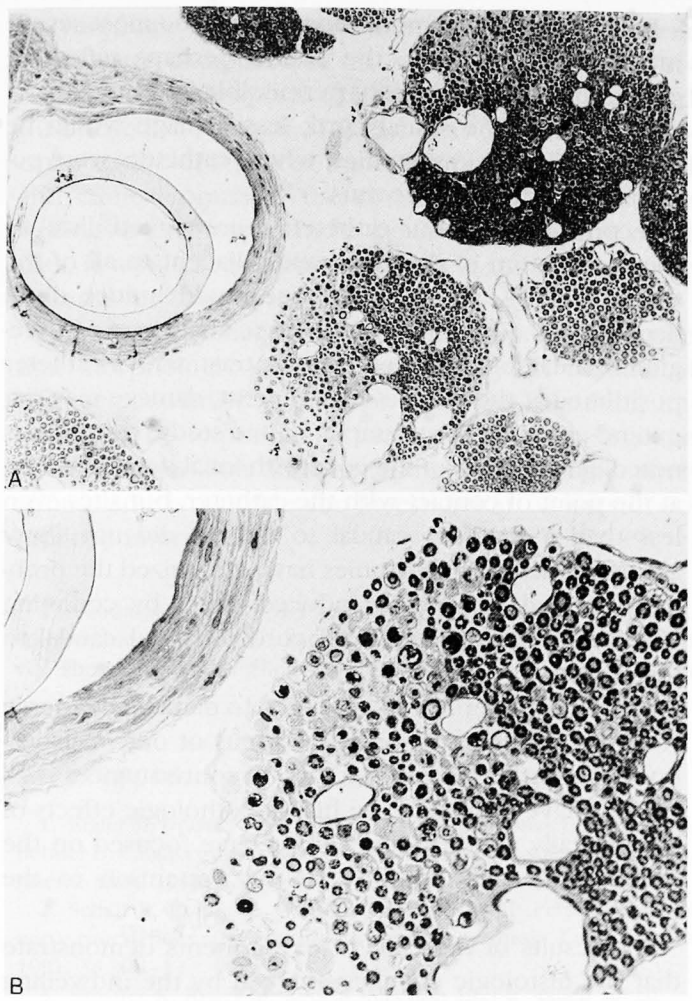

Fig. 3. Transverse sections obtained 7 days after intrathecal administration of normal saline through a 32-gauge polyimide catheter. Stained with toluidine blue: (*A*) 100×; (*B*) 250×. An intense fibrotic reaction encircles the catheter (upper left in both figures). Degeneration and demyelination are evident in multiple fascicles.

unanesthetized animal. In addition, the surgical incision, located at the head of the animal, avoided a more caudal incision and spinal surgery, an added advantage for study of centrally administered agents on spinal cord function.

We recently modified this implantation technique to study the neurotoxic effects of spinally administered anesthetic.[10] Our goal was to develop an *in vivo* model that would closely parallel recent clinical injuries and permit investigation of the mechanism of, and the factors that affect, local anesthetic neurotoxicity. Preliminary experiments had shown that bolus injections through a catheter at the lumbar enlargement produced highly variable and often extensive levels of anesthesia. We found that, by placing the catheter tip among the nerve roots of the cauda equina and by administering anesthetic by continuous infusion, we could produce a more consistent, restricted block. A limited distribution was necessary to model clinical exposure and to minimize the potential hemodynamic, respiratory, and central nervous system effects that might accompany more extensive blockade.

In our initial studies, we demonstrated that local anesthetic administered intrathecally to achieve a restricted distribution could produce persistent sensory deficit,[10] and that toxicity was unaffected by the presence of glucose.[11] However, histologic analysis was not informative because catheterization induced significant mor-

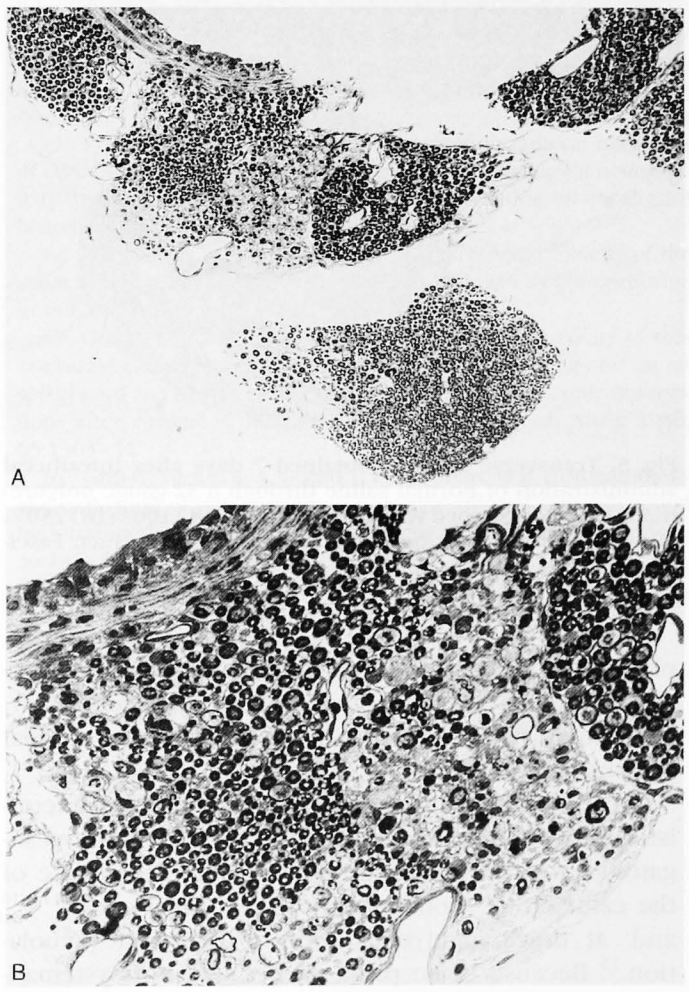

Fig. 4. Transverse sections obtained 7 days after intrathecal administration of normal saline through PE-10 tubing that had been stretched to twice its original length. Stained with toluidine blue: (*A*) 100×; (*B*) 250×. Section of fragmented catheter tract is evident at top of figures. Injury is present in multiple fascicles.

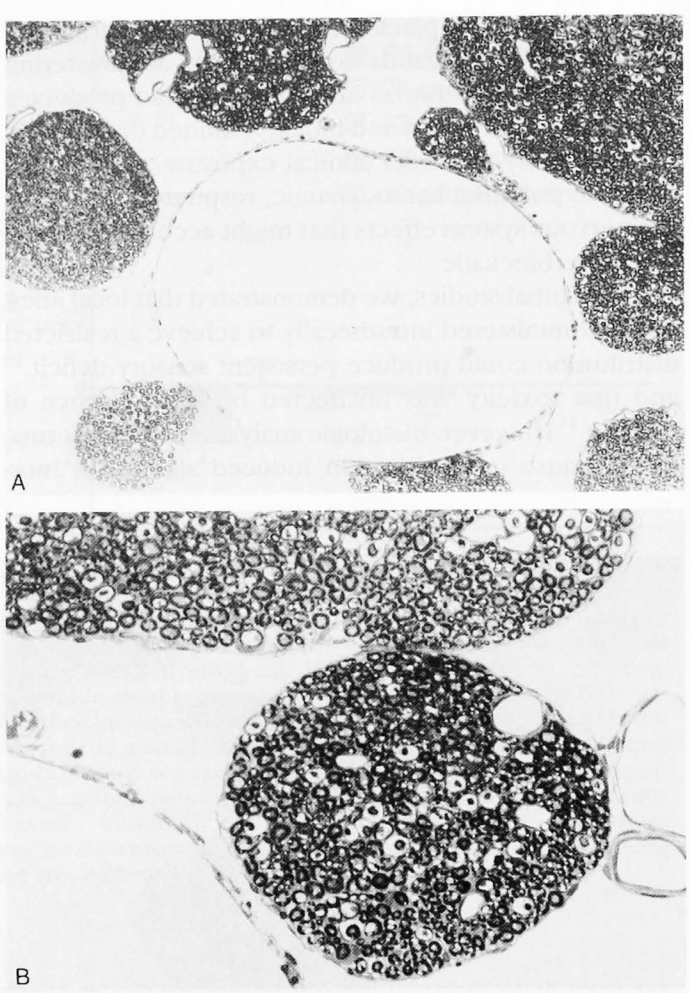

Fig. 5. Transverse sections obtained 7 days after intrathecal administration of normal saline through a 32-gauge polyurethane catheter. Stained with toluidine blue: (*A*) 100×; (*B*) 250×. Minimal fibrotic reaction delineates the catheter tract. Fascicles appear relatively normal.

phologic changes in control animals. In addition, subclinical damage could potentiate drug-induced injury, resulting in an overestimate of the neurotoxic potential of anesthetic solutions.

Chronically implanted intrathecal catheters characteristically induce damage. For example, previous investigators have noted reaction surrounding the course of the catheter,[12-15] compression of the spinal cord,[12,14-16] and, at times, demyelination[12] or neuronal vacuolation.[14] Because these prior studies had not systematically characterized or quantified catheter-induced damage, we could not directly compare our findings; however, the morphologic effects we observed were perhaps more evident, and certainly more problematic, than those encountered by previous investigators:

First, injury in our model appeared to be most severe in the region distal to the conus, perhaps reflecting greater catheter movement permissible in an area unencumbered by the spinal cord; less damage would be expected in previous studies, where catheters were positioned rostral to the conus.

Second, because our catheters were placed distal to the termination of the cord, and adjacent to all of the terminal roots, proximal damage could induce distal degradation, confounding histologic analysis even in regions caudal to the catheter. In contrast, with a catheter positioned at the lumbar enlargement, damage may not extend caudally. For example, in one study, researchers noted deformation of the cord with local demyelination at the point of contact with the catheter, but sites even less than a segment caudal to the tip did not show abnormalities.[12] Some studies have minimized the problems created by catheter-induced injury by confining analysis to sections of spinal cord obtained caudal to the catheter.[17]

Finally, catheterization appeared to damage the nerve roots preferentially, a principal focus of our investigations of anesthetic neurotoxicity. In contrast, most studies that have evaluated the histolopathologic effects of intrathecally administered agents have focused on the spinal cord, with minimal, if any, attention to the roots.[13,16,17]

The results of the current experiments demonstrate that the histologic changes induced by the indwelling catheter can be minimized by the use of 32-gauge polyurethane tubing. Unlike all other catheters tested, this catheter did not produce any moderate or severe fascicle damage (*i.e.*, there was no significant demyelination or fiber degeneration that could obscure assessment of drug-induced damage). Because "subclinical" injury might alter the behavioral response to intrathecal compounds, the use of similar tubing also should be considered for functional studies of intrathecally administered agents.

Our results also suggest that both size and composition are important factors that affect the histologic reaction to an indwelling intrathecal catheter. The 32-gauge polyurethane catheter induced less damage than a larger bore catheter of similar material, and less damage than a catheter of similar size but different composition (*i.e.*, polyimide). Whether this difference is due to the material, *per se,* or a secondary effect, such as the flexibility of the catheter, remains to be determined. Of note, reactivity varied among catheters that have been marketed for continuous spinal anesthesia (*i.e.*, the

polyurethane and polyimide tubing used in the current study were composed of material identical to the Preferred and Microspinal (Richmond Hill, Ontario, and Rüsch Medical, Duluth, GA) catheters, respectively). Because of the differences that exist between our model and clinical practice, the clinical relevance of these findings is unknown. However, in the absence of other data, it would seem prudent to select the least reactive material for clinical use.

In summary, the current experiments demonstrate that morphologic damage induced by an intrathecal catheter depends, in part, on the type of tubing implanted. The experiments identify a tubing, 32-gauge polyurethane, that induces minimal histologic alterations, and is suitable for investigation of anesthetic-induced injury.

The authors thank Linda Prentice, for many helpful discussions, Stephen Brushey of Micor, Inc., for generous supplies, and Winifred von Ehrenburg, for excellent editorial advice.

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