Clinical progression of motor deficit hours or days after an ischemic episode in the central nervous system (CNS) has been termed "deteriorating stroke." The underlying pathophysiologic mechanisms of the deterioration after stroke are poorly understood in part due to lack of experimental models designed to focus on the deterioration.

Several studies suggest that a number of biochemical and metabolic events contribute to progressive and irreversible cell damage as well as to tissue hypoperfusion after ischemia. Recently, it was suggested that eicosanoids may be involved in the secondary damage observed during reperfusion of ischemic brain. These eicosanoids [prostaglandins (PGs), thrombox-

ane A2 (TxA2), and leukotrienes] are potent vasoactive substances that may be involved in regulation of blood flow, microvascular permeability, and inflammatory responses. Although there is considerable species and tissue variability in the response to eicosanoids, it is generally accepted that TxA2 and PGI2 have opposite effects on vascular tone and platelet aggregation and may be involved in the local regulation of blood flow. In all studies, TxA2 is a potent vasoconstrictor and platelet aggregator, whereas PGI2 generally produces vasodilation and inhibits platelet aggregation. PGE2, however, has vasoconstrictor effects on cerebral vessels in rabbits and is involved in inflammatory responses.

The experiments presented in this study explored the potential role of some eicosanoids in a stroke model of progressive motor deterioration after acute ischemia. In this model, ischemia of the rabbit lumbar spinal cord is produced by ligation of the lower abdominal aorta. This model provides a unique opportunity to study motor function in conscious rabbits during ischemia and reperfusion.

We report studies that better characterize this deteriorating stroke model in view of the histopathology, edema formation, and tissue concentrations of PGI2, TxA2, and PGE2. The data presented in this work indicate temporal histopathologic and edema changes associated with selective changes in eicosanoid production throughout the period of functional deterioration following lumbar spinal cord ischemia.
Materials and Methods

Surgical Preparation

Fifty-five male New Zealand albino rabbits (Harle- 
ton Labs, York, Pa.) were anesthetized with 50 mg/kg i.m. ketamine hydrochloride and 40 mg/kg i.v. sodium pentobarbital. Under aseptic technique, a transperito-
nal incision was made to expose the abdominal aorta, and 0.75-mm o.d. polyethylene tubing was placed around the aorta distal to the renal arteries and was threaded through 2 6.0-mm diam. plastic buttons dor-
sal and ventral to the aorta to produce a snare ligature. To prevent movement through the incision site, the ligature was passed through a 6.25-mm o.d. vinyl guide tube that was sutured to the abdominal muscles. A custom-made canvas jacket was then placed around the rabbit to protect the incision site and the externally accessible ligature. The experiments reported were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animals Resources, National Research Council, Department of Health, Education, and Welfare, National Institutes of Health publication number 85-23.

Induction of Experimental Ischemia

Ischemia was induced in the spinal cord using a modification of the stroke model described by Zivin and DeGirolami.14 Approximately 18 hours after surgery, when the rabbits were awake, the aorta was oc-
cluded for 25 minutes by pulling on the snare ligature and clamping with a pair of hemostats. This period of occlusion produced paralysis of the hind-
limbs in 95% of all control rabbits by 48 hours after reperfusion in the work of Faden and Jacobs.15 Motor function was also evaluated after 15 and 20 minutes of occlusion. After occlusion, the ligature was released and removed with the guide tube through the surgical site. A retaining suture that was placed through the abdominal muscles at surgery was then secured. Post-
operative and treatment procedures included monitoring food and water intake, expressing the urinary blad-
ner as needed (Crede’s maneuver), and neurologic scoring of hindlimb motor function. A control group consisted of normal or sham-operated rabbits that were not subjected to ischemia.

Neurologic Examination

Hindlimb motor function was graded at hourly inter-
vals during the first 24 hours after occlusion. The fol-
lowing ordinal grading scale was used: 0, complete paralysis; 1, minimal functional movement, severe pa-
resis; 2, functional movement, cannot hop; 3, hopping, ataxia and paresis; 4, hopping, mild ataxia and/or pa-
resis; and 5, normal. Rabbits were also categorized as hoppers (neurologic score of 3, 4, or 5) or nonhop-
ers (neurologic score of 0, 1, or 2). Two investigators 
evaluated the rabbits independently.

Physiologic Measurements

Mean arterial pressure (MAP) and heart rate (HR) were continuously measured via a catheter placed in an ear artery and connected to a Narco Bio-Systems Mod-
el 1500i pressure transducer (Houston, Tex.) coupled to a Narco Bio-Systems Model MK-JV physiograph. Core body temperature (T) was monitored via a rectal thermistor connected to a telethermometer (Yellow Springs Instruments, Inc., Yellow Springs, Ohio). Pao2, Paco2, and pH were measured at T with an In-
strulab Model 1303 automated blood gas analyzer (Dayton, Ohio).

Biochemical Measurements

At 5 and 30 minutes, 4, 18, and 24 hours after occlusion, rabbits were killed with 100 mg/kg i.v. sodium pentobarbital, and the spinal cord was rapidly removed from the spinal canal within 45 seconds and processed by slicing the lesion area for analysis of biochemistry by freezing on dry ice, edema by immor-
ner in kerosene to prevent water evaporation, and histopathology by immersion fixation in 10% buffered formalin. Venous blood was collected in tubes con-
taining 20 µl of 10^-4 M indomethacin, centrifuged, and frozen for TxB2 and 6-keto-PGF1α analysis by ra-
dioimmunoassay (RIA).

TxB2, the stable metabolite of TxA2, 6-keto-PGF1α, the stable metabolite of PGI2, and PGE2 were extract-
ed from the spinal cord using a technique previously described.9 Tissue concentrations of these metabolites were determined by RIA using specific antibodies pur-
chased from Dr. Lawrence Levine (Brandeis University, Waltham, Mass.) with cross-reactivity of <1.0% with other cyclooxygenase metabolites. [3H]TxB2, 
[3H]6-keto-PGF1α, and [3H]PGE2, 100–200 Ci/mmol, were purchased from New England Nuclear (Boston, Mass.). Samples were incubated with antibody and the corresponding radioligand for 18–24 hours at 4° C. To separate bound and free ligands, 200 µl of 1% activat-
ed Norit-GSX charcoal (Sigma Chemical Co., St. 
Louis, Mo.) coated with 0.1% dextran (Kodak, Roch-
ester, N.Y.) was added to the sample and centrifuged at 1000g for 10 minutes. Five milliliters of Redisol scintillation fluid (Beckman, Palo Alto, Calif.) was added to the decanted sample and radioactivity was determined by liquid scintillation counting (Model 1218, LKB, Gaithersburg, Md.). The sensitivity of the assays was 16 pg/tube. Protein content of the sample was determined by the method of Lowry et al.18 Plasma concentrations of the metabolites TxB2 and 6-keto-
PGF1α were determined with commercially available RIA kits from New England Nuclear.

Microgravimetry for Measuring Tissue Edema

To measure edema in different regions of the spinal cord, the specific gravity (SG) of 1-mm3 tissue seg-
ments (punches) was determined by means of a density gradient column according to a method previously de-
scribed.17,18 Two solutions of different SG were pre-
pared from kerosene and bromobenzene (SG = 0.79 and 1.49, respectively; Fisher Scientific, Silver Spring, Md.). A flask containing 200 ml of Solution A (SG = 0.975) was positioned to slowly siphon down
43 cm of polyethylene tubing into a flask containing 200 ml of Solution B (SG = 1.065). As the two mixed, the mixture was siphoned down 40 cm of polyethylene tubing into the gradient column, which was constantly lowered to maintain a uniform gradient at the surface of the column. In this way, a linear density gradient ranging from SG = 1.065 to 1.020 was established, which encompasses the range of both normal and edematous spinal cord tissue. The column was allowed to stabilize for 1 hour and was then calibrated with 3-μl drops of a previously prepared graded series of NaCl solutions of known SG from SG = 1.025 to 1.057. The drops served as SG reference points and verified the linearity of the density gradient. If a correlation coefficient of r ≥ 0.995 was not reached, the column was discarded.

Immediately after the spinal cord was removed, a 1-mm slice was immersed in kerosene to prevent evaporation of water. Tissue punched from various regions of the spinal cord including the ventral horn (VH) gray matter, lateral (LW), dorsal column (DC), and ventral (VW) white matter tracts using a blunt 15-gauge needle was then carefully placed on top of the density gradient column. After 3 minutes, the samples reached a stable position within the column, and SG of the punches was measured in relation to the NaCl standards.

Light Microscopy for Determining Histopathologic Changes

After removal, the lumbar spinal cord was immersion-fixed in 10% formalin with 10% glycerin (Fisher Scientific) by vol. for at least 72 hours. The tissue was dehydrated in graded alcohols, embedded in paraffin (Fisher Scientific), and cut in 7-μm transverse sections with a Sorvall JB-4A rotary microtome (Wilmington, Del.). Sections were stained with hematoxylin and eosin (Fisher Scientific) and Luxol fast blue (Sigma Chemical Co.). The spinal cord lesions were evaluated by an investigator unaware of reperfusion time using the following criteria: 3, no lesion observed; 2, gray matter contained 1–10 necrotic neurons with 33% of the cross-sectional area involved; 1, gray matter contained 10–20 necrotic neurons with 33–66% of the cross-sectional area involved; and 0, gray matter contained >20 necrotic neurons with >66% of the cross-sectional area involved. Vacuolation of the neuropil was observed in most rabbits with lesions; however, vacuolation was not quantifiable or as sensitive an indicator as neuronal necrosis. Histopathologic changes following ischemic injury are fairly predictive of neurologic outcome and can be readily assessed using ordinal scoring methods.13,19,20

Data Analysis

Data are presented as means ± SEM. Eicosanoid and tissue edema levels in ischemic rabbits were compared with normal rabbits using analysis of variance (ANOVA) followed by the Dunnett’s test.21 Physiologic data were evaluated by repeated-measures ANOVA.21 Eicosanoid concentrations in hopping and nonhopping rabbits were compared using Student’s t test. Frequency analysis was performed using Fisher’s exact probability test.22 Spearman’s rank correlation test was used to measure the association between neurologic and pathologic scores.22 Values for all of the above statistics were considered significant at p < 0.05.

Results

Neurologic Function

A clear correlation between the duration of ischemia and the functional outcome is presented in Figure 1A. Most rabbits subjected to 15, 20, or 25 minutes of ischemia regained hopping function by 4 hours after reperfusion but underwent deterioration of function sometime thereafter. The degree of motor deterioration depended on the duration of the ischemia, with rabbits in the 25-minute group most severely affected (Table 1). The 25-minute group was selected for fur-
Table 1. Effect of Duration of Lumbar Spinal Cord Ischemia on Hindlimb Motor Function as Neurologic Score in Rabbits During Reperfusion

<table>
<thead>
<tr>
<th>Minutes of ischemia</th>
<th>Score</th>
<th>4 hrs after reperfusion</th>
<th>8 hrs after reperfusion</th>
<th>12 hrs after reperfusion</th>
<th>22 hrs after reperfusion</th>
<th>24 hrs after reperfusion</th>
<th>48 hrs after reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>33</td>
<td>—</td>
<td>100</td>
<td>25</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>33</td>
<td>50</td>
<td>40</td>
<td>—</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
<td>25</td>
<td>20</td>
<td>—</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>30</td>
<td>—</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>20</td>
<td>10</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

*Neurologic scores during the 48-hour reperfusion period (see "Materials and Methods"). Numbers are percent of rabbits with each score. N, number of rabbits.

ther analysis because rabbits deteriorated from a hopping (Score 3, 4, or 5) to a nonhopping status (Score 0, 1, or 2).

Within 60 seconds of aortic occlusion, all rabbits had complete hindlimb paralysis, which remained for the duration of the 25-minute ischemic period. Ten rabbits were scored hourly during the 24-hour reperfusion period. Within 4 hours of reperfusion, 70% of the rabbits regained substantial hindlimb function and were capable of hopping (Figure 1B). However, 12–18 hours after reperfusion, a secondary decline in hopping function was observed. By 24 hours after reperfusion, 70% of the rabbits were unable to hop. Hindlimb function changed very little after 24 hours and never improved (unpublished results).

**Physiologic Measurements**

T, MAP, Pao_2, pH, and Paco_2 did not differ from control values during ischemia or the 24-hour reperfusion period (n = 5, Figure 2). HR increased significantly during early reperfusion but was within the physiologic range after 12 hours of reperfusion (repeated-measures ANOVA followed by Dunnett’s test, F = 2.956, p = 0.040, n = 5). The physiologic ranges for HR, T, MAP, Pao_2, pH, and Paco_2 were determined in resting, unrestrained rabbits (range = mean ± SD, n = 15).

**Eicosanoid Tissue Concentrations**

Five minutes after reperfusion (early phase), tissue TxB_2 concentration in the lumbar spinal cord increased almost threefold (ANOVA, F = 8.32, n = 31, p < 0.01; Figure 3). Concentrations of 6-keto-PGF_{1α}, however, did not change during reperfusion. The increase in TxB_2, but not 6-keto-PGF_{1α}, at 5 minutes led to a 2.5-fold increase in the TxB_2:6-keto-PGF_{1α} ratio (ANOVA, F = 7.16, n = 31, p < 0.01). Thirty minutes after reperfusion (early phase), PGE_2 concentra-

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2. Effects of 25-minute aortic occlusion on heart rate (HR), core temperature (T°c), mean arterial pressure (MAP), arterial Pao_2, pH, and Paco_2 in rabbits. Shaded area represents ischemic period. ———— physiologic range for each variable (mean ± SD, n = 15); * statistical significance (p < 0.05; repeated-measures ANOVA followed by Dunnett’s test, F = 2.956, p = 0.040; n = 5 for HR, T°c, and MAP; n = 4 for Pao_2, pH, and Paco_2).**
EARLY PHASE

![Graph showing early phase TXB₂ and 6-keto-PGF₁α concentrations in the lumbar spinal cord of rabbits subjected to 25 minutes of ischemia via aortic occlusion. Number at the base of each bar is the number of animals per group. **TXB₂ concentration and the TXB₂/6-keto-PGF₁α ratio were significantly higher at 5 minutes of reperfusion (early phase) than control (ANOVA followed by Dunnett’s test, p<0.01). *TXB₂ concentrations were also increased at 18 hours of reperfusion (late phase) (ANOVA followed by Dunnett’s test, p<0.05).](image)

LATE PHASE

![Graph showing late phase TXB₂ and 6-keto-PGF₁α concentrations in the lumbar spinal cord of rabbits subjected to 25 minutes of ischemia via aortic occlusion. Number at the base of each bar is the number of animals per group. **TXB₂ concentration and the TXB₂/6-keto-PGF₁α ratio were significantly higher at 5 minutes of reperfusion (early phase) than control (ANOVA followed by Dunnett’s test, p<0.01). *TXB₂ concentrations were also increased at 18 hours of reperfusion (late phase) (ANOVA followed by Dunnett’s test, p<0.05).](image)

Figure 3. TXB₂ and 6-keto-PGF₁α concentrations in the lumbar spinal cord of rabbits subjected to 25 minutes of ischemia via aortic occlusion. Number at the base of each bar is the number of animals per group. **TXB₂ concentration and the TXB₂/6-keto-PGF₁α ratio were significantly higher at 5 minutes of reperfusion (early phase) than control (ANOVA followed by Dunnett’s test, p<0.01). *TXB₂ concentrations were also increased at 18 hours of reperfusion (late phase) (ANOVA followed by Dunnett’s test, p<0.05).

Concentrations were > twofold higher compared with controls (ANOVA, F = 3.42, n = 51, p<0.05; Figure 4). At 18 hours (late phase), the tissue concentration of TXB₂ was again higher than baseline (ANOVA, F = 3.62, p<0.05; Figure 3), whereas no change in 6-keto-PGF₁α concentration was observed. Further analysis revealed that nonhopping rabbits (Scores 0, 1, or 2) had higher tissue concentrations of TXB₂ than hopping rabbits (Student’s t test, t = 6.12, n = 53, p<0.001; Figure 5). Furthermore, rabbits with lumbar spinal cord concentrations of >500 pg TXB₂/mg protein had lower motor function scores than hopping rabbits (Fisher’s exact probability test, n = 53, p = 0.0081). Only 10.7% of the hopping rabbits had TXB₂ levels of >500 pg/mg protein, whereas 87.5% of the nonhopping rabbits had TXB₂ levels of >500 pg/mg protein.

Concentrations of PGE₂ did not change during the late phase (Figure 4).

Assays for TXB₂ and PGs were also performed on spinal cord T10–T12 segments, which did not include the ischemic lesion. No significant changes were observed at these sites, which demonstrates that the TXB₂ and PG increases were confined to the ischemic zone (n = 40, Table 2). Plasma concentrations of TXB₂ were not different from control levels (Table 3). Plasma 6-keto-PGF₁α levels were not detectable.

Tissue Edema

A significant decrease in SG was observed as early as 30 minutes after reperfusion in both gray (VH) and white (DC and VW) matter (ANOVA; F = 11.58 for VH, F = 8.01 for DC, F = 9.12 for VW; n = 53,
**Histopathologic Changes**

The major histopathologic changes observed after 25 minutes of ischemia in the rabbit spinal cord were neuronal necrosis and vacuolation of the neuropil (Figure 7). Neuronal necrosis was first observed in 43% of the rabbits 4 hours after reperfusion and was limited to the intermediate gray area. Eighteen hours after reperfusion, neuronal necrosis was present in the intermediate gray and DH areas, with occasional degenerate neurons in VH of all rabbits. In addition, vacuolation of the neuropil was observed in the intermediate gray and VH areas in 25% of the rabbits. At 24 hours after reperfusion, lesions were similar to those observed at 18 hours, although usually more severe; vacuolation of the neuropil was present in all rabbits (Figure 7). Correlation between the histopathologic changes and hindlimb motor function changes was highly significant (Spearman's rank correlation test, \( r_s = 0.642, p<0.01, n = 23; \) Table 4).

**Discussion**

A distinct advantage of the present model to study events after ischemia is that an integrated CNS function can be monitored. This model has characteristics similar to deteriorating stroke, \(^{23}\) which includes stroke-in-evolution. \(^{24}\) During reperfusion, 70% of the rabbits regained substantial motor function within 4 hours; however, over the next 20 hours, motor function steadied.

**Figure 4.** PGE2 concentrations in the lumbar spinal cord of rabbits subjected to 25 minutes of ischemia via aortic occlusion. Number at the base of bars is the number of animals per group. \(*\) PGE2 concentration was significantly higher than control at 30 minutes after reperfusion (ANOVA followed by Dunnett's test, \( p<0.05 \)).

**Figure 5.** Association of TXB2 tissue concentration and hindlimb motor function in rabbits subjected to 25 minutes of lumbar spinal cord ischemia. Most non-hoppers had higher spinal cord concentrations of TXB2 than hoppers (Student's \( t \) test, \( t = 6.12, p<0.01 \)). Individual rabbit values (\( \bullet \)) --- divides hoppers and non-hoppers. TXB2 tissue concentrations of < or > 500 pg/mg protein (Fisher's exact probability, \( n = 53, p = 0.0081 \)).

<table>
<thead>
<tr>
<th>Time after reperfusion</th>
<th>Control</th>
<th>5 min</th>
<th>30 min</th>
<th>4 hr</th>
<th>18 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>TxB2</td>
<td>521 ± 25</td>
<td>413 ± 30</td>
<td>609 ± 99</td>
<td>389 ± 42</td>
<td>627 ± 90</td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
<td>255 ± 43</td>
<td>233 ± 15</td>
<td>306 ± 23</td>
<td>215 ± 31</td>
<td>327 ± 57</td>
</tr>
<tr>
<td>PGE2</td>
<td>1093 ± 87</td>
<td>928 ± 32</td>
<td>1163 ± 101</td>
<td>763 ± 76</td>
<td>1036 ± 59</td>
</tr>
</tbody>
</table>

Concentrations as pg/mg protein ± SEM at various times after reperfusion were compared by ANOVA. No significant differences at \( p<0.05 \).
Eicosanoids and Spinal Cord Ischemia

Table 3. Plasma Concentrations of TxB₂ and 6-keto-PGF₉α in Rabbits Subjected to 25 Minutes of Aortic Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ischemia</th>
<th>5 min</th>
<th>30 min</th>
<th>4 hr</th>
<th>18 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>TxB₂</td>
<td>92.7 ± 24</td>
<td>82.1 ± 21</td>
<td>67.1 ± 11</td>
<td>70.4 ± 20</td>
<td>109 ± 58</td>
<td>89.2 ± 39</td>
<td>307 ± 220</td>
</tr>
<tr>
<td>6-keto-PGF₉α</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Values as mean ± SEM. ND, not detectable; N, number of rabbits at each time. Statistical analysis was performed by ANOVA; no significant differences at p < 0.05.

At 4 hours after reperfusion, the levels of the PGs and TxB₂ were virtually normal; at that time substantial recovery of neurologic motor functions were also observed. Eighteen hours after the ischemic insult most rabbits had decreased hindlimb function. Analysis of TxB₂ in hopping vs. nonhopping rabbits showed higher TxB₂ levels in the nonhopping rabbits. Also, it appears that rabbits with spinal cord TxB₂ levels of > 500 pg/mg protein had major neurologic deficits. Other studies have reported that carotid infusion of a TXA₂-generating system (platelet suspensions) reduced cerebral blood flow and precipitated a stroke-like condition in rabbits.30

Since AA metabolites are produced de novo, these data suggest that PG levels may represent the functionally declined to where only 30% of the rabbits had minimal hopping function. This model is highly reproducible in producing a consistent functional outcome as observed in previous studies.15,19

The present study demonstrated that spinal cord concentrations of TxB₂ in rabbits increased immediately on reperfusion, similar to reports of brain ischemia models in which TxB₂ tissue concentration was temporarily elevated on reperfusion in several species.6,9,25 At 30 minutes after reperfusion, PGE₂ concentrations were also increased, whereas TxB₂ levels were decreased. The differential changes in eicosanoids in our model vary from recent studies in which reperfusion of ischemic gerbil and rat brain caused an immediate increase in all cyclooxygenase metabolites measured.6,9,25 In the rabbit model, only TxB₂ was elevated while 6-keto-PGF₉α was not changed. The selective increase in TxB₂ may indicate that cellular elements which produce TXA₂, e.g., platelets, macrophages, and neutrophils, are activated. The lack of increase in PGE₂ immediately after reperfusion might be the result of inhibition of PGE-isomerase by the high arachidonic acid (AA) concentration in the tissue at this time.26 On consumption of much of the AA, PGE-isomerase activity is resumed, leading to PGE₂ generation as seen 30 minutes after reperfusion. Since PGE₂ has constrictor effects on rabbit CNS microvessels,13 elevated PGE₂ levels may propagate the initial vasoconstriction produced by TXA₂. The imbalance in the TXA₂:PGL₂ ratio could favor platelet aggregation and vasoconstriction, thus leading to further ischemic damage.3 This possibility is supported by studies showing that supplementing PGL₂ together with blocking TXA₂ formation protects CNS tissue from acute ischemic insults.27,28

At 4 hours after reperfusion, the levels of the PGs and TxB₂ were virtually normal; at that time substantial recovery of neurologic motor functions were also observed. Eighteen hours after the ischemic insult most rabbits had decreased hindlimb function. Analysis of TxB₂ in hopping vs. nonhopping rabbits showed higher TxB₂ levels in the nonhopping rabbits. Also, it appears that rabbits with spinal cord TxB₂ levels of > 500 pg/mg protein had major neurologic deficits. Other studies have reported that carotid infusion of a TXA₂-generating system (platelet suspensions) reduced cerebral blood flow and precipitated a stroke-like condition in rabbits.30

Since AA metabolites are produced de novo, these data suggest that PG levels may represent the functionally impaired state of the tissue at various times during reperfusion. The secondary increase in TxB₂ at 18 hours after ischemia, especially in rabbits with poor functional status, again suggests an increased availability of AA, which may represent the possibility of a secondary ischemic event or membrane damage. However, no data are now available on tissue perfusion in this model. Systemic physiologic variables like blood gases, MAP, HR, and T do not appear to be involved in the secondary motor deterioration phenomenon. Even though HR was significantly increased during the early reperfusion phase, the increments were modest and values were in the normal range when motor deterioration developed. Although the increase in TxB₂ at 18 hours is modest, its effects might be pronounced since the tissue at this time is already damaged and therefore might be more vulnerable to even small additional insults. It is tempting to speculate that the delayed deterioration might be at least partially due to damage produced by the secondary formation of TXA₂, presumably by platelets and/or inflammatory cells such as macrophages and neutrophils. This possibility is supported by recent studies showing platelet and leukocyte accumulation in ischemic brain tissue.20,21 However, an alternative explanation for TXA₂ release is a response to membrane damage where TXA₂ may serve as an indicator of cellular injury.

Edema formation in ischemic tissue is a well-established phenomenon. In the present study, increases in tissue water content in both gray and white matter were observed as early as 5 minutes after reperfusion. At 30 minutes of reperfusion, we found that edema continued to increase. However, by 4 hours, tissue water content was back to normal. An increase in edema between 4 and 24 hours in both gray and white matter correlated well with the secondary deterioration of neurologic function. Several studies suggest that PGs, particularly PGE₂, may be involved in the development of edema after ischemic brain injury.7,8,32 However, in studies in which PG synthesis was blocked by indomethacin, no effect on edema formation after ischemia was seen.6,33,34 However, failure by indomethacin alone to block edema formation might be due to the persistent production of other proedematous eicosanoids through the lipoxigenase pathway and especially the formation of leukotrienes which, together with other 5-lipoxygenase metabolites of AA, increase vascular permeability and promote tissue edema.35 This possibility is supported by recent reports showing elevated leukotriene levels during reperfusion after brain...
ischemia and the increase in 5-HETE (a 5-lipoxygenase metabolite) during reperfusion after spinal cord ischemia in rabbits.

During the 18–24 hour period, we observed an increase in the number of necrotic neurons, which may be responsible for the increased fluid accumulation due to osmotic differences between the blood and tissue. However, the increase in tissue water may also increase tissue pressure, resulting in a concomitant decrease in local microcirculatory blood flow. Interestingly, the number of necrotic neurons increased over time, from the intermediate to intermediate-dorsal to VH regions of the spinal cord. This agrees with studies in which normal spinal cord blood flow was reported to be highest in the intermediate gray region with progressively lower values in the intermediate–dorsal to the VH and may reflect the sensitivity of neurons in

![Figure 6](image-url)

**Figure 6.** A. Tissue edema measured as specific gravity in different regions of the rabbit spinal cord subjected to 25 minutes of aortic occlusion. Tissue water content temporarily increased in ventral horn, dorsal white, and ventral white but returned to normal levels by 4 hours. At 18 hours, tissue water content was significantly increased in the ventral horn followed by significant increases in all regions by 24 hours after reperfusion. (*p<0.05, **p<0.01, ANOVA followed by Dunnett’s test). B. Composite graph of edema (Figure 6A) and neurologic score (Figure 1B) during the 24-hour reperfusion period. The pattern of tissue edema changes in each region of the spinal cord followed the pattern of neurologic changes. Neurologic score as median values.

![Figure 7](image-url)

**Figure 7.** A. The major histopathologic changes after 25 minutes of aortic occlusion in rabbits were neuronal necrosis and vacuolation of the neuropil (edema formation) in the lumbar spinal cord. Lesions at 24 hours in the dorsal and ventral horns and intermediate gray matter. Hematoxylin and eosin stain, bar = 300 μm. B. Neuronal necrosis indicated by arrows. Micrograph of the intermediate gray matter after 24 hours of reperfusion. CC, central canal; hematoxylin and eosin stain, bar = 50 μm. C. Vacuolation of the neuropil indicated by arrows, necrotic neurons indicated by arrowheads. Micrograph of the ventral gray matter after 24 hours of reperfusion. Hematoxylin and eosin stain, bar = 50 μm.
these regions to blood flow changes.39 The histopathologic changes observed in this model during the first 24 hours after ischemia are reported here for the first time. Several rabbits were also followed to 7 days, and the histopathologic changes such as neuronal necrosis, edema, etc., were similar to those reported by DeGirolami and Zivin.20

In summary: This study describes a model with the continuous deterioration of motor function during reperfusion after an initial ischemic insult. This deterioration is associated with increased tissue edema and increased formation of TxA2. However, the relations of the increased TxA2 to tissue edema, histopathologic changes, and late functional deficits need further investigation.

Acknowledgments

We wish to thank Ms. Debbie Anderson and Ms. Sharon Nagle for their skillful technical assistance. We also thank Ms. Leslie S. Watts and Mrs. Laura L. Garza for preparing this manuscript.

References

15. Faden AI, Jacobs TP: Opiate antagonist WIN44.441-3 stereospecifically improves neurologic recovery after ischemic spina1 injury. Neurology 1986;35:1311-1313


Key Words • prostaglandins • thromboxane • stroke • edema • rabbits
Deteriorating stroke model: histopathology, edema, and eicosanoid changes following spinal cord ischemia in rabbits.

T P Jacobs, E Shohami, W Baze, E Burgard, C Gunderson, J M Hallenbeck and G Feuerstein

*Stroke*. 1987;18:741-750
doi: 10.1161/01.STR.18.4.741

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/18/4/741

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/