Brain Tissue Electrolytes and Water Content in Experimental Concussion in the Monkey

Fred H. Faas, M.D., and Ayub K. Ommaya, F.R.C.S.

Biophysics Division, Naval Medical Research Institute, and Surgical Neurology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland

Although a great deal has been written about the physical and physiological changes in experimental head injury, very little of this effort has been directed to the electrolytes of the brain and cerebrospinal fluid. We have found only one report concerning the electrolyte content of the brain following experimental cerebral concussion; Eichelberger, et al., in 1949 performed the analyses on canine cerebral and cerebellar homogenates with no separation of gray and white matter.

Various studies of brain water, blood content, and brain volume after experimental head injury have yielded conflicting evidence as to the presence or absence of cerebral edema. Some of the conflicting data are undoubtedly related to the difficulty in comparing such physiopathological effects when the physical factors of the impact are not defined clearly or are even quite variable. For this reason, initial efforts in an over-all Head Injury Project have been to develop statistically significant correlations between the physical data associated with experimental head injury and the production of experimental cerebral concussion in the Rhesus monkey.

The study reported in this paper was performed to delineate what changes occur in the sodium, potassium, chloride, and water content of the brain after experimental brain concussion and also to examine these data as evidence for or against the existence of cerebral edema. This degree of head injury in the monkey was conceived as the experimental analog of a closed human head injury severe enough to produce loss of consciousness but not associated with skull fracture or significant cerebral contusions and hemorrhage.

Materials and Methods

The analyses of 20 monkey brains (Macaca mulatta) are included in this report. Three animals were sacrificed as normal controls; 15 monkeys in five groups of three were sacrificed at 0.3 hours, 1.5 hours, 6 hours, 24 hours, and 48 hours after experimental concussion; and two were sacrificed at 1 week after concussion. Cerebral concussion was produced with the animal lying on its side with the head free to move. The impact was delivered to the occiput by a modified, rubber-tipped Remington Humane stunner, the tip of the piston starting at 1 inch from the monkey's head. A strain gauge was built into the impacting end of the piston in order to measure the force of impact (Fig. 1). The captive piston was powered by Type IPL 4/2 grain Industrial blank .22 caliber cartridges. The detonation force from these cartridges was shown to be capable of producing cerebral concussion in practically 100% of the animals; it produced impacts with an impulse greater than 0.9 lb/sec in accordance with our previously established criteria.

Severe concussion was consistently produced without skull fracture. The immediate heart rate, a good index of the severity of head injury after impact, was usually about 50/minute compared to about 200/minute in the normal monkey. The head injury thus produced did not result in macroscopic brain damage in the majority of animals. In an occasional monkey, a minor subdural or subarachnoid hemorrhage was found. The results from these animals were not excluded from the series because the values for water content and electrolytes were not significantly different from the concussed animals without such minimal hemorrhages. After the impact, the animals were allowed to recover; they were then maintained until time for sacrifice. No animal showed any evidence of neurological defect before sacrifice.

The technique of fluid and tissue sampling was as follows. The animal was anesthetized with sodium pentobarbital, 3 to 5 cc of blood were drawn from the femoral vein, and 1 cc of cerebrospinal fluid was taken by a cisternal...
puncture. The animal was then placed in a Horsley-Clarke stereotaxic apparatus, the calvarium and dura opened widely, and the cerebral hemispheres rapidly removed at the level of the midbrain while the animal was still alive. The hemispheres were then quickly separated; one was used intact to determine specific gravity and water content, the other to take samples of gray and white matter for electrolyte analysis and water content. Two samples each of gray and white matter, weighing between 200 and 500 mg apiece, were taken from the anterior half of the hemisphere and two each from the posterior half. Thus, each animal provided two gray and two white matter samples for electrolyte analysis. An effort was made to get pure gray and white matter as far as possible. The wet samples were weighed immediately to the nearest 0.2 mg on a torsion balance. The entire procedure from the taking of the blood to separation of the samples took no longer than 20 minutes and the weighing of the samples an additional 5 minutes. The time of midbrain transection was considered the time of sacrifice.

The specific gravity of a hemisphere was calculated by dividing its weight by its volume. The weight was determined to the nearest 0.01 gm on a pharmaceutical, two-pan, platform balance; the volume was determined by volume displacement in a 250 ml graduated cylinder filled with Elliott's solution, reading the meniscus to the nearest 1.0 ml with a cathetometer.

The water content of the hemispheres was determined by drying to a constant weight at 100° C for 48 to 72 hours. The individual samples were weighed in individually made aluminum foil pans and dried at 110° for 16 to 24 hours.

Electrolyte analysis of the serum and cerebrospinal fluid was performed after centrifugation to remove any red cells. The wet brain tissue was homogenized in 4 ml distilled water with ultrasound using a Bronson Instrument S-110 Sonifier. It was noted that white matter samples were more difficult to homogenize thoroughly than gray matter samples; if homogenization was not adequate, falsely low potassium values resulted. The electrolyte...
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content of the homogenate was then determined, and values corrected for the volume of the sample which was added to the original 4 ml, assuming sample volume to be equal to sample weight. The values were expressed in meq/kg fresh tissue.

Since chloride exists in the brain mostly as an extracellular ion, chloride space was calculated as an estimate of the extracellular space. Assuming the chloride space to be in equilibrium with the CSF, we calculated the space by dividing the tissue chloride concentration by the CSF chloride concentration and expressed it as a percentage. All chloride determinations were done by the amperometric titration method of Cotlove\(^4\) with the Instrumentation Associates Cotlove chloridometer. Prior to the chloride titrations, the homogenates were treated according to the modification of Bourke, et al.,\(^3\) of the original method of Cotlove.\(^5\)

The sodium and potassium determinations were done by emission flame photometry with the IL 143 flame photometer. Several experiments were performed in which the arterial blood pressure and cisterna magna CSF pressure were monitored continuously up to 1.5 hours after injury using a fluid system connected to a Statham strain gauge transducer. The CSF catheter was introduced via a laminectomy in the upper lumbar region and threaded up through an opening in the meninges into the cisternal region. Leakage around the dural entry of the catheter was prevented by sealing with “Bio Bond,” a tissue adhesive.

**Results**

All data were expressed as the mean, plus or minus the standard deviation from the mean. There were three animals in each group except the final 1-week group which had only two animals. In the gray matter and white matter data there were two samples from each animal, giving a total of six samples in most groups. Occasional samples were lost, leaving four or five samples in some groups. Statistical differences of the experimental animals from the control were done by the Fisher “t” test, and only those significant at the 1% level were heeded as being significant.

The data for the gray matter samples are shown in Table 1. There were no statistically significant differences between the control group and any of the experimental groups in the water, sodium, potassium, or chloride content, or in the size of the chloride space.

Table 2 gives the results of the white matter samples. The 1.5- and 24-hour experimental results showed a small but statistically significant decrease in the chloride content. The possible implication of this will be discussed later. The remainder of the electrolyte and water values showed no differences between the control and experimental groups.

Table 3 gives the results of the blood and CSF analyses. There were five animals in the control group here and three or sometimes two in the remaining groups. The only important difference in any of the experimental results from the control values was a small but statistically significant decrease in the average CSF sodium in the 48-hour group. This could be a result of slight dehydration in some of the animals because of poor fluid intake after the trauma, although the general behavior of the animals was normal. The wide variation in the serum potassium is probably due to the various levels of anesthesia present when the blood was drawn, which has been shown by Bradbury and Davson\(^4\) to affect the serum potassium.

Table 4 gives the results of the water content and the specific gravity of the whole hemispheres. The only statistically significant finding is a small decrease in water content after 1.5 hours. We feel that this change is probably not real as none of the other groups showed any changes. No change in specific gravity was found. The significance of the specific gravity of the brain is not known, but Alexander and Looney\(^5\) reported a decrease in the specific gravity of some of their edematous brains.

The results of continuous cerebrospinal-fluid-pressure recording revealed no significant change in pressure occurring up to 1.5 hours after the head injury. One animal which was monitored for 9 hours after the experimental cerebral concussion had a maximum increase in pressure to 35 cm saline at 4 hours after injury. Subsequently, the pressure gradually returned toward normality.

**Discussion**

There has never been direct evidence that increased total water content of the brain (cerebral edema) occurs after concussive head injury. All evidence for the existence of cerebral edema after head injury has been indirect
Gray matter electrolytes and water content at intervals after cerebral concussion

<table>
<thead>
<tr>
<th>Time after Concussion</th>
<th>Water (%)</th>
<th>Sodium (meq/kg)</th>
<th>Potassium (meq/kg)</th>
<th>Chloride (meq/kg)</th>
<th>Chloride Space (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.8±0.6</td>
<td>52.7±4.7</td>
<td>99.9±3.8</td>
<td>41.1±4.1</td>
<td>31.0±3.4</td>
</tr>
<tr>
<td>0.3 hr</td>
<td>80.0±0.5</td>
<td>53.6±4.0</td>
<td>102.6±4.2</td>
<td>41.1±3.5</td>
<td>30.4±1.6</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>80.0±0.5</td>
<td>48.6±1.3</td>
<td>103.1±2.6</td>
<td>37.8±0.6</td>
<td>29.2±0.8</td>
</tr>
<tr>
<td>6 hrs</td>
<td>79.1±1.2</td>
<td>50.3±2.6</td>
<td>103.0±3.1</td>
<td>37.8±1.7</td>
<td>27.2±1.1</td>
</tr>
<tr>
<td>24 hrs</td>
<td>79.2±0.9</td>
<td>51.1±1.9</td>
<td>99.4±1.2</td>
<td>38.4±1.9</td>
<td>29.3±1.7</td>
</tr>
<tr>
<td>48 hrs</td>
<td>79.5±0.8</td>
<td>50.5±1.5</td>
<td>98.9±2.9</td>
<td>39.0±2.6</td>
<td>31.0±1.6</td>
</tr>
<tr>
<td>1 week</td>
<td>80.6±0.4</td>
<td>52.5±2.0</td>
<td>98.3±3.6</td>
<td>41.7±3.1</td>
<td>31.5±2.6</td>
</tr>
</tbody>
</table>

White matter electrolytes and water content at intervals after cerebral concussion

<table>
<thead>
<tr>
<th>Time after Concussion</th>
<th>Water (%)</th>
<th>Sodium (meq/kg)</th>
<th>Potassium (meq/kg)</th>
<th>Chloride (meq/kg)</th>
<th>Chloride Space (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.9±2.2</td>
<td>52.8±3.6</td>
<td>87.8±5.0</td>
<td>40.9±2.8</td>
<td>31.0±2.9</td>
</tr>
<tr>
<td>0.3 hr</td>
<td>72.0±1.8</td>
<td>51.2±1.8</td>
<td>92.6±5.2</td>
<td>37.3±1.6</td>
<td>27.6±1.8</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>73.0±1.8</td>
<td>51.3±1.7</td>
<td>91.6±5.6</td>
<td>*36.6±1.8</td>
<td>28.1±1.6</td>
</tr>
<tr>
<td>6 hrs</td>
<td>72.4±1.3</td>
<td>52.9±1.2</td>
<td>94.4±3.2</td>
<td>38.9±1.2</td>
<td>28.8±0.5</td>
</tr>
<tr>
<td>24 hrs</td>
<td>69.8±3.7</td>
<td>52.1±3.4</td>
<td>84.6±4.3</td>
<td>*34.9±1.3</td>
<td>27.7±0.7</td>
</tr>
<tr>
<td>48 hrs</td>
<td>70.5±1.5</td>
<td>50.1±2.2</td>
<td>85.6±1.5</td>
<td>37.6±2.4</td>
<td>29.9±1.3</td>
</tr>
<tr>
<td>1 week</td>
<td>70.6±2.0</td>
<td>51.1±4.3</td>
<td>88.4±1.6</td>
<td>38.6±3.8</td>
<td>29.1±2.7</td>
</tr>
</tbody>
</table>

* Mean significantly different from control ($p<0.01$)

Serum and CSF electrolyte content at intervals after cerebral concussion

<table>
<thead>
<tr>
<th>Time after Concussion</th>
<th>Sodium (meq/L)</th>
<th>Potassium (meq/L)</th>
<th>Chloride (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>CSF</td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>147±3</td>
<td>151±2</td>
<td>4.0±0.9</td>
</tr>
<tr>
<td>0.3 hr</td>
<td>146±5</td>
<td>155±3</td>
<td>4.5±0.8</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>149±4</td>
<td>151±3</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>6 hrs</td>
<td>149±9</td>
<td>152±9</td>
<td>2.8±0.5</td>
</tr>
<tr>
<td>24 hrs</td>
<td>141±8</td>
<td>147±1</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>48 hrs</td>
<td>137±9</td>
<td>*145±2</td>
<td>3.5±0.6</td>
</tr>
<tr>
<td>1 week</td>
<td>144±5</td>
<td>152±5</td>
<td>3.0±0.8</td>
</tr>
</tbody>
</table>

* Mean significantly different from control ($p<0.01$)
and generally has been one of two types: 1) an increase of brain volume without an increase in blood content, as reported by White, et al., and 2) increased cerebrospinal fluid pressure of various degrees occurring any time from a few hours to 24 hours after head injury. Alexander and Looney, after studying many human brains at postmortem, concluded that cerebral edema could not be correlated with water content. However, as Pilcher pointed out, they defined cerebral edema as any increase in brain volume, whereas most subsequent authors have defined it as an increase in water content of the tissues. In human postmortem studies of head injury cases, Shapiro and Jackson concluded that there was a slight decrease in water content of the traumatized brain. Pilcher, in an experimental study, found a small and insignificant increase in water content of the animals receiving head injury. Eichelberger, et al., in another experimental study, found no change in water content in the concussed animals.

We have found no evidence in the present study of any increase in water content of the brain in cerebral concussion. Thus, either cerebral edema does not occur after brain concussion in the experimental animal, or, if it does occur, we are not able to detect it. If an increased water content of the brain does occur after injury and is uniformly distributed, then, because of limitations in methodology, it may be very difficult to detect. As White, et al., pointed out, a 5% increase in brain volume would result only in about a 1% increase in brain water. Thus, it can be seen that this would be difficult to demonstrate because of variations inherent in the methods of determining water content. However, we cannot assume that excess water in cerebral edema would be uniformly distributed.

In most types of experimental cerebral edema such as cold injury, and triethyl tin, edema, it has been shown that the edema fluid collects primarily in the white matter and that major electrolyte changes occur in the white matter. These authors have found an increase in sodium and chloride, a decrease in potassium, and a significant increase in water content in the white matter of edematous brain tissue produced experimentally. Only in osmotically induced brain edema has there been conflicting evidence as to what happens to the brain electrolytes. In all cases of such edema there is an increase in brain water content; van Harreveld, et al., have demonstrated a decrease in sodium, potassium, and chloride content of whole brain tissue of rabbits calculated on a wet weight basis after distilled water infusion. Stern and Coxon have also reported similar changes in sodium and potassium after distilled water infusion in guinea pigs. In contrast to this, Herschkowitz, et al., have demonstrated an increase in sodium and a decrease in potassium after distilled water infusion in monkeys, more marked in white matter than in gray, similar to changes noted in other types of edema. Pappius, on the other hand, using repeated hemodialyses in uremic dogs, was able to produce cerebral edema without any change in the sodium or potassium content of cerebral tissue.

It is even more difficult to get uniform results for water content in white matter than it is in gray matter because of the difficulty in getting samples of pure white matter without gray matter. However, we would like to stress that the water and electrolyte changes demonstrated in the white matter in the common (non-osmotic) types of experimental edema described above have been major changes. This experimentally demonstrable edema has been classified as being of two etiologic types, vasogenic and cytotoxic by Klatzo. Because we found no major electrolyte and water changes, our study indicates that the commonly recognized types of experimental edema of the white matter are not present to

### TABLE 4

<table>
<thead>
<tr>
<th>Time after Concusson</th>
<th>Water (%)</th>
<th>Specific Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78.0 ± 0.2</td>
<td>1.054 ± 0.014</td>
</tr>
<tr>
<td>0.3 hr</td>
<td>77.3 ± 0.5</td>
<td>1.069 (N = 1)</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>*76.8 ± 0.4</td>
<td>1.050 ± 0.017</td>
</tr>
<tr>
<td>6 hrs</td>
<td>77.8 ± 0.3</td>
<td>1.045 ± 0.004</td>
</tr>
<tr>
<td>24 hrs</td>
<td>76.7 ± 0.9</td>
<td>1.039 ± 0.002</td>
</tr>
<tr>
<td>48 hrs</td>
<td>77.5 ± 0.5</td>
<td>1.039 ± 0.004</td>
</tr>
<tr>
<td>1 week</td>
<td>77.9 ± 0.3</td>
<td>1.044 ± 0.001</td>
</tr>
</tbody>
</table>

* Mean significantly different from control (p < 0.01)
any significant extent after experimental cerebral concussion in the monkey.

Thus, either cerebral edema does not occur after concussion, or if it does occur, it is probably a uniformly distributed increase in water content such as the osmotically induced brain edema described above. Moreover, the edema of cerebral concussion does not involve major changes in electrolyte concentration, and is too small to detect by the dry and wet weight method. It should be remembered, however, that these animals received a moderate head injury which, although producing cerebral concussion, was followed by rapid recovery and normal responses within a period of a few minutes after the trauma. In no case was there a severe head injury with macroscopic lesions associated with prolonged unconsciousness. The results of comparable experiments with more complex head injury might be quite different.

If cerebral edema as experimentally defined does not occur after uncomplicated cerebral concussion, this does not mean that there are no chemical changes in the brain after concussion. In fact, it would be surprising if there were no changes. Although a few enzymatic and metabolic studies have been reported, existing data on electrolyte and water content are not complete, and much further work is required. We must realize that current methods are relatively crude, and it is difficult to measure redistribution of water and electrolytes among the various compartments of the brain without a change in total quantity.

The only evidence for something of this nature in our experiments was the small but significant decrease in chlorides of the white matter after 1.5 hours and particularly after 24 hours (Table 2). One could argue that functional changes in exchange mechanisms for water might well lead to the decrease in chloride content that we have seen, but direct evidence for this is very tenuous. In unpublished experiments by Steinwall, et al., the minimal interference with the blood-brain barrier produced by unilateral intracarotid injection of mercuric chloride in cats caused a decrease of whole brain tissue chloride in that hemisphere, without a change in the water content as compared to the contralateral control hemisphere. This is comparable to our findings after concussion. However, when a maximal blood-brain barrier lesion was similarly induced by a stronger concentration of mercuric chloride, it was found that the brain tissue chloride as well as the total water content of that hemisphere increased significantly, as one would expect. This chemical evidence for brain edema was also confirmed by the gross demonstration of extravasation of acid dyes in the affected white matter.

In any discussion of cerebral edema, it is wise to remember that, even if there is no significant or measurable edema, brain swelling (increase in volume of intracranial contents excluding cerebrospinal fluid and meninges) still may occur. Some investigators, because of the lack of any direct evidence of increased water content, have felt that the brain swelling that does occur after concussion is probably due to vascular engorgement in the brain substance. Because of clinical impressions and the work of White, et al., others have felt that an increase in the brain volume must be due to an increased water content of the brain. A true understanding of the role of cerebral edema and brain swelling after head injury cannot be suitably obtained from observations in man, primarily because of the almost complete absence of precise correlations between the biomechanical data and the initial extent of brain injury. Experimental observations in animals are thus absolutely necessary; and these, too, may be vitiated by lack of attention to correlations between the mechanics of the experimental trauma and its pathological effects.

Many investigators have noted increased cerebrospinal fluid pressure after head injury although without statistical correlations between the mechanics of the trauma and the severity of head injury. We have not found any significant increase of cisternal CSF pressure up to 1.5 hours after concussion. One animal monitored for a longer period of time did show a moderate increase in pressure at 4 hours. Since the head is essentially a closed space, it is apparent that an increased pressure must be due either to increased volume of the brain, intracranial blood, or CSF. The CSF volume can only change because of increased production or decreased absorption. Intracranial blood volume changes are best known to occur as a result of increased blood pCO2. However, various other things including trauma could also cause vasodilation.

The only known way that the volume of the brain excluding the blood vessels can increase
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is through increased water content. We feel that the brain swelling that occurs is probably due largely to vascular factors in these cases and takes a period of hours to develop. However, it may be that even the slow onset of brain swelling after uncomplicated concussion may be of little clinical significance. None of the animals that were observed up to 1 week after concussion showed any deleterious effect of the head injury. Our further experiments will attempt to define more accurately both the nature and degree of brain edema as well as brain swelling after increasing degrees of experimental head injury in the monkey.

Summary

Careful measurements have been made of the electrolyte and water content of frontal and parietal cortical gray and subjacent white matter samples in 20 monkey brains at intervals of 0.3, 1.5, 6, 24, 48 hours, and 1 week after experimental cerebral concussion. Compared to suitable controls, there was no significant change in sodium, potassium, or water content in any of the samples. The white matter samples taken at 1.5 and 24 hours after the standardized head injury showed a statistically significant decrease of chloride ion without concomitant change in water content.

We have interpreted our results in the light of current knowledge concerning the biochemical correlates of brain edema, and suggest that uncomplicated cerebral concussion is not followed by any significant degree of brain edema except insofar as it may be due to a uniformly distributed water increase not detectable by the dry and wet weight method. Our evidence suggests that, although some brain swelling does occur after a concussion, it is of relatively slow onset and is probably not of clinical significance in uncomplicated cases.

Acknowledgments

Pilot experiments developing some of the techniques used in this project were conducted by Dr. R. Bourke in the Laboratory of Neurochemistry, NINDB, under the direction of Dr. Donald Tower. We are grateful for the continuing encouragement and advice given by Dr. Tower and for the earlier work of Dr. Bourke.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

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The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, NAS-NRC.

References


