Discussion

Grades of recovery and outcome of patients with "stroke" have been widely reported by different authors. There is reasonable agreement on the "poor" outcome of a certain number of surviving "stroke" patients; their number is widely estimated to vary from 19% to 35%.

With the growing need for physical medicine and rehabilitation treatment and the associated mushrooming financial expenditures for facilities, questions about the proper selection of patients for therapy, the economy of the methods used, and the practicality of the results of therapy are coming to the foreground more and more. It is for those patients who after the initial acute cerebral insult appear to remain in a sort of limbo, not deteriorating but not progressing in any direction in their functional recovery, that questions like "How long do we treat this type of patient?" and "When do we stop treatment?" usually arise and beg for an answer.

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 brain-blood 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 in the compression edema, 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. Klatzo 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. 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

References


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variation in body weight. The rats were maintained at approximately 20°C in the cage and fed on commercially supplied pellets and tap water ad libitum.

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>
<thead>
<tr>
<th>Table 1</th>
<th>Epidural Compression Brain Edema and Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. no. Insults</td>
<td>Water content ( % )</td>
</tr>
<tr>
<td>1 None</td>
<td>79.19 ± 0.23 (10)</td>
</tr>
<tr>
<td>2 Compression</td>
<td>R: compressed side</td>
</tr>
<tr>
<td>L: control side</td>
<td>79.39 ± 0.11 (5)</td>
</tr>
<tr>
<td>3 Compression + steroid*</td>
<td>R: compressed side</td>
</tr>
<tr>
<td>L: control side</td>
<td>79.28 ± 0.53 (5)</td>
</tr>
</tbody>
</table>

*5 mg of hydrocortisone (Solucotef®) was injected eight times intraperitoneally every six hours. Total doses: 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 (48 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

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Carotid Ligation and Steroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insults</td>
<td>Water content</td>
</tr>
<tr>
<td>BLCL*</td>
<td>80.16 ± 0.30 (8)</td>
</tr>
<tr>
<td>BLCL + HC(45 mg, i.p.)</td>
<td>80.17 ± 0.43 (8)</td>
</tr>
<tr>
<td>BLCL + DX(1 mg, i.p.)</td>
<td>80.42 ± 0.54 (8)</td>
</tr>
</tbody>
</table>

*BLCL: Bilateral carotic ligation. †HC: Hydrocortisone (Solucotef®), total doses: 45 mg/48 hours. ‡DX: Dexamethasone (Decortin®), total doses: 1 mg/48 hours. Each value shown in the water content column is mean ± SD. Number of hemispheres.
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>Exp. no.</th>
<th>Insults</th>
<th>Water content</th>
<th>Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>79.19 ± 0.23 (10)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Ligation of R-carotid artery</td>
<td>79.30 ± 0.38 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>BLCL</td>
<td>80.16 ± 0.23 (8)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>4</td>
<td>BLCL + R-compression</td>
<td>81.74 ± 0.15 (3)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

*: 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
brain water contents. 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. Wexler10 reported the bilateral carotid ligation procedure as a model of ischemic brain damage in the rat. Eklof and Siesjö11 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.7 On the other hand, staining with Evans blue was not observed in the BLCL brain edema specimens. Hossman and Olsson12 showed that ischemia reduced the blood-brain barrier leak caused by inorganic mercury compounds. Hossman 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.13 reported that steroid had no beneficial effect on the brain edema model produced by microembolism. Kahn and co-workers14 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.15 and Harrison et al.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.

Meining et al.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 alterations with steroid administration.

In our studies, ischemia did not entirely prevent the blood-brain barrier leak produced by compression. The report by Hossmann and Olsson12 of the prevention of mercury-induced blood-brain barrier leak by ischemia13 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,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 classification1 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.

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11. Hossmann KA, Olsson Y: Functional aspects of abnormal protein
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 apnea, is important clinically and has been extensively researched since the late 1930s. Until the elaborate studies by Plum et al. the answer to this question was not clear, since conflicting results of earlier investigations could not be resolved due to significant differences in experimental design. Plum's group, basing their conclusions in part on jugular venous outflow increases of twofold to fourfold and constant metabolite levels in sampled arterial and venous blood, has indicated that the metabolic demand is met in the idealized case where there is no muscular involvement in the seizure and apnea does not occur. However, the mechanisms by which the cerebral microvasculature reacts to the sudden increase in metabolism accompanying seizures have not been clearly demonstrated or completely explored in these investigations.

Various autoregulatory and vascular control mechanisms exist which have been shown to have differing degrees of influence on control of cerebral circulation in physiological and pathological states. These mechanisms may be classified in three major groups: (a) Bayliss or myogenic control — increasing arterial pressure causes an intrinsic and compensatory decrease in vascular diameter to maintain flow constant as if vascular smooth muscle tension was regulated, (b) neurogenic control — centrally mediated sympathetic and parasympathetic influences on vascular smooth muscle act to control arterial diameter in response to autonomic influences, and (c) metabolic control — products of metabolism act directly or indirectly on vascular smooth muscle to change arterial diameter and thus alter flow to maintain a constant metabolic environment around the microvasculature. The percentage contribution of these autoregulatory/control mechanisms is debated, but it is generally agreed that the vascular response to CO₂ accumulation is the dominant control factor with the myogenic effect exerting a lesser influence in physiological states and the neurogenic contribution being of least magnitude.

While the intense cerebrovascular effects of metabolites are well known, and have been hypothetically presented 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-
Ischemic Brain Edema and Compression Brain Edema: Water Content, Blood-Brain Barrier and Circulation

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