Brain edema was produced in cats by a standardized cortical freezing lesion. With a careful microsurgical technique, the injured cortex was removed as a single piece, either immediately after induction or at 2, 4, or 8 hours after lesion production. The injured brain was either discarded or replaced in its bed. Brain edema and the defect in the blood-brain barrier were assessed by determining percent dry weight, increase in volume of white matter, and spread of Evans' blue by planimetry. The results indicate that 1) if the lesion is removed immediately after production, formation of the expected vasogenic brain edema is completely abolished; 2) replacement of the frozen brain is unable to induce significant increase in permeability of the surrounding blood-brain barrier or a significant amount of brain edema; and 3) if the lesion is removed at 2, 4, or 8 hours with or without replacement, advancement of the edema front and increase in the amount of edema is stopped. It appears that an intact vascular bed is necessary for the extracellular fluid component of brain edema, and that no edemagenic factors exist within the injured brain in this model that influence either the production or propagation of the increased extracellular fluid volume.

**Key Words**  - vasogenic brain edema  - cold-lesion dynamics

Brain edema may be classified into vasogenic, cytotoxic, and interstitial types. Invasogenic brain edema an incompetent blood-brain barrier allows extravasation of a plasma-like fluid into the extracellular space. Normally the functional extracellular space of the brain measures between 17% and 20% of the total volume. In vasogenic brain edema the extracellular space is expanded to a much larger volume. Once the fluid is in the brain extracellular space, further advancement of the edema front is primarily by bulk flow rather than diffusion; extravasated compounds with different molecular weights and diffusion coefficients travel together under the influence of hydrostatic pressure. There seems to be a free communication between the ventricular cerebrospinal fluid (CSF) and extracellular space of the brain, and much of the clearance of edema fluid apparently is into the ventricular system. Clearance is dependent on the hydrostatic pressure gradient between the capillaries of cortex and ventricular CSF. In cytotoxic brain edema the extracellular space is reduced, and there is cellular swelling probably due to the lack of functioning Na/K/Cl pumps and normal cellular metabolism. This seems to be due to a break in cellular energy production. The blood-brain barrier to protein is intact in cytotoxic brain edema. In interstitial brain edema there is fluid expansion of the periventricular extracellular space caused by increased ventricular pressure as in hydrocephalus.

There is much written about the dynamics of cryogenic vasogenic brain edema, but the exact pathogenesis of the defect in the blood-brain barrier and involvement of any active or neurohumoral factors in this process is not clear. These studies were undertaken to explore the effects of an intact capillary bed upon the development of brain edema and to begin to explore the possibility of edemagenic factors in the genesis of vasogenic edema.

**Materials and Methods**

**Experimental Design**

Mongrel male and female cats weighing 2 to 4 kg were anesthetized with ketamine (40 mg/kg) and placed in a head-holding frame. Through a midline sagittal incision, the skull was exposed and periosteum reflected laterally. With the aid of a hand trephine, a
1.5-cm bone button was removed 3 mm behind the coronal suture and 2 mm off the midline. A metallic device, $3 \times 10$ mm in area, was cooled to $-50\degree$ and placed on the medial ectosylvian gyrus for 60 seconds with the dura intact. After this lesion was produced the animals were randomly subdivided into six different experimental groups (Table 1):

Group 1: Sham-operated animals: skin and bone incisions only were made

Group 2: By the most atraumatic microsurgical technique, the cryogenic lesion was excised immediately after production and discarded; animals were sacrificed after 48 hours (Fig. 1)

Group 3: The lesion was excised en bloc using microtechniques immediately after production and then replaced in the excision bed; the experiments were terminated after 48 hours (Fig. 2)

Group 4: The lesion was left intact for 48 hours; edema developed without interference (Fig. 3)

Group 5: The lesion was excised microsurgically at 2, 4, or 8 hours after production and the animals were sacrificed at 24 hours

Group 6: The animals were sacrificed at 2, 4, 8, or 24 hours following lesion production without prior excision.

Shortly after the termination of each experiment the animals were infused with 1 ml/kg of a 2.5% solution of Evans' blue. Sacrifice of the animals was by intrathoracic exsanguination.

**Operative Details**

**Excision of the Cortical Lesion.** Under $\times 25$ to $\times 40$ magnification, the dura was opened and the cortical lesion was visualized. The pia mater around the lesion was incised circumferentially using fine microsurgical techniques. The entire pial vasculature of the lesion was interrupted using microscissors with microbipolar cautery. The lesion ($4 \times 11 \times 5$ mm in size) was removed from the subjacent white matter en bloc. When the lesion was out, the bed was washed, immaculate hemostasis obtained, and the dura sutured with 8-0 Prolene. The bone button was replaced, the scalp closed with 3-0 black silk suture, and the animal taken back to its cage without special care. If the lesion was to be replaced, the mass of excised tissue was immediately returned to the excision bed in proper anatomical position.

**Sham-Operated Animals.** Five animals had a bone button removed; the dura opened longitudinally and then sutured back microsurgically. The bone button was left in place and the scalp closed. These animals were sacrificed at 48 hours for percent dry weight determination of the subjacent white matter beneath the exposed cortex.

**Control Animals.** The white matter of the opposite untouched hemisphere was used for determination of the normal water content of cortex and white matter.
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Edema Determination

Determination of Water Content of White Matter. Samples of the white matter, 0.5 mm distant from the lesion’s margins and 2 × 2 × 2 mm in volume, were taken and their wet weight determined. After placing them in a 50°C drying oven for 24 hours, the samples were again weighed. The percent dry weight (dry weight/wet weight × 100) was the measure of water content of the samples. The swelling of the tissue was computed by the Elliot and Jasper formula (ΔH₂O/% dw × 100), where ΔH₂O is the difference between the percent water content of the experimental and control specimens and % dw is the percent dry weight of the experimental samples.

Planimetry. Photographs were made of standardized mid-lesion coronal sections. The cross-sectional area of the brain white matter was evaluated by planimetry. Under projector magnification, the exact surface area covered by Evans' blue was calculated in square millimeters. This surface area was taken as representative of the progression of the edema front.

Group 1

In the sham-operated group, there was no extravasation of the blood-brain barrier marker and the degree of swelling was insignificant (4.32 ± 4.54%).

Group 2

In Group 2, the lesion was immediately excised and discarded. Forty-eight hours after excision the area covered by Evans blue was 5.51 ± 1.64 sq mm and the degree of white matter swelling was 7.08 ± 6.99%. This is not significantly different from the findings in the control or sham-operated animals (Table 2 and Figs. 1 and 4).

Group 3

Replacement of the cryogenic lesion following microsurgical excision induced no change in the degree of Evans blue spread (6.59 ± 0.65 sq mm) or the extent of white matter swelling (10.27 ± 3.24%) (Table 2 and Figs. 2 and 4).

Group 4

Leaving the lesion intact caused swelling of white matter to 43.82 ± 3.01% of total volume and a spread of Evans blue over 63.48 ± 6.49 sq mm. This increase is highly significant as compared to Groups 1 and 2 (p < 0.001), and corresponds to that seen in similar experiments in our laboratory (Table 2 and Figs. 3 and 4).

Groups 5 and 6

In Group 5 cats, excision of the lesion at 2, 4, or 8 hours after production was followed by cessation of the spread of Evans' blue, and no further increase in the amount of edema was noted after 24 or 48 hours. These animals were contrasted with Group 6 cats where sacrifice at 2, 4, 8, or 24 hours following the lesion production was carried out without excision of the lesion. Comparison of these two groups indicated that the extravasation of Evans’ blue stopped at the time of excision. The spread of Evans’ blue is essentially stopped at the same time, and the progression of the edema front across the brain and its increase in volume were both stopped (Table 3 and Fig. 5).

Discussion

In this study, two aspects of cryogenic vasogenic brain edema were investigated: 1) evolution of vasogenic brain edema after microexcision of the cryogenic lesion, and the accumulation, spread, and resolution of the edema, and 2) the capability of the microscopically isolated cortical lesion to induce a

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**TABLE 2**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Cats</th>
<th>Evans' Blue Spread in White Matter (sq mm)</th>
<th>Dry Weight of White Matter (%)</th>
<th>Increase in White Matter Volume (Swelling) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>none</td>
<td>31.13 ± 1.37</td>
<td>4.32 ± 4.54</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5.51 ± 1.64</td>
<td>29.48 ± 1.71</td>
<td>7.08 ± 6.09</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6.59 ± 0.65</td>
<td>28.33 ± 0.8</td>
<td>10.27 ± 3.24</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>63.48 ± 6.49</td>
<td>21.68 ± 0.48</td>
<td>48.32 ± 3.01</td>
</tr>
</tbody>
</table>

Fig. 3. The cryogenic cortical lesion was left intact following induction (arrowhead) and the animal sacrificed after 48 hours. The extent of vasogenic brain edema is visualized by extravasation of Evans' blue (outlined).
FIG. 4. Histograms illustrate the spread of Evans blue by planimetry (left) and increase in volume of white matter (right) in 15 animals randomly divided into three subgroups: 1, the cryolesion is left intact after induction; 2, the cryolesion is excised and replaced; and 3, the lesion is excised and discarded. There was no significant difference between Subgroups 2 and 3 but when Subgroup 1 was compared with either 2 or 3 the difference was highly significant (p < 0.001).

After a cryogenic cortical lesion there is a break in the integrity of the blood-brain barrier. The exact mechanism of this process is still ill defined. Accumulation, spread, and clearance of the edema fluid is highly dependent on the capillary hydrostatic pressure, hydraulic conductivity of the damaged capillaries and extracellular space, and to a lesser extent on the combination of reflection coefficient, an indicator of a solute's osmotic effectiveness in a particular membrane system, and concentration of the pathological break in blood-brain barrier and brain edema, allowing evaluation of the presence of possible neurohumoral factor(s).

### TABLE 3

| Spread of Evans' blue in control and in experimental cats* |
|-----------------|-----------------|-----------------|-----------------|
| Hours after Lesion | No. of Cats | Evans' Blue Spread in Control Cats (sq mm) | Evans' Blue Spread after Excision of Lesion (sq mm) |
|                  |                |                               |                               |
| 2                | 5              | 19 ± 2.07                     | 17.9 ± 3.89                   |
| 4                | 5              | 20.26 ± 4.79                  | 14.96 ± 2.68                  |
| 8                | 5              | 32.25 ± 4.22                  | 24.86 ± 2.53                  |
| 24               | 5              | 51.06 ± 4.64                  | ---                          |

*Control cats were sacrificed 2 to 24 hours after cryoinduction. In the experimental cats, the lesion was excised 2 to 8 hours after cryoinduction, and the cats were sacrificed at 24 hours.

FIG. 5. Histogram shows the spread of Evans' blue by planimetry in 35 animals subdivided into two groups. In one group the experiments were terminated at 2, 4, 8, and 24 hours following cryoinduction of vasogenic edema (A, B, C, D, with five animals in each subgroup). In a second group the cryolesion was excised at 2, 4, and 8 hours after induction and the animals were sacrificed at 24 hours (A', B', C', with five animals in each subgroup). The differences between two related subgroups (e.g., A and A') were not statistically significant. However, the spread of edema between 8 and 24 hours was clearly stopped by excision.
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solutes in the edema fluid and plasma. Preliminary data indicated that excision of the cryogenic lesion can reduce the extent of accumulation of edema fluid. These expanded experiments substantiate this view (Fig. 4).

Excision of the lesion with partial development of vasogenic edema at 2, 4, and 8 hours after cryoinjury was undertaken to assess the importance of an intact vascular bed in the evolving process. It was postulated that if there were significant nonhydrostatic factors, there should be further evolution of the edema front even in the absence of the generating forces of capillary hydrostatic pressure. The results indicate that following elimination of the lesion the spread of the edema front comes to a halt. This strongly supports the theory of the spread of edema by bulk flow which is dependent on the hydrostatic pressure. If diffusion or an active process were important factors in the spread of the edema front, there would have been further progression of the edema front in the 24 hours after excision. Actually the area covered by Evans' blue was less in experimental than in control animals, which may indicate some absorption of edema fluid by transcapillary exchange and shrinkage of the edematous white matter after excision.

There is much controversy regarding the exact mechanism by which the cold lesion causes a break in blood-brain barrier. Mechanical injury to cortical vessels is generally thought to be important. Virtually nothing is known about the possible participation of any neurohumoral factor(s), such as monoamines and prostaglandins. Microisolation of the cryogenic lesion can bring the evolution of vasogenic brain edema to a halt. Two main components are involved in these events: 1) the capillary bed, and 2) the cortical cellular elements, their processes, biochemical contents, and interstitial fluids. One way to evaluate the effect of tissue factors of the injured brain on intact capillaries is to replace the dissected lesion in its bed and evaluate the progression of edema front. Any factor(s) able to disrupt the blood-brain barrier would do so by diffusing into the intact cortex and white matter. These results indicate that when the cryogenic lesion is isolated from its capillary bed, it is unable to induce brain edema. There is no significant difference between the edema obtained when the lesion is discarded or replaced in its bed. Nor is there any change in vascular permeability brought about by the isolated brain returned to its previous location. It seems unlikely that any active factors in the injured brain are important in the production of the increased white matter extracellular fluid volume.

However, this does not indicate that there are no edemagenic factors important in the process of cellular swelling well known to accompany the more impressive increase in white matter extracellular fluid volume. The dysfunction that accompanies brain edema may not all be related to this volume abnormality and subsequent brain shifts. Great improve-

References


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