Acute changes in regional brain water content following experimental closed head injury

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A Remington humane stunner was used to deliver blows to the skulls of anesthetized cats. The animals were sacrificed at 30 minutes or 1, 2, or 6 hours after trauma and selected for data collection on the basis of the following two categories of gross intracranial pathology: 1) unilateral contusion, with subarachnoid hemorrhage (SAH); or 2) SAH only. For selected cats, specific gravity was measured in 5- to 10-mg samples of uncontused tissue taken from coronal slices at the level of the frontoparietal suture. The regions tested included dorsal cerebral cortex, subcortical white matter, deep white matter, and caudate nucleus. Specific gravity data from injured animals were compared with those from similar areas in uninjured anesthetized cats to test for cerebral edema. At 30 minutes after head injury, contused hemispheres had significant edema of all areas tested except the caudate nucleus. Edema of the subcortical and deep white matter increased with time after the injury. Increase in water content of the cerebral cortex was transient and appeared unrelated to contusion. The caudate nucleus was edematous only at 6 hours, suggesting movement of fluid from the deep white matter compartment into that nucleus. The hemispheres opposite the contusion and those related to SAH had, with one exception, an absence of edema in the white matter and caudate nucleus, but a transient increase in water content of the cerebral cortex. These findings suggest that, in the presence of contusion, cerebral edema can contribute to brain swelling as early as 30 minutes after closed head trauma. In addition, a transient and minimal cortical edema, perhaps related to ischemia, occurred in all groups of hemispheres examined.

KEY WORDS • experimental head injury • cerebral edema • cerebral contusion • specific gravity • cerebral ischemia

RECENT widespread use of computerized tomography has provided evidence that brain swelling can occur very shortly after severe head injury. Indeed, diffuse brain swelling, as evidenced by collapse of the ventricular system, has been reported as early as 20 to 30 minutes after closed-head trauma.16,24 The basis for early brain swelling has yet to be well defined. Acute intracranial vascular dilatation, cerebral edema, or a combination of the two are the most likely causes.16,17,24

Although data accumulated from the use of cold injury have suggested that it takes several hours for measurable traumatic edema fluid to collect,4,5,7,24,28 examinations for the presence or absence of brain edema shortly after mechanical insult to the brain have been sparse. In a report on the use of a fluid percussion injury in the cat, brain edema was not found at 5 and 30 minutes after trauma.5

In the present study, four regions of the brain were tested for the presence of brain edema at 30 minutes to 6 hours after experimental closed-head trauma. The model used involved mechanical impact to the closed skulls of anesthetized cats from a Remington humane stunner. Due to differing skull characteristics of individual animals, this injury resulted in variable intracranial pathology. In previous studies with this model, animals were selected on the basis of gross pathological findings of unilateral contusion. In the present investigation, data were collected from animals suffering unilateral contusion and also from animals with subarachnoid hemorrhage (SAH) only, in order to determine what effect may be due to this component of severe head trauma.

Animals were tested for regional brain edema at 30 minutes or 1, 2, or 6 hours after cranial impact. Brain edema was measured by determination of change in
brain density from values of uninjured anesthetized control animals.

**Materials and Methods**

**Animal Preparation**

Adult mongrel cats of both sexes, 2.2 to 4.6 kg in weight, were subjected to cranial impact. Preparation for injury included sedation with ketamine hydrochloride (20 mg/kg, administered intramuscularly), reflection of the skin, fascia, and temporalis muscles from the dorsum of the skull, and intravenous injection of Evans blue dye (2.5% in distilled water) in a dose of 1.0 cc/kg. Each animal was placed in a Plexiglas holder containing a fixed Remington humane stunner.* This device contains a piston fitted at one end with an impacting disc, with characteristics that have been described previously. The animal's head was placed on a compressed aluminum foil support that permitted movement of the head in response to impact. The positioning of the cat's head before impact differed from that described previously, in that the head was rotated along the rostrocaudal axis approximately 10°, with the right side of the head positioned superior to the left. In addition, the head was rotated to the left approximately 15° using the frontoparietal suture as a pivot point. With these rotations, the center of the striking disc was above the medial aspect of the right side of the skull and 3 mm rostral to the frontoparietal suture prior to impact. The animal's head was then impacted by detonation of the stunner disc, using a .22-caliber blank cartridge.

The time interval between cranial impact and the next breath of the animal was measured in order to evaluate length of respiratory arrest with this model. Animals with apnea of greater than 5 minutes were excluded from the study. Following cranial impact, each animal was maintained at a temperature of 38°C, using a rectal thermometer, temperature controller, and heating pad, until the time of sacrifice. At 30 minutes or 1, 2, or 6 hours after head injury, animals were sacrificed under ketamine anesthesia by immersion of the head in liquid nitrogen.

In a separate group of head-injured cats, preparation included monitoring for arterial pressure, intracranial pressure (ICP), and blood gases. Arterial pressure and ICP were monitored before head injury and for 6 hours after. Blood gases were measured before impact, at 5 minutes following trauma, and at hourly intervals until sacrifice.

Control animals for this study consisted of uninjured cats subjected to ketamine sedation, exposure of the skull, and intravenous injection of Evans blue dye. These animals were sacrificed after 30 minutes or 6 hours of ketamine anesthesia by immersion of the head in liquid nitrogen.

The frozen heads of all animals were sliced coronally at 5-mm intervals, using a band saw. Five slices, at levels described previously, were cleaned while frozen, and immediately placed in a Petri dish containing kerosene to permit thawing without evaporation of water. Each slice was photographed and then examined for gross pathology using a dissecting microscope.

**Animal Selection**

Head-injured animals were rejected from the study prior to sacrifice on the basis of early death, postimpact apnea of greater than 5 minutes, and poor respiratory patterns following injury. Further selection of the sacrificed animals was made at the time of inspection of brain slices for gross pathology, with inclusion of only those animals that demonstrated one of the following: 1) unilateral contusion, as evidenced by the presence of contusion hemorrhage; or 2) SAH only.

**Data Collection**

All brain slices taken from each selected animal were examined with a dissecting microscope for location of extravasation of plasma protein, as evidenced by visible parenchymal Evans blue dye. From the brain slice taken at the level of the

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FIG. 1. Coronal section through the frontoparietal suture in a cat sacrificed 6 hours after cranial impact. The large contusion on the left extends caudal to the frontoparietal suture and involves the cerebral cortex, subcortical white matter, and deep white matter. Evans blue dye stains the lateral area of the head of the caudate nucleus. Sampling sites for density determinations are represented by paired black dots.
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frontoparietal suture, bilateral duplicate samples of 5 to 10 mg were removed from the following areas and tested for specific gravity after a 5-minute drop in an organic density gradient: cerebral cortex of the gyrus lateralis; subcortical white matter deep to the gyrus lateralis; deep white matter of the centrum semiovale; or head of the caudate nucleus. Samples were taken at the level of the frontoparietal suture because, for many animals with unilateral contusion, the caudal limit of contused tissue was at that level. Thus, routine sampling of uncontrolled tissue was usually possible in that area. The precise position of sampling is demonstrated in Fig. 1.

For all animals in this study, all samples tested were without evidence of parenchymal hemorrhage. One animal had contusion of the gyrus lateralis, and samples from that area were excluded from the study.

Data Analysis

Specific gravity data from control animals sacrificed at 30 minutes and 6 hours after anesthesia were compared to determine the effect of prolonged anesthesia on regional brain density. Data from head-injured animals sacrificed at 30 minutes, 1 hour, and 2 hours were compared with those from 30-minute control cats to determine regional change in brain water content from control values. Data from 6-hour head-injured cats were compared with those from six-hour control animals.

A single density value for each area measured was determined for each animal with SAH only, by averaging bilateral density values for that point. For animals with unilateral contusion, data from affected and unaffected hemispheres were evaluated separately.

Data concerning change in brain density from control values (Table 1) were converted to change in tissue volume as water (Table 2), using the Nelson equation:

\[
\% \text{ change in tissue volume as water} = \frac{(SG_t - 1) \text{ con} - (SG_t - 1) \text{ exp}}{(SG_t - 1) \text{ exp}} \times 100,
\]

where SG_t = mean value, specific gravity of brain tissue, con = control group, and exp = experimental group.

With this equation, change in tissue volume is calculated from the density data, with the presumption that change in brain density is due to the addition of water only. The volume changes reported in Table 2, therefore, represent minimal changes in tissue volume.

The Wilcoxon nonparametric test was used to evaluate differences between groups.

Results

Postimpact Responses

Immediately following cranial impact, almost all animals in this study demonstrated temporary respiratory arrest. Excluding animals with apnea of greater than 30 minutes, the following respiratory responses were observed.

| TABLE 1 |
| Regional specific gravity in uninjured control and head-injured cats* |

<table>
<thead>
<tr>
<th>Source of Tissue Tested</th>
<th>Time After Injury or Anesthesia</th>
<th>No. of Cats</th>
<th>Area Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lateral Gyrus (cortex)</td>
</tr>
<tr>
<td>anesthetic control</td>
<td>30 min 7</td>
<td>1.0488 ± .0004</td>
<td>1.0496 ± .0003</td>
</tr>
<tr>
<td></td>
<td>6 hrs 7</td>
<td>1.0485 ± .0005</td>
<td>1.0491 ± .0004</td>
</tr>
<tr>
<td>SAH only</td>
<td>30 min 4</td>
<td>1.0485 ± .0006</td>
<td>1.0495 ± .0004</td>
</tr>
<tr>
<td></td>
<td>1 hr 4</td>
<td>1.0478 ± .0005</td>
<td>1.0476 ± .0007</td>
</tr>
<tr>
<td></td>
<td>2 hrs 3</td>
<td>1.0470 ± .0002†</td>
<td>1.0481 ± .0008</td>
</tr>
<tr>
<td></td>
<td>6 hrs 4</td>
<td>1.0479 ± .0008</td>
<td>1.0487 ± .0004</td>
</tr>
<tr>
<td>unilateral contusion</td>
<td>30 min 9</td>
<td>1.0472 ± .0006†</td>
<td>1.0478 ± .0006†</td>
</tr>
<tr>
<td>contused side</td>
<td>1 hr 6</td>
<td>1.0459 ± .0010†</td>
<td>1.0471 ± .0006†</td>
</tr>
<tr>
<td></td>
<td>2 hrs 8</td>
<td>1.0463 ± .0009†</td>
<td>1.0467 ± .0009†</td>
</tr>
<tr>
<td></td>
<td>6 hrs 8</td>
<td>1.0474 ± .0007</td>
<td>1.0454 ± .0013†</td>
</tr>
<tr>
<td>uncontused side</td>
<td>30 min 9</td>
<td>1.0478 ± .0004</td>
<td>1.0487 ± .0005</td>
</tr>
<tr>
<td></td>
<td>1 hr 6</td>
<td>1.0472 ± .0004†</td>
<td>1.0484 ± .0001</td>
</tr>
<tr>
<td></td>
<td>2 hrs 8</td>
<td>1.0473 ± .0005</td>
<td>1.0487 ± .0005</td>
</tr>
<tr>
<td></td>
<td>6 hrs 8</td>
<td>1.0483 ± .0007</td>
<td>1.0482 ± .0006</td>
</tr>
</tbody>
</table>

* Each value reported is a mean for the group ± SEM. SAH = subarachnoid hemorrhage.
† Significantly different from control values for the same region, p < 0.05.

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TABLE 2
Percentage increase in regional tissue volume from control values

<table>
<thead>
<tr>
<th>Source of Tissue Tested</th>
<th>Time after Injury</th>
<th>Area Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cerebral Cortex</td>
</tr>
<tr>
<td>SAH only</td>
<td>30 min</td>
<td>0.6%</td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>2.1%</td>
</tr>
<tr>
<td></td>
<td>2 hrs</td>
<td>3.8%</td>
</tr>
<tr>
<td></td>
<td>6 hrs</td>
<td>1.3%</td>
</tr>
<tr>
<td>unilateral contusion</td>
<td>30 min</td>
<td>3.4%</td>
</tr>
<tr>
<td>contused side</td>
<td>1 hr</td>
<td>6.3%</td>
</tr>
<tr>
<td></td>
<td>2 hrs</td>
<td>5.4%</td>
</tr>
<tr>
<td></td>
<td>6 hrs</td>
<td>2.3%</td>
</tr>
<tr>
<td>uncontused side</td>
<td>30 min</td>
<td>2.1%</td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>3.4%</td>
</tr>
<tr>
<td></td>
<td>2 hrs</td>
<td>3.1%</td>
</tr>
<tr>
<td></td>
<td>6 hrs</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

* Values calculated from density data reported in Table 1, using the Nelson equation. SAH = subarachnoid hemorrhage.

than 5 minutes, which were categorized as "instant deaths," the mean duration of respiratory arrest was 58.9 ± 8.1 seconds. There was no correlation between length of respiratory arrest and either gross intracranial pathology or brain density data.

For animals subjected to blood gas and pressure monitoring, neither hypoxia nor hypercapnia was seen at 5 minutes after head injury, or up to 6 hours after trauma. Immediately following impact, a transient and substantial increase in ICP was seen, generally accompanied by a modest decrease in systemic arterial pressure. Later, ICP responses were variable. Substantial decreases in cerebral perfusion pressure, as determined by the difference between mean systemic arterial pressure and ICP, were not seen. The effects of impact to the skull on ICP, arterial pressure, and blood gases will be described in detail in a separate communication.

Gross Pathology

Thirty-one head-injured animals demonstrated unilateral contusion, as defined by gross evidence of contusion hemorrhage in both the cerebral cortex and underlying white matter. For most of these animals, contusion hemorrhages were located in the frontal area of the right cerebral hemisphere, including the cerebral cortex of the sigmoid, coronal, anterior suprasylvian, and anterior ectosylvian gyri, and the white matter deep to these gyri. In 15 animals, the caudal extent of contusion was near the level of the frontoparietal suture. In six animals, the injury was limited to the rostral frontal lobe. In all animals with unilateral contusion, there was also gross evidence of SAH. Fifteen injured cats demonstrated SAH only.

Evans Blue Data

Visible parenchymal Evans blue dye was seen primarily, but not exclusively, in relation to contusion hemorrhage.

For the contused hemispheres, the cerebral cortex adjacent to foci of cortical contusion was usually stained with Evans blue. The distance of spread of the dye within cerebral cortex was small, usually approximately 1 mm at 30 minutes after trauma and up to 2 to 3 mm by 6 hours. In the white matter, the peripheral spread of Evans blue from white matter foci of hemorrhage was greater than that seen in the cerebral cortex at all time periods tested. At 30 minutes after head injury, visible dye had spread as far as 3 mm from the edge of isolated white matter contusion. By 6 hours after trauma, the dye had spread from foci of contusion throughout much of the deep white matter compartment and adjacent subcortical white matter at the rostrocaudal level of contusion. At that time, there was also up to 9 mm of caudal spread of the dye in the deep white matter compartment from the caudal edge of contusion.

In addition to Evans blue staining related to contusion hemorrhage, a number of contused hemispheres showed isolated small wedges of cerebral cortex that were deeply stained with Evans blue dye, usually located at the crest of one of the lateral gyri.
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Thorough dissection of these areas of cortical staining failed to reveal evidence of petechial hemorrhage.

For most hemispheres opposite contusion, or with SAH only, Evans blue dye could not be detected anywhere in the cerebral hemispheres. An exception to this finding was the presence, in a small number of hemispheres of both groups, of isolated wedges of Evans blue stain in the cerebral cortex, similar to the foci of cortical stain seen in contused hemispheres, that appeared unrelated to hemorrhage.

Specific Gravity Data

Specific gravity values for uninjured control animals subjected to ketamine anesthesia for 6 hours were statistically similar for all areas tested to those of animals anesthetized for 30 minutes prior to sacrifice.

Hemispheres with contusion demonstrated a modest but significant decrease in density (increase in tissue volume) as compared to control values for all areas tested except the caudate nucleus at 30 minutes after head injury (Tables 1 and 2). At 1 and 2 hours after head injury, contused hemispheres showed data that were statistically similar to those seen at 30 minutes, with a trend toward increase in tissue volume for subcortical and deep white matter. At 6 hours following cranial impact, density of the cortex of the gyrus lateralis was similar to control values, the deep white matter showed a substantial increase in tissue volume, and the caudate nucleus demonstrated significant edema for the first time (Tables 1 and 2).

For the uncontused hemispheres of animals with unilateral contusion, there was no change in tissue volume of any area tested at any time period examined, with the following exceptions: the deep white matter demonstrated a slight but significant increase in tissue volume at 30 minutes after head injury, and the gyrus lateralis was slightly edematous at 1 hour after impact (Tables 1 and 2).

Tissue volume was similar to control values for all areas tested at all time periods examined in animals suffering SAH only, with the exception of a small but significant increase in tissue volume of the gyrus lateralis at 2 hours following head injury (Tables 1 and 2).

Discussion

Many features of "traumatic" cerebral edema have been described from experimental studies involving a freezing insult to the cerebral cortex. The cold-injury model for vasogenic brain edema causes vascular damage that results in movement of fluid from the vascular compartment into the parenchyma of the brain. Edema fluid of cold origin is rich in protein and electrolytes, and travels from the area of cortical injury into the white matter compartment with preferential accumulation in the interstitial spaces of the white matter.

The relationship between "traumatic" brain edema resulting from a thermal insult and that caused by mechanical injury is unclear. Cold-injury edema, presumed to model vascular damage of contusion, results from a single focal insult that is not accompanied by the violent intracranial events of mechanical trauma. In the present study, acute brain edema was evaluated with a model involving impact to the closed skull, the type injury most frequently seen in human head trauma.

Evans blue dye was injected intravenously before injury to detect, by visualization of blue stain of brain tissue, extravasation of plasma protein into brain parenchyma resulting from vascular dysfunction. This simple technique provides clear evidence of vasogenic edema territory during the early time periods following trauma.

We usually saw a close correlation of Evans blue stain to areas of contusion hemorrhage, with preferential staining in white matter. These findings suggest a spread of vasogenic edema fluid from areas of contusion that is similar to that seen with the cold lesion. Additional staining was seen in isolated patches of uncontused cortex, most frequently in hemispheres with contusion and least frequently in hemispheres with SAH only. Although the precise basis for this edema remains speculative, these data suggest that following closed head trauma, vasogenic edema can develop outside areas of contusion hemorrhage.

Tissue density was measured in order to quantitate magnitude of edema at four fixed points in the cerebral hemisphere. The points chosen included superficial gray matter (gyrus lateralis), superficial white matter (subcortical), deep white matter (centrum semiovale), and deep gray matter (caudate nucleus). The rostrocaudal level chosen for sampling was, for many hemispheres with contusion, the level of the caudal limit of contused tissue. The gyrus lateralis was chosen for cortical sampling because, with one exception, that gyrus was outside areas of contusion.

The white matter areas tested in hemispheres with contusion had an increase in tissue volume at all time periods tested. These samples were usually stained with Evans blue dye that could be traced to areas of contusion, suggesting that edema fluid of the white matter compartment originated from damaged vessels of contusion sites. This conclusion is supported by findings of an absence of edema of the white matter in hemispheres with SAH, seen both in this study and in an investigation by Corales, et al.
The isolated finding of slight but measurable edema in the deep white matter contralateral to contusion is difficult to explain. White matter edema has been demonstrated contralateral to a cold injury in mice, and results from spread of fluid across the small corpus callosum of these animals. In the present study, edema fluid contralateral to contusion was not due to spread across the corpus callosum, since the density of the corpus callosum was similar to control values. It is concluded that this isolated finding results either from technical error or from an insult unrelated to contusion.

The density of the caudate nucleus was normal at 30 minutes to 2 hours after trauma. At 6 hours, this nucleus was edematous in contused hemispheres and was often stained with Evans blue dye that could be traced to contusion hemorrhage. These findings, as well as the absence of edema at 6 hours in hemispheres without contusion, suggest that edema of the caudate nucleus was due to spread of edema fluid from areas of contusion.

The gyrus lateralis was edematous at variable early time periods in all groups of hemispheres examined. This cortical edema was transient, of a small magnitude, and was usually associated with lack of Evans blue staining. In contused hemispheres, this gyrus was not near foci of contusion hemorrhage. These findings suggest that the edema of the gyrus lateralis resulted from insults independent of contusion.

The basis for the cortical edema seen in the present study remains speculative. We suspect that it may result from transient cortical ischemia. Ischemic brain edema is noteworthy for very rapid development, being measurable as early as 5 minutes after an ischemic insult. In the cat, rapid regression of ischemic edema occurs, with return to normal tissue water content within 2 to 4 hours following reperfusion.

It should be mentioned that substantial changes in cerebral perfusion pressure were never seen in animals monitored for systemic arterial pressure and ICP. Foci of ischemic damage following head trauma, with suggestion of adequate cerebral perfusion pressure, however, have been reported in both experimental and clinical studies.

We conclude that the brain edema of mechanical trauma is more widespread than that associated with the cold lesion and that further investigations using mechanical injuries are necessary in order to identify the specific factors involved in the pathogenesis of traumatic cerebral edema. It is also concluded that, at least in hemispheres with contusion, cerebral edema contributes to early brain swelling following closed head injury.

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References

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