Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats

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There have been few investigations of brain edema formation after intracerebral hemorrhage (ICH), despite the fact that mass effect and edema are important clinical complications. The present study was designed to investigate the time course for the formation and resolution of brain edema and to determine how changes in cerebral blood flow (CBF) and blood-brain barrier (BBB) permeability are temporally related to edema formation following ICH.

Anesthetized adult rats received a sterile injection of 100 μl of autologous blood into the caudate nucleus. Water and ion contents were measured immediately, at 4 and 12 hours, and daily to Day 7 (10 time points, six rats at each time) after experimental ICH. The water content of the ipsilateral basal ganglia increased progressively (p < 0.002) over the first 24 hours, then remained constant until after Day 5, when the edema began to resolve. Edema was most severe in the tissue immediately surrounding the hemorrhage; however, it was also present in the ipsilateral cortex, the contralateral cortex, and the basal ganglia. Measurements of local CBF (using [14C]-iodoantipyrine) and BBB permeability (using [3H]-α-aminoisobutyric acid) were obtained in separate groups of six to eight rats at various time intervals between 1 and 48 hours after ICH. Cerebral blood flow was reduced to 50% of control at 1 hour, returned to control values by 4 hours, but then decreased to less than 50% of control between 24 and 48 hours after ICH. The BBB permeability increased significantly prior to the occurrence of significant edema in the tissue surrounding the clot. However, BBB permeability in the more distant structures remained normal despite the development of edema.

These results demonstrate a time course for the formation and resolution of brain edema following ICH similar to that observed during focal ischemia. Brain edema forms in the immediate vicinity of the clot as a result of both BBB disruption and the local generation of osmotically active substances and then spreads to adjacent structures. While local ischemia, due to the mass effect of the hemorrhage, may play a role in producing cytotoxic and vasogenic edema, the release of toxic substances from the clot should also be considered. Since edema is nearly maximal by 24 hours after ICH, therapy directed at reducing edema formation must be instituted within the 1st day.

Key Words · intracerebral hemorrhage · brain edema · brain water · cerebral blood flow · blood-brain barrier permeability · rat

Brain edema is an important clinical complication of intracerebral hemorrhage (ICH). Despite much effort directed at clarifying the roles of surgical and medical therapy for ICH, the most appropriate management is still controversial. This may be due, in part, to an incomplete understanding of the factors that lead to the development of brain edema following ICH.

During the past two decades, various studies in monkeys, dogs, cats, rabbits, and rats have focused on the acute effects of experimental ICH on pressure-volume relationships, cerebral blood flow (CBF) in the region, and related ischemic changes (see the review by Kaufman and Schochet17). Few studies, however, have examined the sequelae of hemorrhage over a period of days and the dynamic forces that cause secondary injury after the primary event. There has also been little attention to brain edema formation, one of the most significant complications of ICH.

Although the development of brain edema is recognized as a major cause of delayed deterioration following spontaneous ICH,31 the mechanism for its formation is poorly understood. Local ischemia may play a role since experimental studies have shown an immediate dose-dependent reduction in local and distant CBF following injection of autologous blood into the
caudate nucleus of rats. However, this initial decrease is short-lived and CBF returns to near-normal by 10 minutes after ICH. While blood-brain barrier (BBB) permeability increases during the first 30 minutes after ICH, it is not significantly different from that observed in sham-operated animals.

In the present study, we investigated the accumulation of brain edema following ICH both adjacent to and distant from the hemorrhage. Our studies were designed to determine how changes in CBF and BBB permeability relate to brain edema formation following experimental ICH in the rat.

**Materials and Methods**

*Rat Intracerebral Hemorrhage Model*

The procedures in this study using laboratory animals were approved by the University Committee on the Use and Care of Animals. Adult male Sprague-Dawley rats, each weighing 250 to 350 gm, were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg). A polyethylene (PE-50) catheter was introduced into the femoral artery in order to monitor arterial blood pressure and to obtain blood for analysis of blood gases, blood pH, hematocrit, and blood glucose concentration and for production of the intracerebral hematoma. The rat’s body temperature was maintained at 37.5°C with a heating pad. Blood gases were analyzed once during the operation. The animals were given a 30% oxygen/70% nitrogen gas mixture via an inhalation mask to maintain a PaO2 level of 90 mm Hg or higher. The blood glucose concentration was estimated using a commercial test.*

The model of intracerebral hematoma was a modification of the method described by Masuda, et al. Briefly, rats were placed in a stereotactic frame, the scalp was incised longitudinally in the midline, and a 2-mm burr hole was made in the skull using a dental drill. A No. 30 sterile needle was then lowered vertically into the right caudate nucleus at coordinates A 6.8, L 3.0, and H 1.0. Fresh autologous blood was drawn from the femoral artery catheter into a 1-ml syringe which was immediately mounted in an infusion pump. Then, 100 µl of non-heparinized fresh blood was infused at a rate of 20 µl/min. After completion of the infusion, cyanoacrylate glue was placed around the burr hole and the needle was withdrawn quickly. The arterial catheter was removed, incisions in the skin were closed, and the animals were allowed to recover. Some animals underwent a sham procedure consisting of removal of arterial blood through a femoral artery catheter and insertion of a needle into the caudate nucleus; however, blood was not injected into the brain. Instead, after 5 minutes, the needle was removed and the skull sealed.

**General Experimental Protocols**

Groups of six rats each were sacrificed by decapitation immediately, at 4 or 12 hours, or each day between Day 1 and Day 7 after the injection of blood. The brains were quickly removed and two coronal slices (each 2 mm thick, with the first slice 5 mm from the frontal pole) were cut with a sharp blade (Fig. 1). The anterior slice contained the fresh blood clot and is designated as the hemorrhagic section, while the posterior slice is designated as the adjacent section. At early time points, when it was still semisolid, the clot was removed and discarded.

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*Glucotix and Glucometer manufactured by Miles Laboratories, Elkhart, Indiana.*

![Diagram of rat brain with injection site and blood flow](https://example.com/diagram.png)

**Fig. 1.** Drawing demonstrating the procedure for sampling brain tissue for water and ion content measurement. Two coronal sections, each 2 mm thick, were cut through and immediately posterior to the hemorrhage. Each section was divided into four areas: ipsilateral cortex, ipsilateral basal ganglia, contralateral cortex, and contralateral basal ganglia. The tissue samples were then processed for measurement of water, sodium, potassium, and chloride contents as described in the Materials and Methods section.

**Water, Sodium, Potassium, and Chloride Content**

The tissue samples were immediately weighed on an electronic analytical balance to the nearest 0.1 mg to obtain the wet weight. The tissue was then dried in a gravity oven at 95°C for 24 hours and reweighed to obtain the dry weight. The water content, expressed as a percentage of the wet weight, was calculated as (wet weight − dry weight)/wet weight × 100. When expressed in milliliters/gm dry tissue weight, the water content was calculated as (wet weight − dry weight)/dry weight.

The dehydrated samples were digested in 1 ml of 1 N nitric acid for 1 week. Then, a 0.2-ml aliquot was removed and diluted to 2 ml with deionized water and 3 mM of CsCl solution. The sodium and potassium concentrations of this solution were measured by flame photometry. Flame conditions and detection wavelengths were optimized for sensitivity and linearity. Chloride was measured using a digital chloridometer.
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Magnetic Resonance Imaging

Standard T₁-weighted magnetic resonance (MR) imaging was performed using a 2.0-Tesla/31-cm imaging spectrometer.† The head of the rat was fixed to a 2-cm receiver coil by means of ear posts and a nose prop, and mounted within a 13.6-cm birdcage transmit coil. Fast gradient spin-echo MR images were obtained in orthogonal planes to confirm desired positioning (acquisition parameters for T₁-weighted images: TR = 2500 msec; TE = 80 msec, 256 × 128 matrix; field of view = 30 mm; 2 excitations averaged; and four 1-mm coronal slices with 1.5 mm center-to-center spacing).

Cerebral Blood Flow Measurement

Cerebral blood flow was measured in groups of six rats each, at 1, 4, 24, and 48 hours after the ICH or the sham procedure. The rats were reanesthetized with ketamine and xylazine shortly before termination of the experiment, intubated, and ventilated with a rodent ventilator to maintain the PCO₂ between 35 and 45 mm Hg. Catheters (PE-50) were inserted into a femoral artery and a vein.

The CBF was measured in the four sampled regions by the indicator fractionation technique.27 The method uses an intravenous bolus injection of CF indicator followed by a constant rate of blood withdrawal through a femoral artery catheter to obtain the integral of the arterial isotope concentration. The withdrawal was started 15 seconds before intravenous injection of a 0.1-ml bolus of saline containing 10 μCi of 4-[N-methyl-14C]-iodoantipyrine. Exactly 10 seconds later, the animals were decapitated and blood withdrawal was stopped. The sample of withdrawn arterial blood and the brain tissue samples were digested in methylbenzenethionox hydrioxide. Blood samples were bicircated with H2O and then the radioligand concentration of both the blood and tissue samples was determined using a two-channel scintillation counter.

The blood flow rates for the individual pieces of tissue were calculated using the following equation:

\[
F_b = \frac{Q_a(T)F_a}{M_b} \times 100,
\]

where \(F_b\) = the brain blood flow; \(M_b\) = the brain mass (in gm); \(Q_a\) = the quantity of indicator present in the tissue at time T; \(F_a\) = the rate of blood withdrawal into the syringe from t = 0 to t = T; and \(Q_a(T)\) = the quantity of indicator present in the syringe at time T. The resulting CBF is expressed as ml/100 gm/min.

Permeability of BBB and Brain Plasma Volume

Blood-brain barrier permeability was measured in groups of six rats each, at 4, 12, 24, and 48 hours after the ICH or sham procedure. The rats were reanesthetized with ketamine and xylazine shortly before termination of the experiment, intubated, and ventilated with a rodent ventilator to maintain the PCO₂ between 35 and 45 mm Hg. Catheters (PE-50) were inserted into a femoral artery and a vein.

The small, nontransported amino acid analog α-aminoisobutyric acid (AIB) was used to measure BBB permeability as described previously.28 After an intravenous bolus injection of [3H]-AIB, the amount of tracer that moved into the extravascular compartment of the brain was divided by the integral of the plasma concentration of the tracer determined by continuous withdrawal of arterial blood between the time of injection and the killing of the rat. The extravascular compartment of the brain was calculated by subtracting the amount of tracer in the plasma compartment (plasma volume measured using 14C-inulin) multiplied by the terminal plasma concentration) from the total radioactivity in the brain. The results were expressed as a rate constant for brain uptake which, for a compound of low BBB permeability such as AIB, is equal to the product of the capillary permeability and the surface area of the exposed vascular bed. The rats received 35 μCi of [3H]-AIB, injected 10 minutes before the end of experiment, then 20 μCi of [14C]-inulin as a second injection 2 minutes before the end. Both simultaneously with the first injection, a peristaltic pump was started and blood was withdrawn at a constant rate into an arterial cannula. At the end of the experiment, a terminal plasma sample was obtained and the rat was killed by decapitation. The entire contents of the arterial cannula were emptied and a portion was pipetted for scintillation counting. Blood samples were digested in methylbenzenethionox hydrioxide, bleached with H2O2, and counted in an aqueous-based liquid scintillation cocktail. Brain tissue samples were also digested in methylbenzenethionox hydrioxide prior to preparation for liquid scintillation counting.

Statistical Analysis

Differences in water and ion contents between the time points were evaluated using analysis of variance (ANOVA), and the level of significance between the value immediately after ICH and the other time points was determined using a one-tailed Student t-test for unpaired samples and Bonferroni's correction for multiple comparisons.39 Significant differences in CBF and BBB permeability between the ICH and sham-operated groups at each time point were identified using a two-tailed Student t-test. A p value of less than 0.05 indicates a significant difference.

The relationship between ion and water content changes was assessed by linear regression analysis using the StatView II program.§ The hypothesis that the slope was significantly different from a value of 1 was tested by determining whether the 95% and 99% confidence intervals overlapped with this value.

Results

Table 1 shows the mean arterial blood pressure, blood gases, blood pH, hematocrit, and blood glucose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean arterial blood pressure (mm Hg)</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.01</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>112 ± 7</td>
</tr>
<tr>
<td>HCO₃ (mmol/liter)</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>hematocrit</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>228 ± 13</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for 60 rats. There were no significant differences between study groups, so the combined data are presented.

‡ Omega CSI imaging spectrometer manufactured by Bruker Instruments, Fremont, California.


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concentrations for the animals undergoing edema measurement. When mean values for the animals within each study group were compared by ANOVA, there were no significant differences. Thus, combined average data are shown.

Magnetic resonance T2-weighted images obtained within 1 hour and at 48 hours after ICH showed that initially the clot was well localized with little increase in tissue water except along the needle track and immediately surrounding the hematoma (Fig. 2A and B). By 48 hours, however, extensive edema appeared to have spread across the corpus callosum (Fig. 2C) and to sites distant from the hemorrhage such as the hippocampus and contralateral cortex (Fig. 2D). The signal intensity of the clot also changed, consistent with studies in humans.\footnote{Changes in Water Content Following ICH}

The net edema formation, calculated as the difference in water content in the animals sacrificed immediately after ICH (Time 0) and those sacrificed at later time points, is displayed for the hemorrhagic section in Fig. 3A. Compared with the Time 0 group, the water content was significantly increased in the animals sacrificed at 4 hours (77.29\% vs. 78.47\%) in the ipsilateral basal ganglia. In the animals sacrificed at 12 hours after ICH, the water content was significantly increased in all regions. The degree of increased water content was more severe (4.7\%) in the ipsilateral basal ganglia in the animals sacrificed at Time 0 and those sacrificed at 24 hours; in the contralateral basal ganglia, the difference in these groups was 2.9\%. The accumulation of water was at its peak between Day 1 and Day 4. After Day 4, edema in the ipsilateral tissues gradually lessened and the water content of the contralateral hemisphere returned toward normal.

Changes in Ion Content Following ICH

The differences in sodium content between Time 0 and later time points after ICH are displayed for the hemorrhagic section in Fig. 3B. Compared with the Time 0 group, the sodium content increased in the animals sacrificed after 4 hours of ICH in the ipsilateral and contralateral basal ganglia although the increase was most severe in the ipsilateral basal ganglia, where it changed by 44 \( \mu \)Eq/gm dry weight in the group sacrificed at 4 hours and by 172 \( \mu \)Eq/gm dry weight in the group sacrificed at 24 hours. In the contralateral basal ganglia, the difference between the Time 0 group and the group sacrificed at 24 hours was 51 \( \mu \)Eq/gm dry weight. The accumulation of sodium was similar...
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**Fig. 3.** Graphs showing changes in water (A), sodium (B), potassium (C), and chloride (D) levels in the hemorrhagic section following injection of 100 μl of autologous blood. Water and ion contents were measured in groups of six rats immediately (Time 0) and at various intervals between Day 1 and Day 7 following intracerebral hemorrhage. The values shown are the means ± standard error of the mean differences between values at Time 0 and those at subsequent times. Measurements were made in four brain regions: ipsilateral basal ganglia (solid squares), ipsilateral cortex (solid circles), contralateral basal ganglia (open squares), and contralateral cortex (open circles).

The changes in potassium content in the hemorrhagic section at intervals from immediately after ICH to Day 7 are displayed in Fig. 3C. Compared with the Time 0 group, the potassium content was essentially unchanged in the ipsilateral basal ganglia in the group sacrificed at 24 hours during the first 12 hours but then decreased by 62 μEq/gm dry weight. The potassium content also tended to decrease in the ipsilateral cortex; however, this change was only significant at Day 2 and Day 3. At all time points, loss of potassium was less than the accumulation of sodium and, consequently, there was a net increase in brain cations.

The changes in chloride content in the hemorrhagic section at intervals from immediately after ICH to Day 7 are displayed in Fig. 3D. Compared with the Time 0 group, the chloride content increased in the ipsilateral basal ganglia by 36 μEq/gm dry weight in the animals sacrificed at 4 hours and by 93 μEq/gm dry weight in the animals sacrificed at 24 hours. Similar to the pattern of sodium levels, the increase in chloride content was more severe in the ipsilateral basal ganglia than in the ipsilateral cortex and the accumulation of chloride was at its peak between Day 1 and Day 3, but then gradually subsided. Significant changes in the chloride content of the contralateral hemisphere were also observed during the period of peak edema formation.

**Correlation Between Changes in Water and Changes in Ions**

To assess the relationship between the development of edema and the shifts in cation content, we performed a linear regression analysis of brain [Na⁺] + [K⁺]/plasma [Na⁺] + [K⁺] versus brain [H₂O]. This analysis compares the brain content of cations relative to their concentration in plasma to the brain water content. If the increase in brain water concentration is accompanied by an iso-osmolar influx of ions from blood, the slope of this relationship should have a value of 1.

A scatterplot of the relative brain cation content versus water content for the early and later time periods following ICH in the ipsilateral and contralateral basal ganglia can be seen in Fig. 4. Data from the hemorrhagic section and the adjacent section were combined. Values for the slopes of the regression analysis are shown in Table 2. During both the early phase of edema formation (range 0 to 12 hours) and the later phase (≥ 24 hours) the slope is significantly less than 1 in the ipsilateral tissue but not in the contralateral basal ganglia. This indicates that only 64% to 84% of edema

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in the ipsilateral tissue can be accounted for by the change in ions, while all edema in the contralateral tissue is accounted for by the cations (with the exception of the contralateral cortex at the longer time points, where the data were more scattered).

Cerebral Blood Flow

The CBF was measured in a separate group of rats using tissue samples that corresponded to those used for the edema measurements. When measured 1 hour after the ICH, CBF was reduced globally, varying from 50% of the value in sham-operated rats in the ipsilateral basal ganglia to 73% in the contralateral cortex (Fig. 5 upper). The CBF values returned to nearly that in the sham-operated rats by 4 hours after ICH and remained fairly constant at 24 hours. However, by 48 hours, all brain regions of the experimental animals once again demonstrated significantly reduced CBF with both the ipsilateral basal ganglia and cortex levels about 48% of the corresponding levels in the sham-operated animals. The contralateral tissues were not as severely affected.

Blood-Brain Barrier Permeability

The BBB permeability was measured using AIB, a small neutral amino acid that crosses the normal BBB very slowly. Although the BBB was intact at 4 hours in all regions, permeability was increased in the ipsilateral basal ganglia by 12 hours and to an even greater extent by 48 hours after ICH (Fig. 5 lower). Despite significant edema in the contralateral basal ganglia, BBB permeability remained normal.

In the ipsilateral cortex, BBB permeability was modestly increased in both the sham-operated and the experimental animals. This probably represents local damage from the needle, which passed through the cortex to reach the caudate nucleus. However, by 48 hours, BBB permeability in the ICH group was approximately twice that of the sham-operated group and that in the contralateral cortex was unaffected by the ICH.

In order to quantitate BBB permeability using radiotracers, it is necessary to correct total brain radioactivity for the portion of radiotracer present within the cerebral vasculature. This requires separate measurements of the plasma volume using a high-molecular-
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TABLE 2

Regression analysis of brain [Na] + [K]/plasma [Na] + [K] vs. brain water content

<table>
<thead>
<tr>
<th>Time Group &amp; Region</th>
<th>( r^2 ) Value</th>
<th>Slope</th>
<th>( p ) Value for Slopes Different From 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>early period (immediately &amp; 4 &amp; 12 hrs)</td>
<td>0.854</td>
<td>0.84 ± 0.06</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ipsilateral basal ganglia</td>
<td>0.796</td>
<td>1.11 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>contralateral basal ganglia</td>
<td>0.642</td>
<td>0.77 ± 0.10</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ipsilateral cortex</td>
<td>0.743</td>
<td>0.90 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>contralateral cortex</td>
<td>0.848</td>
<td>0.79 ± 0.04</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>maintenance period (≥ 24 hrs–4 days)</td>
<td>0.789</td>
<td>0.97 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>ipsilateral basal ganglia</td>
<td>0.470</td>
<td>0.64 ± 0.10</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>contralateral basal ganglia</td>
<td>0.393</td>
<td>0.73 ± 0.12</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ipsilateral cortex</td>
<td>0.470</td>
<td>0.64 ± 0.10</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>contralateral cortex</td>
<td>0.393</td>
<td>0.73 ± 0.12</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

* Brain [Na] + [K]/plasma [Na] + [K] was regressed against brain water content as shown in Fig. 4. Values for \( r^2 \) and the slope (means ± standard errors of the means) were calculated. The \( p \) values indicate the level of significance for the null hypothesis that the slope is different from 1. NS = not significant.

A weight tracer such as \( ^{14} \text{C} \)-inulin, which remains within the vascular space. Unfortunately, when the BBB is damaged, some intravascular tracer may escape into the brain. This problem can be minimized but not eliminated by using short circulation times for the tracer. However, even under the best circumstances, the plasma volume is usually overestimated when the BBB is grossly disrupted. The plasma volumes measured in the present experiments illustrate this problem (Table 3). Apparent increases in plasma volume are seen in samples showing increased BBB permeability. While a true increase in plasma volume may have occurred, it is more likely that the plasma volume in these samples was overestimated and, consequently, the calculated BBB permeability data were underestimated. Therefore, the actual changes in BBB permeability may be greater than those shown in Fig. 5 lower.

![Graphs showing changes following intracerebral hemorrhage (ICH) in samples of tissue from the basal ganglia (left) or cortex (right) that were ipsilateral (squares) or contralateral (circles) to either injection of 100 \( \mu \)l of autologous blood (solid symbols) or a sham procedure (open symbols). Values shown are means ± standard error of the means for six animals. Significance of difference: * = \( p < 0.05 \), † = \( p < 0.01 \), and ‡ = \( p < 0.001 \), comparing the ICH group with the sham-operated group at the same time point based upon a two-tailed Student t-test. Upper: Cerebral blood flow measured using \( ^{14} \text{C} \)-iodoantipyrine. Lower: Measurement of blood-brain barrier (BBB) permeability to \( ^{3} \text{H} \)-\( \alpha \)-aminoisobutyric acid (AIB).](image)

**Discussion**

Spontaneous ICH is a relatively common neurosurgical emergency associated with significant morbidity and mortality. The pathophysiological mechanisms are thought to include an increase in intracranial pressure (ICP) as a result of the hematoma mass, with a sub-

TABLE 3

<table>
<thead>
<tr>
<th>Group &amp; Region</th>
<th>4 Hrs</th>
<th>12 Hrs</th>
<th>24 Hrs</th>
<th>48 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham-operated</td>
<td>6.69 ± 1.03</td>
<td>6.85 ± 0.80</td>
<td>7.45 ± 1.09</td>
<td>6.40 ± 0.78</td>
</tr>
<tr>
<td>ipsilateral basal ganglia</td>
<td>6.39 ± 0.34</td>
<td>6.14 ± 0.54</td>
<td>6.29 ± 0.51</td>
<td>5.86 ± 0.50</td>
</tr>
<tr>
<td>contralateral basal ganglia</td>
<td>7.15 ± 0.76</td>
<td>6.19 ± 0.70</td>
<td>7.25 ± 0.79</td>
<td>6.99 ± 0.63</td>
</tr>
<tr>
<td>ipsilateral cortex</td>
<td>7.04 ± 0.82</td>
<td>6.70 ± 0.69</td>
<td>7.55 ± 0.78</td>
<td>6.53 ± 0.39</td>
</tr>
<tr>
<td>contralateral cortex</td>
<td>7.03 ± 0.58</td>
<td>8.71 ± 1.12</td>
<td>10.89 ± 1.92</td>
<td>12.47 ± 1.58†</td>
</tr>
<tr>
<td>intracerebral hemorrhage</td>
<td>6.67 ± 1.00</td>
<td>6.65 ± 0.53</td>
<td>6.96 ± 0.64</td>
<td>6.71 ± 0.64</td>
</tr>
<tr>
<td>ipsilateral basal ganglia</td>
<td>8.06 ± 0.68</td>
<td>8.84 ± 0.76</td>
<td>8.95 ± 0.33</td>
<td>11.06 ± 1.13†</td>
</tr>
<tr>
<td>contralateral basal ganglia</td>
<td>6.54 ± 0.53</td>
<td>7.58 ± 0.67</td>
<td>7.22 ± 0.36</td>
<td>6.84 ± 0.54</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for six rats in each group.
† Significance of difference: \( p < 0.01 \), comparing sham-operated and intracerebral hemorrhage groups using a two-tailed Student t-test.


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sequent focal reduction in blood flow producing ische-

mia; however, toxic substances that accentuate the tis-
sue injury may also be released from the hematoma. 

The patient who survives the initial event may later show deterioration as brain edema develops and the ICP increases further. While aggressive removal of the blood clot is advocated by some, supportive care without clot removal is recommended by others. This dichotomy in therapeutic approaches undoubtedly derives from a poor understanding of the pathological mechanisms that injure brain following ICH. Thus, animal models of ICH have been devised so that injury mechanisms can be identified and specific therapy developed.

Experimental models of ICH have been available since the 1960's and commonly involve the injection of autologous blood into the frontal lobe of anesthe-
tized dogs, cats, or monkeys. Intracranial pressure, edema, and histology were initially studied. More recently, rodents have been found to provide equally convenient and suitable models. They are more desirable because of the lower cost of rodents and procedures, the relative homogeneity within strains owing to inbreeding, the close resemblance of the cerebrovascular anatomy and physiology to that of higher species, and a small brain size well suited to rapid fixation procedures for biochemical analysis.

Several different approaches to the development of rodent models of ICH have been described. The ICH has been produced by connecting a femoral artery catheter to a needle inserted into the caudate nucleus. When the catheter is opened, blood flows into the caudate nucleus until the flow is arrested by the increase in pressure. This model was designed to mimic the natural events that occur with spontaneous ICH in humans; however, it produces hematomas of varying size, making quantitation of injury difficult. Nath, et al. used rapid injection (approximately 10 seconds) of a measured amount of blood at a fixed pressure of 100 mm Hg to simulate spontaneous hemorrhage. Others have used a microballoon inflated to 50 μl to simulate the mass effect caused by ICH injury in rats. Because the microballoon can be deflated, it can also be used to study the possible benefit of removal of the mass. More recently, Rosenberg, et al. reported collagenase-induced ICH in rats and found that hematomas were formed by 4 hours, the size of the hematomas depending on the amount of collagenase injected. Collagenases are proteolytic enzymes that digest the col-
gen present in the basal lamina of blood vessels; while dissolution of the basal lamina of blood vessels may contribute to the hemorrhages that occur in the germinal matrix of premature infants, this is probably not the mechanism that produces ICH in adults.

We chose to inject fresh autologous blood into the caudate nucleus as described by Masuda, et al., because it produces a controllable and reproducible lesion that lends itself to quantitative measurement. Furthermore, the injurious effects of ICH may result from more than simple mass effect, so whole blood is preferred over a microballoon. Our injection rate was slower and at a lower pressure than that used by Nath, et al.; however, we found that a more rapid injection rate resulted in a variable reflux of blood along the needle track and poorly reproducible lesions.

Brain edema is a clinically important consequence of ICH and its presence may determine the patient’s ultimate fate. However, the formation of edema following ICH has not been well studied. Brain edema is generally divided into cytotoxic and vasogenic types, depending upon whether the BBB is intact or disrupted. In the presence of an intact BBB, however, increased tissue water content can occur even though there is no direct cytotoxicity. For example, edema may spread from an area where the BBB is open to an area where it is intact, or the local generation of osmolar substances that cannot escape from the brain may lead to an influx of water. These different forms of intact BBB edema can be distinguished by examining the changes in tissue cation contents and their relationship to the changes in water content. Cytotoxicity leads to impairment of energy metabolism and a loss of the normal ion gradients between the intracellular and extra- cellular compartments. As extracellular potassium increases, it is lost across the BBB in exchange for sodium. However, since this exchange is mediated by Na,K-adenosine triphosphatase, three sodium ions are taken up for every two potassium ions that are lost, resulting in a net increase in brain cations that causes water accumulation. Thus, brain edema that results from cytotoxicity is characterized by an increase in sodium and a loss of potassium, with the water accumulated accounted for by the overall change in cation content. In contrast, edema that results from opening of the BBB is similar to plasma in its composition (that is, high in sodium and low in potassium). Thus, if va-

sogenic edema flows into an area where the BBB is intact, it will be accompanied by an increase in sodium content but there will be no loss of potassium. Finally, edema that results from the generation of osmolytes other than ions is characterized by an increase in water content that is not accompanied by changes in the ion content.

Our results indicate that the amount of edema increases progressively in hemorrhagic brain tissue during the first 24 hours after ICH. Edema also develops with some delay in more distant structures such as the ipsilateral cortex and the contralateral basal ganglia; even the contralateral cortex is affected to a mild degree. Edema formation is accompanied by a large and progressive increase in tissue levels of sodium and chloride but little change in the potassium content. We found that edema formation was maximum by 24 hours after ICH and remained relatively constant for 4 days before beginning to resolve.

The time course of edema formation following ICH in the rat bears a general resemblance to that of edema formation during focal ischemia. Menzies, et al., showed that following middle cerebral artery occlusion in rats, brain edema develops progressively over 24 hours, remains relatively constant for 3 days, and then resolves over a period of several weeks. Edema formation during focal ischemia is caused entirely by net changes in cations. During the cytotoxic phase (the first
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4 to 6 hours), there is a large increase in sodium as well as a large decrease in potassium content; however, the gain in sodium exceeds the loss of potassium, resulting in a net gain in cations, which accounts for the edema formation. After the BBB breaks down (the vasogenic phase), the gain in sodium becomes even more important relative to potassium loss. Although there is a substantial influx of albumin during the vasogenic phase, the change in cations continues to account for the edema completely.

Despite the similarity in time course, the mechanism of edema formation following ICH appears to be more complex. In tissue immediately surrounding the hemorrhage, osmoles other than cations play a role in the development of edema as shown by the scatterplots in Fig. 4. This probably represents the generation of osmotically active molecules as the clot is degraded. During the first 12 hours, the increase in brain sodium content without a loss of potassium is similar to the pattern observed in ischemia after the BBB breaks down, suggesting the presence of vasogenic edema. This was confirmed by the increased BBB permeability in the tissue immediately surrounding the clot. By 24 hours, there is a significant loss of potassium from the tissue. Since massive loss of potassium is commonly seen during the cytotoxic phase of ischemic edema, these results suggest that ischemia or cellular toxins may contribute to edema formation at later times after ICH. Thus, edema that forms in the immediate vicinity of the clot appears to have both vasogenic and cytotoxic components as well as contributions from the local generation of osmoles. In contrast, edema seen in the more distant structures, such as the contralateral basal ganglia, appears to result from the spread of vasogenic edema fluid since it is totally accounted for by changes in sodium while the permeability of BBB remained normal. Edema in these areas appears to result from infiltration of fluid from areas with leakage in the BBB.

Remaining questions include the cause of local BBB injury and the mechanism of cytotoxicity following ICH. Ischemia could play a role in both of these since continuous incomplete ischemia produces an early intact BBB type of edema and a later open BBB form of edema. Transient incomplete ischemia results in the delayed development of edema and BBB disruption. Nath, et al., showed an immediate but short-lived reduction of CBF to ischemic levels (< 25 ml/100 gm/min) both in areas surrounding the clot and in more distant regions following the injection of 25 to 100 µl of blood. Our results also show an early transient decrease in CBF (Fig. 5 upper). While the lowest CBF values obtained in our study would not generally be considered ischemic, they represent the average values for a 60- to 100-cu mm sample of brain, which is likely to be quite heterogeneous and include areas of very low flow and areas of nearly normal flow. Furthermore, our earliest time point was 1 hour and the lowest flow may have occurred before this time. However, by 3 to 4 hours the CBF was nearly normal in our study and in that of Nath, et al. Transient ischemia lasting 1 hour or more could account for the later appearance of brain edema and BBB disruption, while ischemia lasting less than 30 minutes probably would not account for large changes seen in the animals sacrificed at 24 hours in our study. Thus, it is possible that the period of transient ischemia seen immediately after ICH does not explain the large amount of edema present in the animals sacrificed at 24 hours.

We also observed a second decrease in CBF following a period of near normal flow (Fig. 5 lower). In the area immediately surrounding the clot, this delayed reduction in CBF appears to be a consequence rather than a cause of the edema development since the water content did not increase further in response to reduced blood flow. However, delayed ischemia could have contributed to further damage in the ipsilateral cortex as edema appears to worsen and the BBB opens (Fig. 5 lower) when the CBF falls at 48 hours.

Instead of (or in addition to) ischemia, toxic substances could be released from the clot and cause local cytotoxicity and BBB disruption. Clot-derived vasoconstrictors could also be responsible for the reduced CBF. Indeed, studies of CBF following the inflation of a microballoon in the caudate nucleus suggest that an equal volume of blood produces a greater effect. However, the microballoon does not appear to mimic the mass effect of the hemorrhage perfectly since CBF deteriorates progressively following microballoon inflation, whereas it returns to normal after the injection of blood. Furthermore, the mass effect of a hemorrhage is probably not static as is the case with the microballoon because, as blood coagulates, it forms serum as well as a thrombus. The serum should be able to flow away from the clot. Thus, identification of a role for toxic substances in brain edema formation following ICH will require the development of an inert mass that closely simulates the mechanical properties of a blood clot and/or the use of pharmacological agents to block the effects of putative toxins.

In summary, these results demonstrate a time course for the formation and resolution of brain edema following ICH. Besides local increases in water content near the hemorrhage, there is considerable edema in distant brain regions. The edema that forms in the immediate vicinity of the clot is caused by BBB disruption, cytotoxicity, and the generation of osmotically active substances, while that in more distant regions results from spread of vasogenic edema fluid.

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References

3. Blasberg RG, Fenstermacher JD, Patlak CS: Transport of α-aminoisobutyric acid across brain capillary and cellular
haemorrhage induced at arterial pressure in the rat. Part 1: description of technique, ICP changes and neuropathological
findings. Neurosurg 6:184–188, 1984
5. Debbin J, Crockard HA, Ross-Russell R: Transient blood-brain barrier permeability: following profound temporary
global ischaemia: an experimental study using 14C-AIB. J
1975
7. Goldstein GW: Pathogenesis of brain edema and hemor-
rhage: role of the brain capillary. Pediatrics 64:357–360,
1979
hematomas: imaging by high-field MR. Radiology 157:
87–93, 1985
following occlusion of the middle cerebral artery in the rat.
I: The time courses of the brain water, sodium and potassium
contents and blood-brain barrier permeability to 14C-albu-
10. Hatashita S, Hoff JT: Brain edema and cerebrovascular
permeability during cerebral ischemia in rats. Stroke 21:
582–588, 1990
11. Ito U, Ohno K, Nakamura R, et al: Brain edema during is-
chemia and after restoration of blood flow. Measurement of
water, sodium, potassium content and plasma protein per-
of ultra-early operation for hypertensive intracerebral
hypertensive intracerebral hematoma. A comparative study of
305 nonsurgical and 154 surgical cases. J Neurosurg 61:
1091–1099, 1984
model of intracerebral hematoma. Acta Neurochir 65:
315–321, 1985
safety of tissue plasminogen activator. Neurosurgery 20:
403–407, 1987
16. Kaufman HH, Schochet SS: Pathology, pathophysiology,
and modeling, in Kaufman HH (ed): Intracerebral Hema-
intracerebral mass: time-related effects on local cerebral
file of serum albumin extravasation following cerebral isca-
emia in a newly established reproducible gerbil model for
vasogenic brain edema: a combined immunohistochemical
dye tracer analysis. Acta Neuropathol 82:164–171,
1991
19. Klatzo I: Neuropathological aspects of brain edema. J Neu-
ropathol Exp Neurol 26:1–14, 1967
of the blood-brain barrier to proteins following temporary
middle cerebral artery occlusion. Acta Neuropathol 68:
122–129, 1985
activity in experimental intracerebral hematoma. J Neuro-
surg 68:274–278, 1988
22. Mendelow AD, Bullock R, Nath FP, et al: Experimental in-
tracerebral haemorrhage: intracranial pressure changes and
cerebral blood flow, in Miller JD, Teasdale GM, Rowan JO
(eds): Intracranial Pressure VI. Berlin: Springer-Verlag,
1986, pp 515–520
haemorrhage induced at arterial pressure in the rat. Part 2: short-term changes in local cerebral blood flow measured
24. Menzies SA, Betz AL, Hoff JT: Contributions of ions and
albumin to the formation and resolution of ischemic brain
edema. J Neurosurg 78:257–266, 1993
25. Nath FP, Jenkins A, Mendelow AD, et al: Early hemody-
namic changes in experimental intracerebral hemorrhage.
J Neurosurg 65:697–703, 1986
intracerebral hemorrhage on blood flow, capillary perme-
intracerebral hemorrhage: early removal of a spontaneous
mass lesion improves late outcome. Neurosurgery 27:
674–682, 1990
intracerebral hemorrhage: progression of hemodynamic
changes after production of a spontaneous mass lesion.
30. Ropper AH, King RB: Intracranial pressure monitoring in
catamnose patients with cerebral hemorrhage. Arch Neurol
41:725–728, 1984
ase-induced intracerebral hemorrhage in rats. Stroke 21:
801–807, 1990
32. Schielpke GP, Moises HC, Betz AL: Blood to brain sodium
transport and interstitial fluid potassium concentration dur-
ing early focal ischemia in the rat. J Cereb Blood Flow
Metab 11:466–471, 1991
intracerebral hemorrhage: effects of a temporary mass le-
34. Sussman BJ, Barber JB, Goald H: Experimental intracere-
bral hematoma. Reduction of oxygen tension in brain and
35. Takanugi S, Ueda S, Matsumoto K: Chronological changes
in spontaneous intracerebral hematoma — an experimental
36. Van Uitert RL, Levy DE: Regional brain blood flow in the
tracerebral hematomas. A new proposal about the usefulness
and limits of surgical treatment. Neurosurgery 15:
663–666, 1984
38. Wallenstein S, Zucker CL, Fleiss JL: Some statistical meth-
39. Warner DS, Smith ML, Siesjö BK: Ischemia in normo-
and hyperglycemic rats: effects on brain water and electrolytes.
40. Whisnant JP, Sayre GP, Millikan CH: Experimental intra-