We do not have sound clinical criteria or objective laboratory tools to scientifically answer these questions. It is evident from the results reported in this study that, despite the initial lack of progress and what, for all practical reasons, was the initial "failure" during the first few months following a stroke, a good number of "poor" patients could still be helped. The results also show that a certain number of patients require longer periods of therapy in order to reach their maximum potential.

What is more relevant, however, is the observation that for certain patients it takes a longer time before they start to show any evidence of favorable change.

**References**


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**Ischemic Brain Edema and Compression Brain Edema**

**Water Content, Blood-Brain Barrier and Circulation**

**Michio Yamaguchi, M.D.,* Seiya Shirakata, M.D., Syun Yamasaki, M.D., and Satoshi Matsumoto, M.D.**

**SUMMARY** Two experimental models of brain edema have been produced in rats. Some animals underwent bilateral carotid artery ligation (BLCL), while others received extradural compression by a rubber bar. The characteristics of these conditions were compared by Evans blue injection and by the colloidal carbon perfusion method. Although both models produced an increase in the water content of brain tissue, a blood-brain barrier leak to Evans blue was observed only in the compression edema model. The steroid drug, hydrocortisone, diminished the water content of the edematous brain caused by compression but had no effect on that produced by BLCL. The cerebral circulation, as studied by colloidal carbon perfusion, revealed a vasodilation pattern in those animals who underwent bilateral carotid artery ligation, while vascular damage was observed in the compression edema model.

**SINCE BRAIN EDEMA** is a very important clinical problem, a number of clinical and experimental studies have appeared. Several models of brain edema have been experimentally produced by different methods and under varying circumstances. In the absence of a classification properly established on an understanding of the mechanisms involved in brain edema, confusion besets the study of its etiology, pathological course, and treatment. Klatzo1 proposed a useful classification of the brain edemas, dividing them into (1) cytotoxic and (2) vasogenic types. This definition contributed greatly to the clinical and experimental study of the pathophysiology of brain edema. In this laboratory, the authors have developed two experimental models of brain edema in rats: (1) brain edema by the bilateral carotid ligation and (2) compression brain edema. The former can be considered a suitable model to elucidate the effect of an ischemic insult on brain edema. The latter is a modification of the model developed by Ishii et al.4 using the rat brain. When the integrity of the blood-brain barrier was examined in these two models, an apparent discrepancy was observed in the staining of Evans blue. Sensitivity to the steroid, hydrocortisone, was also different in the two models. In addition, studies with the colloidal carbon perfusion method showed that there were two patterns of change in the cerebral circulation under these experimental brain insults. These findings are of interest to the study of the characteristics of brain edemas.

**Methods**

Wistar strain rats of both sexes and weighing 100 to 120 gm were used in this experiment. The authors felt the variation in the brain water content might be influenced by the
TWO MODELS OF EXPERIMENTAL BRAIN EDEMA

Experimental brain edemas were produced by (1) the bilateral carotid ligation (BLCL) and (2) the extradural compression methods. The former model was produced under light anesthesia with ether in the supine-positioned rat fixed on the operating table, with a transverse incision in the animal's neck. The common carotid arteries were exposed bilaterally and ligated under the operating microscope. Care was taken to avoid any slight injury to the vagus nerves, because trauma to the vagus nerves seemed to produce an occasional fatal result within a few hours after surgery. As compared to the midline skin incision method, the transverse neck wound could be more easily closed without tense narrowing of the trachea after the application of sutures. Forty-eight hours following ligation, the animals were killed by guillotine, and the brains were immediately removed to examine the water content. The survival rate up to 48 hours was 55.5%.

Production of extradural compression brain edema was carried out under moderately deep anesthesia with sodium pentobarbital. A burr hole of approximately 2 mm in diameter was made in the right parietal bone using a dental drill. A rubber bar 4 to 5 mm long with a width slightly larger than the diameter of the hole was inserted into the hole. The depth below the bone edge was approximately 1.5 to 2 mm. Since these procedures were done under the operating microscope, dural laceration during the surgical process was prevented. Twenty-four hours after compression, the rubber bar was removed under light anesthesia with ether, and the animal was returned to the cage. The rat was decapitated 24 hours after decompression, and the brain was removed and examined.

In studies of the effect of the steroid drug on brain water content, hydrocortisone or dexamethasone was administered as described in the legends of tables 1 and 2. The first injection was always started at the beginning of the insult (tables 1 and 2).

**TABLE 1** Epidural Compression Brain Edema and Steroids

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Insults</th>
<th>Water content</th>
<th>Versus control</th>
<th>Versus compression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>79.19 ± 0.23 (10)</td>
<td>—</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>Compression</td>
<td>80.26 ± 0.15 (5)</td>
<td>P &lt; 0.001</td>
<td>—</td>
</tr>
<tr>
<td>R: compressed side</td>
<td>79.39 ± 0.11 (5)</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>L: control side</td>
<td>80.42 ± 0.54 (8)</td>
<td>NS</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Compression + steroid*</td>
<td>79.31 ± 0.31 (5)</td>
<td>NS</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>R: compressed side</td>
<td>79.28 ± 0.53 (5)</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>L: control side</td>
<td>78.17 ± 0.43 (8)</td>
<td>NS</td>
<td>55.5</td>
<td></td>
</tr>
</tbody>
</table>

*5 mg of hydrocortisone (Solucotef®) was injected eight times intraperitoneally every six hours. Total dose: 40 mg/48 hours. ( ): number of hemispheres. NS: not significant.

**ESTIMATION OF THE WATER CONTENT**

The brain was removed quickly from the calvarium and placed into a pre-weighed clean glass container with a stopper. Immediate decapitation without any violent handling of the animal was intended to avoid venous congestion in the brain tissue. The container was stoppered as soon as possible (average time was within 30 seconds) after decapitation to avoid evaporation of water from the tissue to the air. The water content was estimated by a slight modification of the method of Elliot and Jasper. After the precise estimation of the wet weight of the brain tissue in a covered and completely pre-dried container, the tissue was minced in 2 to 3 ml of reagent grade acetone with a pre-weighed clean glass bar in the container. Then, the container, glass bar, and slurry of the brain-acetone mixture were placed in a preheated oven at 110°C. When the samples were placed in the oven, the oven switch was turned off to avoid explosion. After the acetone was completely evaporated, the electric circuit was closed again. After overnight drying at 110°C, the dry weight was determined, and the water content was expressed as grams per 100 gm of wet weight. Handling with the naked finger was avoided throughout this procedure. Statistical significance of difference between samples was estimated by Student's t test of unpaired samples.

**STUDY OF THE BLOOD-BRAIN BARRIER**

Two milliliters of 1% Evans blue solution were injected via the tail vein two hours prior to decapitation (46 hours after the initial insult). After the removal of the dura mater, the brain was inspected macroscopically and photographed. Brain cutting also was done to observe sections of the hemispheres.

**COLLOIDAL CARBON PERFUSION**

The method of Ames et al. was employed with slight modifications. Forty-eight hours after the insult, a laparotomy was performed under moderately deep anesthesia with sodium pentobarbital. A polyethylene cannula was inserted into the abdominal aorta. The tip of the catheter was placed at the aortic arch. The external jugular veins were bilaterally exposed and held with silk strings. At the same time as the veins were cut, approximately 5 ml of a commercially supplied suspension of colloidal carbon (Fueki
Bokudyu, for stationery use) were introduced via the aorta under constant pressure. The colloidal carbon perfusion was continued for at least two minutes after the venous outflow turned completely black. The brain was then removed and fixed in 10% formalin for one week. Using a cryostat, the fixed brain was sliced into 50 to 100 μ sections and treated with xylene. The colloidal carbon suspension used in this experiment required no addition of gelatin.

**Results**

**WATER CONTENT IN THE BRAIN**

As shown in table 3, the water content did not increase in either hemisphere after unilateral carotid ligation. However, the bilateral carotid ligation procedure always produced an increase in brain water content. Epidural compression by a rubber bar in the right parietal region also produced a statistically significant increment in brain water. Moreover, both bilateral carotid ligation and extradural compression were performed on some animals, and the water contents in the brain increased to a greater extent than with either insult alone (table 3). In table 1, the effect of the steroid on water contents in the brain compression model is listed.

**BLOOD-BRAIN BARRIER LEAK**

When Evans blue was injected via the tail vein, the edematous brain caused by the extradural compression always showed dye staining just beneath and around the compressed area. The pale but apparent blue color was observed around the necrotic point which marked the place where the strongest compression was applied with the rubber bar. This hollow-like faint blue area could represent a state of compression brain edema with evident blood-brain barrier damage (fig. 1).

On the other hand, no blue staining could be observed after dye infusion in the animal group which underwent bilateral carotid ligation (no picture shown). The authors were unable to find any evidence of staining in the brain, even in thin slices. In order to clarify whether the dye was unable to reach the ischemic area because of the obstruction of the carotid arteries, the following experiment was done. Under light anesthesia with ether, bilateral carotid ligation and compression by rubber bar were performed at the same time. After 24 hours, the decompressive removal of the rubber bar was carried out. Forty-six hours after the initial operation, 2 ml of 1% Evans blue was injected via the tail vein. The animal was killed by decapitation two hours after the dye injection, and the brain was inspected for dye staining. Since some light staining was observed around the compressed area, it is apparent that the injected dye could reach the brain tissue and permeate through the vessels in spite of the bilateral carotid ligation (fig. 2). It is of interest that the staining was weaker in the right parietal lobe of the animals which received both bilateral carotid ligation and compression as compared with that in the simple compression group. Neither systemic nor local blood pressure in the brain circulation was determined during this experiment.

**COLLOIDAL CARBON PERFUSION**

A second trial, using colloidal carbon perfusion, was employed to evaluate the presence of effective cerebral blood flow under the bilateral carotid ligation procedure. As shown in figures 3 and 4, the normal brain was well perfused with colloidal carbon. After bilateral carotid ligation, the filling of the vessels with colloidal carbon was greater than

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Brain Edema by Carotid Ligation</th>
</tr>
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<tbody>
<tr>
<td>Exp. no.</td>
<td>Insults</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Ligation of R-carotid artery</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BLCL</td>
</tr>
<tr>
<td>4</td>
<td>BLCL + R-compression</td>
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<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1): number of hemispheres.
*Difference versus control.
FIGURE 2 Evans blue staining in the edematous brain region (extradural compression and bilateral carotid ligation).

that of the normal control group (figs. 5, 6). The compression edema group showed a different pattern of microcirculation in the brain. As shown in figures 7 and 8, the filling of the vessels was not satisfactory, and some extravasation was observed in the most strongly compressed area. The narrowing of the vessels and the extravasation of colloidal carbon suggested the presence of local vascular damage and extensive swelling of the perivascular tissues.

EFFECT OF STEROID ON BRAIN EDEMA

The effect of the steroid drugs, hydrocortisone or dexamethasone, on brain water content was tested in the two experimental edema groups. As seen in table 2, no anti-edema effect of either steroid was observed in the bilateral carotid ligation group. In the compression edema group, however, a decrease in water content was apparent when the steroid drug was given. In terms of sensitivity to steroid treatment, therefore, one can distinguish between at least two types of brain edema.

In evaluating the therapeutic action of the steroid by its effect on brain water content, one must consider the possibility that an insufficient amount of the drug reached the lesion because the carotid arteries were occluded. The carbon perfusion studies, however, clearly demonstrate that the cerebral circulation remains open after bilateral carotid ligation.

Discussion

Numerous methods of production of experimental brain edema have been studied in various species. Levine previously reported an "anoxic-ischemic" model of brain edema produced by subjecting rats with unilateral carotid ligations to systemic hypoxia. Unilateral carotid ligation alone failed to produce brain edema in this species unless the hypoxia was added. This model, however, lacks reproducibility in that the rate and intensity of the hypoxic insults are difficult to control. Survival is often unpredictable, and those animals which do survive often show normal
When Levine's original method was employed in our laboratory (M. Yamaguchi, unpublished data), the inconsistency of the brain lesions produced rendered the model unsatisfactory for comparison with other models and for the evaluation of a steroid effect. More recently, Plum et al. have introduced modifications of the anoxic-ischemic preparation and have improved its reproducibility. Other workers have reported experimental infarction or edema in the gerbil brain produced by unilateral carotid ligation. The cerebral circulation of the rat, however, is different anatomically, and presumably functionally, from that of the gerbil.

The method of bilateral carotid ligation employed in this study resulted in a reproducible increase in the water content of the brain. As noted by Levine, unilateral carotid ligation had no such effect on brain water. The pattern of circulatory injury in the bilateral carotid ligation method also differed fundamentally from the infarction pattern produced by unilateral carotid ligation in the gerbil; the microcirculation in the brain remained open, with apparent vasodilatation. Macroscopically, the brain appeared swollen and congested, with a reddish tone. The authors are confident that the internal jugular veins were not ligated by mistake. The enlarged diameter of the vessels may have been caused by local accumulation of carbon dioxide which could not be efficiently removed by the cerebral circulation. If the local pH, Pco, around the lesion, and the local circulating blood pressure could have been estimated, more fruitful information might...
have been uncovered. Wexler\textsuperscript{10} reported the bilateral carotid ligation procedure as a model of ischemic brain damage in the rat. Eklof and Siesjö\textsuperscript{11} also described the use of bilateral carotid ligation. They estimated some enzyme activities, labile substances, and other compounds in the brain. However, those workers were not primarily interested in this method as an experimental model of brain edema. They also did not examine the question of a blood-brain barrier leak. The increased water content seen in this preparation permits did not examine the question of a blood-brain barrier leak.

When experimental brain edema was produced by the extradural compression method, vascular damage and blood-brain barrier leak were always observed. The condition therefore might be considered a vasogenic brain edema as classified by Klatzo.\textsuperscript{1} On the other hand, staining with Evans blue was not observed in the BLCL brain edema specimens. Hossmann and Olsson\textsuperscript{12} showed that ischemia reduced the blood-brain barrier leak caused by inorganic mercury compounds. Hossmann and Olsson's observation might appear, in our experiment, to explain the poor staining of the dye in the edematous region of the brain. Our animal which underwent both BLCL and extradural compression showed very slight staining in the edematous area. Ischemia thus appeared to reduce but not abolish the blood-brain barrier leak produced by compression. The mechanism of this effect of ischemia on the blood-brain barrier system is not clear.

When steroid was administered to the bilateral carotid ligation group, no effect was observed on the brain water content. Siegel et al.\textsuperscript{13} reported that steroid had no beneficial effect on the brain edema model produced by microembolism. Kahn and co-workers\textsuperscript{14} also reported the ineffectiveness of steroid in the treatment of experimental brain infarction in gerbils: there was no effect on either the morbidity or the mortality. On the other hand, Bartko et al.\textsuperscript{15} and Harrison et al.\textsuperscript{16} reported a beneficial effect of steroid drugs on the morbidity and mortality from ischemic brain insults. The result obtained in this laboratory showed no effect of the steroid in the reduction of the water content of edematous brain tissue. As steroid drugs are thought to exert a beneficial effect on brain edema through changes in vascular permeability, the lack of effect in the BLCL model, in which no blood-brain barrier leak was demonstrated, seems predictable.

Meinig et al.\textsuperscript{17} reported the effects of changes in the local arterial blood pressure at the site of a damaged vascular wall. In their work, the blood-brain barrier leakage was explained by the concept of filtration edema. According to this explanation, when bilateral carotid ligation is performed, the blood pressure in the brain is decreased, and the blood supply is insufficient to produce any edema by filtration alone. Even if the blood-brain barrier were damaged, therefore, insufficient leak might be present to show any alteration with steroid administration.

In our studies, ischemia did not entirely prevent the blood-brain barrier leak produced by compression. The report by Hossmann and Olsson\textsuperscript{12} of the prevention of mercury-induced blood-brain barrier leak by ischemia\textsuperscript{11} thus is not fully confirmed by our studies. It is of interest, however, that the Evans blue staining around the compressed area in animals which underwent both carotid ligation and compression was paler in color and smaller in area than that in the animals which underwent compression alone.

By Klatzo's conception,\textsuperscript{1} brain edema can be defined as a state of abnormally high water content in the brain tissue. Statistically significant water increases in brain tissue were observed after both bilateral carotid ligation and extradural compression. However, the studies of Evans blue leak and of sensitivity to steroid administration suggest that the two models of brain edema might differ fundamentally in their origins and pathogenesis. The state of the cerebral microcirculation, as studied by colloidal carbon perfusion, also differed markedly in the two edematous conditions.

The two experimental models studied in this report bear some resemblance to clinical conditions; i.e., BLCL is similar to major cerebral artery occlusion in man, while the compression model is analogous to the postoperative state after the removal of intracranial mass lesions. It remains unclear why the edematous condition with an apparent blood-brain barrier leak is sensitive to the steroid drug, while the BLCL model is refractory. If steroids exert their beneficial effect by stabilizing vascular walls or altering vascular permeability, however, a lesser effect would be expected in the ischemic type, in which a blood-brain barrier leak is not evident.

From this information, it can be concluded that the term "brain edema" must include varieties with different origins, developmental processes, and characteristics. Although Klatzo's classification\textsuperscript{1} is greatly useful in the neuropathological sense, clinical practitioners should be encouraged to study brain edema in their own cases to contribute to the further classification and elucidation of the pathophysiology, clinical course, and sensitivity to treatment of the different types of brain edema.

**Acknowledgment**

The authors acknowledge the help of Dr. Howard S. Kirshner in preparing this manuscript.

**References**

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Brain Microvascular Hemodynamic Responses to Induced Seizures

ROBERT R. MYERS, PH.D., AND MARCOS INTAGLIETTA, PH.D.

SUMMARY Arteriolar diameters and venular erythrocyte velocities in the small pial vessels on the surface of the cat brain were measured by TV methods during induced epileptic seizures through a cranial window. Grand mal seizures maximally dilated arterioles and increased venular erythrocyte velocity up to 400%. High positive correlation existed between changes in CSF hydrogen ion concentration and pial arteriolar diameter, suggesting metabolic regulation of CBF through CSF/interstitial fluid hydrogen ion alterations during the seizure.

IN BOTH IDIOPATHIC and symptomatic epilepsy, which is manifested as generalized seizures without focal onset or partial or focal seizures with generalization, consciousness is lost at the onset and apnea may develop secondary to tonic contraction of the respiratory muscles. The ability of the cerebral circulation to meet the increased metabolic demands imposed by the seizure, especially in the presence of apnea, is important clinically and has been extensively researched since the late 1930s. Until the elaborate studies of Plum’s group, basing their conclusions in part on jugular venous outflow increases of twofold to fourfold and pathological states. These mechanisms may be classified as a mechanism of vasodilatation during the ictus, no direct evidence has been presented to elucidate the role of metabolites and therefore pH in microvascular control during the ictus. Similarly, no attempts have been made to separate these metabolic effects from blood pressure effects. Plum’s group presented data indicating that the increase in cerebral blood flow (CBF), measured by techniques which only yield average values over extended time intervals, can be accounted for by a corresponding increase in systemic pressure, suggesting that cerebral autoregulation might be suspended in these circumstances. In order to study these effects, we developed a method for quantifying cerebrospinal fluid (CSF) pH and pial microvascular hemodynamic responses to induced seizures in the cat at constant systemic pressure, believing that the increase in blood flow in the absence of systemic pressure changes must ultimately be a consequence of substantial readjustments in the brain microvasculature.

Methods

The method for quantifying brain electrographic and circulatory hemodynamic events in a physiologically viable exposed cortex is based on the implantation of skull screws for recording the electrocorticogram (ECOG) and the substitu-

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