Numerous studies have shown that antioxidant treatment prior or subsequent to central nervous system (CNS) injury ameliorates functional and histologically identified damage.\textsuperscript{15,37} Oxidative stress is thought to play a major role in physiological dysfunction and cell death following various types of trauma.\textsuperscript{31} Cells contain endogenous free-radical scavengers such as superoxide dismutase and glutathione to protect against damage from free radicals generated by normal cellular respiration.\textsuperscript{16} Under traumatic conditions, excessive free radicals are formed, overwhelming cellular defense mechanisms. Free radicals attack polyunsaturated fatty acids of the lipid membranes, producing various aldehydes (for example, malonaldehyde and 4-hydroxynonenal [HNE]).\textsuperscript{11} These substances damage proteins by forming an aldehyde–protein complex, compromising protein function by altering its secondary and tertiary structure. Unlike free radicals, aldehydes are long lived and can diffuse from the site of origin to attack distant intra- and extracellular proteins.\textsuperscript{40} One of the most toxic of these aldehydes is HNE, which may be a more specific parameter for lipid peroxidation than malonaldehyde.\textsuperscript{19,33,40}

Endothelial cells of the CNS vasculature form a continuous wall joined by tight junctions, with a high reflective coefficient and electrical resistance that prevent virtually all water-soluble substances from entering the brain or spinal cord parenchyma. This unique vascular characteristic provides protection for the CNS from neurotoxins circulating in the blood as well as maintaining disparate ion concentrations in the extracellular space of the brain/spinal cord and vascular plasma.\textsuperscript{18,24} Breach of the blood–spinal cord barrier (BSCB) is one of the hallmarks of spinal cord trauma and has been described in animal models.\textsuperscript{25,26,29} Compromise of the BSCB may exacerbate oxidative stress to injured spinal cord tissue by creating isch-
emic conditions and allowing toxic levels of plasma glutamate and iron-bearing molecules into CNS parenchyma, all of which have been shown to play a role in the generation of free radicals. 

In this study we used a widely accepted animal model for spinal cord trauma and a spinal cord injury device developed at New York University (NYU) to examine the timing and distribution pattern of possible HNE/protein complex and immunoglobulin G (IgG) by conducting immunocytochemical studies of tissue sacrificed after a moderate spinal cord contusion. Horseradish peroxidase (HRP) was injected into the animals to visualize the time course of relative BSCB compromise after contusion by using the NYU model. Breach of the blood-CNS barrier induces lipid peroxidation, and free radicals may in turn exacerbate this breach by damaging vascular endothelial cells. In light of prior experimental evidence of the relationship between blood-CNS barrier breach and oxidative stress, the hypothesis tested in the present study is that there will be evidence of oxidative stress and that a similar staining pattern between HNE/protein complexes and extravasated IgG will be seen.

**Materials and Methods**

**Animal Preparation**

Young adult female Fischer 344 rats, each weighing 200 to 225 g, were anesthetized with sodium pentobarbital (50 mg/kg) and received a spinal cord contusion at the T-10 level. Before injury a T-10 laminectomy was performed to expose the dorsal surface of the spinal cord without disturbing the dura mater. The animals were placed in an NYU spinal cord injury device, which consisted of a free-falling 10-g rod with a flat 2.5-mm-diameter brass tip that was dropped from a height of 25 mm, impacting the spinal cord. After injury the rats were removed from the device and the wound was sutured closed.

Four groups of animals were injured. The first three groups were perfused with 4% paraformaldehyde and the fourth group was killed but not perfused. The first group received 160 mg/kg HRP Type II and the animals were killed at 1, 2, and 6 hours, and 1, 2, and 7 days (five animals/time point). Five additional animals were injected with HRP 10 minutes before contusion and killed 1 hour postinjury. Control animals (five rats) received HRP 10 minutes prior to a sham procedure (T-10 laminectomy with no contusion injury). These animals were killed 1 hour after the laminectomy. To distinguish HRP reaction product from possible endogenous plasma peroxidases, 50-μm-thick horizontal sections from injured animals that did not receive HRP were tested for reaction to 3,3′-diaminobenzidine for 5 minutes. Spinal cords were cut in horizontal sections spanning T6–L1 at multiple levels of the gray matter (Fig. 1). The HRP-injected spinal cord and control sections were coded and evaluated in a blind fashion, each animal receiving a score of 0 to 5, with 0 indicating no stain and 5 denoting maximum stain.

The second group of animals was used for HNE/protein and IgG immunocytochemical examination. The rats were killed at 1, 3, and 6 hours, and 1, 2, and 7 days (five animals/time point). Thirty-micrometer horizontal sections spanning T6–L1 were assessed by using the well-characterized monoclonal anti-HNE/protein complex antibody 1g4h7 (1:100) and an anti–rat IgG biotinylated monoclonal antibody (1:40,000). The IgG antibody was used for the 2-day time point only. A standard immunocytochemical protocol was performed and is described elsewhere.

The third group of animals (five injured and five sham-injured rats) was killed 2 days postinjury and used in immunocytochemical evaluations for HNE/protein and IgG after their spinal cords had been sectioned transaxially (50-μm slices) at T-6, T-8, T-9, T-11, T-12, and L-1. Care was taken to ensure that segments from each spinal cord level were equidistant from the contusion epicenter rel-

**Image Analysis**

Transaxial sections were obtained in animals killed 2 days after spinal cord injury, stained with anti-HNE/protein complex and anti–rat IgG antibodies, and analyzed by using software developed at the National Institutes of Health (NIH) (Image 1.57; NIH, Bethesda, MD). At the beginning of each analysis session, the microscope lighting and NIH program were calibrated by using a densitometry slide standard to ensure consistent lighting conditions and linearized densitometry readings. For analysis, the gray matter was divided into three regions: Areas 4, 5, and 6 contained dorsal, ventral, and lateral white matter tracts, respectively. Three sections from each of the six spinal cord levels (T-6, T-8, T-9, T-11, T-12, and L-1) were analyzed for intensity of the antibody stain by using multiple 625-μm² zones within each area (Fig. 2). The dot blots were scanned into an image file and individual mean densitometry readings were taken for each dot. The doublets for each spinal cord section were averaged and used for statistical analysis.

**Cell Counts**

The HNE/protein positive neurons were counted in three sections...
Results

All injured animals manifested bilateral hindlimb paralysis with modest improvement of function by 7 days postinjury. Computer-recorded parameters from this weight-drop model revealed that the mean velocity at the impact site was 0.668 ± 0.003 m/second (± the standard error of the mean [SEM]). The mean compression was 1.918 ± 0.035 mm and the mean compression ratio was 0.462 ± 0.006. No animal undergoing the sham procedure (T-10 laminectomy with no contusion) showed obvious hindlimb motor impairment postsurgery.

Immunocytochemical Evaluation Using HNE

One hour after contusion, immunolabeling in horizontal sections was evident at the T-10 level throughout both gray and white matter. A few neurons were observed above control levels in the T-10 region. By 6 hours postinjury, the stain had spread to T-9 and T-11, showing an increase in HNE/protein–positive neurons throughout these spinal cord levels. Some axonal staining was observed and a few immunopositive axonal retraction balls were evident at the T-10 level. By 1 day postinjury the noncellular stain had spread to T-8 and T-12. There was an increase in the number of axons and axonal retraction balls at T9–11 (Fig. 3) as well as stained neurons in T8–12. Maximum stain intensity at the time points tested was evident in sections obtained in animals killed at 2 days, although the spatial distribution of the immunolabeling was the same as in the 1-day spinal cord tissue. Small cells that were morphologically consistent with glia were evident in both white and gray matter at this time point. By 7 days postinjury small portions of the T-10 area were missing because of histological processing artifacts of the frozen sections. Staining was found only in the area of T-10. A few HNE/protein–positive neurons were seen in this region, although no axon or glia staining was observed. The staining pattern at levels T-6 and L-1 was the same for all time points and was identical to that found in sham-injured animals (Fig. 4C).

On Day 2 spinal cords in sham-injured animals as well as control animals showed very light neuronal staining throughout all levels of the cord (T6–L1) without remarkable staining of nonneuronal tissue. There was no obvious difference between sham-injured and control rats. This indicates either some nonspecificity of the HNE/protein antibody or modest levels of naturally occurring HNE/protein complexes caused by low levels of lipid peroxi-

Sources of Supplies and Equipment

The HRP Type II, ethylenediamine tetraacetic acid, and 3,3′-diaminobenzidine were obtained from Sigma Chemical Co., St. Louis, MO, and the antirat IgG antibody from Vector Laboratories, Burlingame, CA. The dot-blot apparatus and nitrocellulose paper densitometry slide from Olympus Instruments, Melville, NY; and the 4-

Statistical Analysis

For densitometry readings and cell counts, a series of one-way analysis of variance (ANOVA) tests were conducted, and Neuman–Keuls’ posthoc analyses (p < 0.05) were performed only if the appropriate ANOVA was significant (p < 0.05). The same statistical tests were used for dot-blot data analysis. The HRP data were analyzed by using a Kruskal–Wallis ANOVA followed by Mann–Whitney U-tests.

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Transaxial sections showed numerous immunopositive neurons throughout the gray matter (Figs. 4A and 5). In all white matter regions at the T-9 and T-11 levels, darkly stained small round structures (3–5 μm) were evident (Fig. 3). Sections from these areas were counterstained with cresyl violet, which revealed that these dark round structures were not glia. On the basis of the axonal staining pattern seen in longitudinal sections, these dark structures are probably crosscut axons.

The image analysis results and statistical analysis are found in Table 1. The most intensely staining spinal cord level was T-11 for all regions examined (p < 0.05). Evaluation by spinal cord level revealed less stain intensity at T-8 and T-12 when compared with levels closer to the con-

Fig. 3. Upper: Photomicrograph showing HNE/protein–positive staining in axons and axon retraction balls (arrow) in a longitudinal section of lateral white matter tracts of the T-10 level 2 days after spinal cord contusion. Lower: Photomicrograph of a transaxial section manifesting numerous cross-cut HNE/protein–positive axons in the ventral white matter tracts (white arrows) at the T-11 level 2 days postinjury. The antibody also stains some white matter glia cells (black arrows). The HNE/protein complexes in the axons indicate possible impairment of protein functions such as ion pumps or key metabolic enzymes, possibly leading to physiological dysfunction. Bar = 100 μm.
tusion site (T-9 and T-11, p < 0.05). Both T-11 and T-12 manifested more intense staining when compared with T-9 and T-8 (p < 0.05), indicating an asymmetrical pattern of oxidative stress relative to areas rostral and caudal to the impact site. Levels most distal to the point of contusion (T-6 and L-1) were indistinguishable from those in sham-injured animals. Comparing the six areas within the T-11 spinal cord level revealed the most intense stain in Area 1 (p < 0.05). At levels T-9 and T-12 the staining in Area 1 was modest although not significantly greater than in Areas 2 and 3. In general, gray matter (Areas 1–3) and dorsal white matter (Area 4) stained significantly more intensely than ventral (Area 5) and lateral white matter (Area 6) at levels T-9 and T-12 (p < 0.05).

There was a significantly greater number of HNE/protein–positive neurons (p < 0.05) in all three regions examined in the spinal cord levels T-9 and T-11 compared with T-8 and T-12 (p < 0.05, Table 2). However, levels T-6 and L-1 were indistinguishable from sham-injured animals. The percentage of cresyl violet–stained neurons labeled with the HNE/protein antibody also indicates a proximal–distal graduated oxidative stress response relative to the injury site as well as rostral–caudal asymmetry. Immunodot-blot assay revealed that all three levels in the injured animals stained significantly more intensely than in sham-injured rats (p < 0.05) and both T-10 and T-11 were significantly darker than T-9 (p < 0.05, Table 3).

**Horseradish Peroxidase Staining**

Kruskal–Wallis ANOVA tests revealed a significant statistical effect among the postsurgery time intervals (p < 0.05). Assessment of HRP staining is summarized in Fig. 6.

**Immunocytochemical Examination Using IgG**

Transaxial sections obtained in animals killed 2 days postinjury and stained with the anti–rat IgG antibody showed a diffuse staining pattern throughout the neuropil as well as some immunopositive neurons and glia (Fig. 4B). The image analysis results of the IgG spatial intensity staining pattern (Table 4) were very similar to that seen with HNE staining.
Staining With HNE/Protein and IgG Antibody in Control Animals

Horizontal sections from nonperfused, immersed fixed tissue stained with anti–rat IgG revealed intense reaction product throughout the vasculature as well as staining in the neuropil immediately surrounding all blood vessels. The HNE/protein antibody–treated sections exhibited no vascular stain and were identical to those from sham-injured perfused animals. This result indicates that the HNE/protein antibody does not cross react with endogenous IgG. It also indicates that there is no detectable level of naturally occurring HNE/protein in plasma with this antibody. The immunopositive axons and axon retraction balls observed in the HNE/protein–stained sections were absent from the IgG-stained sections. This indicates that the anti–rat IgG antibody does not cross react with the HNE/protein complex.

Discussion

The HNE/Protein Reaction Product

The progressive nature of the HNE/protein reaction product from 1 hour to 2 days indicates an ongoing lipid peroxidation cascade during this time interval and possible secondary injury to the spinal cord tissue rostral and caudal to the injury site. The HNE/protein immunoposi-

Table 1: Optical density of HNE/protein stain in areas within the spinal cord of rats 2 days after a T-10 contusion

<table>
<thead>
<tr>
<th>Spinal Cord Level</th>
<th>T-6</th>
<th>T-8</th>
<th>T-9</th>
<th>T-11</th>
<th>T-12</th>
<th>L-1</th>
<th>Sham T-11†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1</td>
<td>127 ± 3</td>
<td>107 ± 21†</td>
<td>137 ± 16</td>
<td>262 ± 22‡</td>
<td>154 ± 10‡</td>
<td>71 ± 2‡</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Area 2</td>
<td>73 ± 2</td>
<td>101 ± 9‡</td>
<td>116 ± 12</td>
<td>186 ± 14‡</td>
<td>134 ± 5‡</td>
<td>64 ± 2‡</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>Area 3</td>
<td>71 ± 2</td>
<td>101 ± 10‡</td>
<td>126 ± 16</td>
<td>232 ± 23‡</td>
<td>144 ± 6‡</td>
<td>66 ± 3‡</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Area 4</td>
<td>71 ± 4</td>
<td>95 ± 9</td>
<td>99 ± 13</td>
<td>132 ± 6‡</td>
<td>118 ± 4‡</td>
<td>66 ± 3‡</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>Area 5</td>
<td>53 ± 2</td>
<td>82 ± 11</td>
<td>86 ± 8</td>
<td>137 ± 3‡</td>
<td>117 ± 1‡</td>
<td>60 ± 1‡</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>Area 6</td>
<td>58 ± 1</td>
<td>89 ± 10</td>
<td>139 ± 17‡</td>
<td>NA</td>
<td>148 ± 6‖</td>
<td>66 ± 2‡</td>
<td>60 ± 3</td>
</tr>
</tbody>
</table>

* Values are presented as the mean ± SEM. Abbreviation: NA = not available; tissue in this region of the spinal cord was too damaged to measure optical density.
† Sections obtained at T-11 from sham-injured control animals were not significantly different from T-6 or L-1 sections from injured animals.
‡ p < 0.05; indicates difference from rostral spinal level: for example, within Area 1, T-8 is significantly greater than T-6.
§ p < 0.05; in Areas 1, 2, 3, 5, and 6, T-12 readings were significantly higher than T-8.
‖ p < 0.05; indicates difference from previous area: for example, within T-11 Area 4 is significantly less than Area 3.
tive neurons, axons, glia, and neuropils, which may include synapses, indicate that those structures have undergone oxidative stress and may be functionally impaired. This may play a role in observed motor deficits in rats.

The presence of HNE in neurons has been shown to make them more vulnerable to excitotoxicity. Whether the HNE-positive neurons seen in the spinal cord eventually die or recover remains an open question. By 7 days after contusion, the HNE/protein–reaction product was absent except in areas immediately proximal to the injury site, indicating abatement of free-radical generation and lipid peroxidation in those regions. This may signal a return to normal operations for cellular proteins.

TABLE 2

| Neurons, HNE-positive neurons per square millimeter, and percentage of HNE-positive neurons in three areas of spinal cord gray matter 2 days after a T-10 contusion* |
|------------------|------------------|------------------|
| T-6              | T-8              | T-9              |
| HNE-Pos Neurons/ | Neurons/ | Neurons/ | Neurons/ |
| mm               | mm               | mm               | mm       |
| 1                | 2                | 3                | 4        |
| 404 ± 20         | 289 ± 13†       | 204 ± 10         | 192 ± 11 |
| Area mm²         | 1                | 2                | 3        |
| 0                | 0                | 0                | 0        |

* Values are presented as the mean ± SEM. Abbreviations: NA = not available (tissue in this region of the spinal cord was too damaged for neuron counts); pos = positive.
† Significant difference (p < 0.05) from the rostral spinal cord level in a given area: for example, within Area 1 for neurons/mm², T-8 is significantly less than T-6.
‡ The percentage of neurons stained with the HNE/protein antibody was significantly higher (p < 0.05) in T-12 when compared with T-8 in all three areas.
§ Significant difference (p < 0.05) from previous area: for example, within T-8, Area 2 is significantly less than Area 1 for HNE-positive neurons/mm².

TABLE 3

| Optical density readings from protein blots at Day 2 postinjury* |
|-------------------|-------------------|-------------------|
| Spinal Cord Level | Group            |                |
|                   | T-9              | T-10            | T-11            |
| injured           | 42 ± 1†          | 55 ± 1†         | 50 ± 3†         |
| sham injured      | 26 ± 1           | 25 ± 1          | 26 ± 2          |

* Values are presented as the mean ± SEM. Values for all injured animals were significantly different from those in sham-injured animals.
† Significant difference (p < 0.05) from sham-injured animals.
‡ Significant difference (p < 0.05) from T-9 spinal level.

Fig. 6. Bar graph displaying HRP staining after spinal cord injury. Spinal cord sections from three groups of animals were graded on a scale of 0 to 5 by observers who were blinded with respect to treatment and time interval (0 = no stain; 5 = maximum stain). Group 1 consisted of injured animals injected with HRP at the seven time points seen in the graph and killed 1 hour later. Group 2 contained injured animals receiving no HRP and killed at similar points as in Group 1. Group 3 was composed of animals injected with HRP 10 minutes before receiving a laminectomy but no injury (sham-injured) and killed 1 hour postsurgery. A significant decrease in staining was seen by 1 hour postinjury, and a further decrease was observed by 2 days postsurgery; the levels were no different from those seen at the 7-day time point and in injured animals receiving no HRP. This indicates that the BSCB is not significantly permeable to macromolecules by 2 days after an NYU-type weight drop from a height of 25 mm. There was no significant time-dependent difference within the non-HRP-injected animal group, which is presented in the graph as a simple mean of all time points. No staining was seen in the sham-injured animals. *p < 0.05 compared with the prior time point.

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### TABLE 4

<table>
<thead>
<tr>
<th>Area</th>
<th>T-6</th>
<th>T-8</th>
<th>T-9</th>
<th>T-11</th>
<th>T-12</th>
<th>L-1</th>
<th>Sham T-11†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43 ± 2</td>
<td>95 ± 19‡</td>
<td>215 ± 8‡</td>
<td>283 ± 23‡</td>
<td>252 ± 24‡</td>
<td>35 ± 5‡</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>41 ± 2</td>
<td>81 ± 24</td>
<td>150 ± 10‡</td>
<td>237 ± 34‡</td>
<td>150 ± 13§</td>
<td></td>
<td>36 ± 5‡</td>
</tr>
<tr>
<td>3</td>
<td>38 ± 1</td>
<td>71 ± 23</td>
<td>162 ± 10‡</td>
<td>222 ± 13‡</td>
<td>128 ± 14§</td>
<td></td>
<td>50 ± 14‡</td>
</tr>
<tr>
<td>4</td>
<td>38 ± 1</td>
<td>47 ± 9</td>
<td>98 ± 12§</td>
<td>182 ± 17‡</td>
<td>101 ± 23§</td>
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<td>40 ± 8‡</td>
</tr>
<tr>
<td>5</td>
<td>38 ± 1</td>
<td>52 ± 10</td>
<td>91 ± 16‡</td>
<td>168 ± 3‡</td>
<td>69 ± 9‡</td>
<td>36 ± 5‡</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>37 ± 1</td>
<td>52 ± 12</td>
<td>160 ± 5§</td>
<td>NA</td>
<td>159 ± 14§</td>
<td></td>
<td>35 ± 5‡</td>
</tr>
</tbody>
</table>

* Values are presented as the mean ± SEM. Abbreviation: NA = not available; tissue in this region of the spinal cord was too damaged to measure optical density.
† Sections obtained at T-11 from sham-injured control animals were not significantly different from T-6 or L-1 sections from injured animals.
‡ p < 0.05; indicates difference from rostral spinal level: for example, within Area 1, T-8 is significantly greater than T-6.
§ p < 0.05; in Areas 1, 2, 3, 4, and 6, T-12 readings were significantly higher than T-8.
|| p < 0.05; indicates difference from previous area: for example, within T-9, Area 4 is significantly less than Area 3.

allowing some recovery of motor function. Using identical NYU injury device parameters and contusion location as in the present study, Basso, et al., demonstrated significant improvement in hindlimb function by 7 days compared with 2 days after surgery.

The reaction product seen in areas immediately adjacent to the contusion site at 7 days postinjury may be an indicator of ongoing oxidative stress. This is possibly caused by red blood cells that are still visible at that time point, undergoing degradation and releasing iron into the region. It is likely that the T-10 level contains ferritin and a ferritin-derived protein, hemosiderin, both of which have been shown to generate free radicals.

Free-radical production and lipid peroxidation have been shown to be induced by HNE, thus potentiating oxidative cascades. This aldehyde may be the most cytotoxic one and could be largely responsible for cytopathological effects observed during oxidative stress. Once generated, it binds to the lysine, histidine, serine, and cysteine amino acids of proteins, causing protein dysfunction.

Cells exposed to HNE manifest rapid depletion of glutathione; disturbance of calcium homeostasis; dysfunction of potassium channels; inhibition of DNA, RNA, and protein synthesis; induced lysis of red blood cells; inhibition of respiration and glycolysis; dysfunctions of key metabolic enzymes (for example, glyceraldehyde-3-phosphate dehydrogenase); and decreased sodium/potassium-adenosine triphosphatase and protein kinase C activity. Compromise in mitochondrial membrane fluidity as a result of HNE interaction with membrane phospholipids has been demonstrated. This aldehyde can induce cellular apoptosis, which has been observed as a mechanism of cell death after spinal cord injury, and HNE is toxic to cultured neurons at concentrations as low as 0.1 μM. Synaptosomes exposed to HNE manifest impairment of glutamate uptake, glucose transport, and mitochondrial function. This signals a deleterious effect of this aldehyde on synaptic function, which may contribute to functional deficits.

### Immunoglobulin G Staining

Immunoglobulin G staining shows the location of extravasated plasma proteins and areas possibly exposed to plasma toxins. We infer from the data that there are graduated IgG levels relative to the contusion site with the greatest IgG staining in regions closer to the injury. The greater intensity of IgG staining in spinal cord levels caudal to the contusion site when compared with equidistant rostral levels is not without precedent. Noble and Wrathall observed a larger BSCB breach in spinal cord levels caudal to the site of transection when compared with equidistant rostral levels. They speculated that spinal cord damage of white matter tracts in higher-level neural centers controlling vascular permeability may play a role. We have previously reported a greater astrocytic reactivity in the caudal spinal cord levels relative to the contusion site when compared with equidistant rostral levels.

This observation of rostral–caudal asymmetrical damage after spinal cord contusion has not been observed in all spinal cord injury studies. Differences in the severity of injury or experimental models may be a factor.

Neurons and some glialike cells also stained positively. This has been observed in other CNS studies in which IgG was used. Like IgG, additional plasma proteins, for example, fibronectin, fibrinogen, and albumin, are taken up by cells after breach of the blood-CNS barrier. It has been suggested that cellular IgG staining is artifactual, although some investigators speculate that such a staining pattern may indicate cell membrane damage.

This observed similarity between IgG and HNE/protein conjugates is consistent with the hypothesis of a causal relationship between BSCB breach and oxidative stress. Studies have confirmed that extravasated blood in tissue engenders free-radical formation. The relationship between BSCB breakdown and oxidative stress may not be simply a one-way cause–effect relationship. Other studies have shown that free radicals themselves can compromise CNS vascular integrity. Thus, the putative cause–effect relationship between BSCB and oxidative stress may involve one exacerbating the other in a feed-forward cascade.

### Horseradish Peroxidase

Noble and Wrathall report HRP permeability lasting up to 7 days posttrauma in animals with more severe spinal cord injuries administered using a weight-drop model. In light of the size of HRP molecules (approximately 50
kD), lack of HRP staining does not necessarily mean that the BSCB is reestablished, only that the barrier is impermeable to proteins of that size and larger. The mechanisms leading to the rapid reduction of HRP product seen in this study could either be repair of the damaged endothelial cells or abated blood flow. In a very elaborate study in which the Ohio State University spinal cord injury device was used, Popovich, et al., have demonstrated long-term BSCB permeability 28 days after contusion injury by using a small (104-D) radioactively labeled protein (aminoisobutyric acid). However, the results of the present HRP study indicate a rapid relative decline in the BSCB permeability less than 2 days after a T-10 contusion from a height of 25 mm in the NYU model. From the HRP-staining time course seen in this study we hypothesize the presence of a window of opportunity to administer pharmacological factors intravenously that may not normally cross the BSCB. Depending on the size of the protein, the window of opportunity to administer such compounds may be fewer than 2 days at this level of injury in this model.

Conclusions

Of the time points examined, maximum HNE/protein complex staining occurred 2 days postinjury, resulting in immunopositive neurons, axons, neurorips, and some glia. These data indicate possible compromise of neuronal, axonal, glial, and synaptic function, which may be a factor in motor deficits seen in animals after spinal cord contusion administered by means of the NYU device. The colocalization of the IgG stain with the HNE/protein stain is consistent with the hypothesis of a mutual cause–effect relationship between BSCB and oxidative stress in CNS trauma.

References

29. Oliver H, Bayley ED, Povlishock JT, et al: The morphopatho-

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