Fluorescein Studies of Cerebral Edema Produced by the Cryogenic Probe*

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COLD lesions have become a standard laboratory model for the study of cerebral edema. The usual technique for producing these lesions has been application of an extra-cerebral cold reservoir to the brain. This method produced both cortical and subcortical destruction and edema. However, it was extremely difficult to distinguish between edema of the gray matter and that of the white matter. Therefore, a method has been sought to determine whether a distinction existed between edema in the gray and white matter.

The liquid nitrogen cold probe has been used clinically to produce discrete lesions of deep brain structures. By using a cold probe placed stereotactically, it was thought possible to study edema from profound cold lesions in white matter or in deep gray structures discriminatively. Because the cold probe produces discrete small lesions, the evolution of the edema in either gray or white matter could be determined by sacrificing the experimental animals at different times.

It has been shown that the passage of fluorescein into cerebral tissue is indicative of an alteration in the blood-brain barrier and that the degree of this fluorescence closely parallels that of the edema of the cerebral tissue as determined histologically. Therefore, the passage of fluorescein was used to identify the area of edema.

Methods and Materials

Twenty-two cats of both sexes varying in weight from 2.5 kg. to 4.5 kg. were used in this study. Each animal was anesthetized with intraperitoneal pentobarbital (57 mg./kg.) and the head was fixed in a Horsley-Clark stereotactic frame. Using a standard atlas of the cat brain, coordinates corresponding to the subcortical white matter of the frontal lobe and the thalamus were selected for lesion placement. With a Lee liquid nitrogen cold probe 2 mm. in diameter, a lesion was made in the subcortical white matter in 15 cats and in the thalamus in 7 cats.

A uniform set of parameters of $-40^\circ$C. for 3 minutes was used to produce both the thalamic and subcortical lesions and the temperature was carefully monitored by a thermocouple at the probe tip (Fig. 1).

All animals were then allowed to recover from anesthesia. At varying times animals with lesions in the subcortical white matter and animals with thalamic lesions were sacrificed. The time intervals and number of animals is summarized in Table 1.

Five minutes prior to sacrifice, each animal was again anesthetized and given 2.5 cc. of 20 per cent fluorescein intravenously. The calvarium was rapidly opened and the brain removed. A coronal section was made through the lesion and the maximum dimensions determined. Photographs were made on Ektachrome or Kodachrome film using both standard light and ultraviolet light ($2500\text{Å}$). Sections of selected lesions were submitted for histologic examination and hematoxylin and eosin, Klüver, and periodic acid Schiff (PAS) preparations were made.

Results

1. Lesion Dimensions. Using the cold

![Fig. 1. Thermocouple recording from cold probe tip during the making of a lesion.](image-url)
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**TABLE 1**

*Time at which animals were sacrificed*

<table>
<thead>
<tr>
<th>Time</th>
<th>Subcortical White</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1 day</td>
<td>4</td>
<td>2</td>
</tr>
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<td>2 days</td>
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<td>7 days</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

probe parameters of $-40^\circ$C. for 3 minutes, lesions of dimensions varying from 4 mm. by 4 mm. to 6 mm. by 7 mm. were made. The mean of all lesions was 4.9 mm. $\pm$ 0.6 mm. by 5.4 mm. $\pm$ 1 mm. The mean measurements of the thalamic lesions were 5.3 mm. $\pm$ 0.9 mm. by 5.7 mm. $\pm$ 1 mm. The mean of the subcortical lesions was 4.7 mm. $\pm$ 0.7 mm. by 5.3 mm. $\pm$ 1.0 mm.

2. *Gross Appearance of Lesions.* The lesions themselves were generally hemorrhagic with 88 per cent of the subcortical and 100 per cent of the thalamic lesions showing gross hemorrhage. In all but one instance the hemorrhage was confined to the lesion per se with no extension into the surrounding tissue. In one thalamic lesion, hemorrhage was found along the tract of the probe and extended into the subarachnoid space.

3. *Subcortical White Matter Lesions.* Gross examination of the brains with subcortical white matter lesions at 3 days showed that the entire hemisphere was enlarged, and a shifting of the ventricles away from the lesion was apparent. In animals sacrificed at

![Fig. 2. Low power photomicrograph at edge of cold lesion in subcortical white matter. The center of the cold lesion (A) is necrotic while the edge (B) is sharply defined. The swollen cells and sponginess of the adjacent white matter is seen (C). Klüver stain; X31.](image)

![Fig. 3. Higher power view of Fig. 2. The necrotic center (A) is sharply separated (B) from the surrounding edematous white matter (C). The swollen astroglia are apparent. The sharpness of the lesion is well demonstrated Klüver stain; X64.](image)