BASIC STUDIES IN CEREBRAL EDEMA
ITS CONTROL BY A CORTICOSTEROID (SOLU-MEDROL)*

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The interchangeable use of the terms “cerebral edema” and “cerebral swelling” is confusing and, to us, seems incorrect. Cerebral “edema” implies a specific state of the cerebral parenchyma, whereas cerebral “swelling” is not specific. We should, therefore, like to define cerebral edema as “that state of the brain, wherein there has been an increase of fluid in brain substance, excluding the vascular bed.” In view of conflicting concepts of the cellular structure of the brain, we do not propose to enter into the controversy of whether this fluid may be intracellular, extracellular, or both. Our definition of cerebral edema seems to be limited enough, yet comprehensively inclusive to allow a common knowledge of what is being discussed. The general term “cerebral swelling” does not satisfy these criteria.

In studying cerebral edema, two difficulties are encountered. First, a technique is needed to produce consistently, in the experimental animal, edema of the brain. Secondly, some parameters that are indicative of the presence of cerebral edema must be constantly reproducible. A review of the literature of cerebral edema (swelling) would lead one to believe that edema is one of the uniform responses of the brain to any noxious stimulus. Edema is inconstant in degree and determined by many factors. These include maturity, the degree and nature of the stimulus as well as the physiologic background to which the stimulus is applied.

The technique of Edstrom (injecting vegetable oil into the internal carotid arteries to produce cerebral edema) has proved a satisfactory method of studying the problem. In the first part of this study (Blinderman and Graf), the water content and osmolarity of whole brain tissue were chosen as parameters, for it was felt that these reflected best the physiologic state of the edematous brain. These, moreover, were readily adaptable to measurement; this approach to the problem is different from those employed by others.

METHOD

Male mongrel dogs weighing between 9 and 18 kg. were anesthetized by using Nembutal, 0.5 cc./kg., intravenously. Four control and 5 test dogs were used. Four independent determinations were made on each animal.

The dog’s head, neck and thighs were shaved, and an intravenous infusion of normal saline was given. Throughout the experiment the animals received 300 cc. of normal saline intravenously over a period of 3-4 hrs.

Both internal carotid arteries were isolated, and the common carotid arteries were ligated. In the test group 0.1 cc./kg. of cottonseed oil was injected, being washed into the internal carotid artery with 10 cc. of normal saline. In the control dogs, arterial puncture was made and 10 cc. saline only were injected.

Three to 4 hrs. after the injection, the animals were sacrificed by means of an overdose of intravenous Nembutal. The calvarium was opened quickly, the brain was transected at the level of the colliculi, and the cerebral hemispheres were removed. The right and left hemispheres then were each divided into approximately 10 equal parts.

Four parts were selected at random from each hemisphere, placed into each of four tared vessels, and weighed. The parts were then placed into liquid N₂ and transferred to the drying vessels of the Fisher Freeze-Dry apparatus (Model 6-380).
The time consumed for this part of the experiment (from sacrificing the animal to freeze-drying of specimens) was approximately 10 to 15 min.

The tissue was then subjected to a sublimation water-extraction process at 10–15 microns of Hg pressure for 24 hrs. and then reweighed. Previous experience had shown that if drying were adequate, the brain tissue could be ground to a fine powder; but if any water remained, such grinding was impossible. Specimens dried inadequately were rejected.

The four specimens of brain from each animal were treated separately. The dry tissue was ground to a fine powder, and weighed again. Fifty cc. of distilled water were added to each specimen and shaken well.

Aliquots of each specimen were transferred to centrifuge tubes and spun at 2500 rev./min. for 40 min. Two cc. of the supernatant material were then transferred to tubes of a Fiske osmometer (Model G) and the freezing-point depression was determined. From this depression the osmolarity of the solution was calculated. No correction for activity coefficients was made in the calculation. The biological interpretation of this parameter will not be discussed at this time.

RESULTS

The results of the control and test groups are shown in Table 1. Column A shows the per cent dry weight (weight after drying divided by weight of wet brain before drying). Column B gives the milliosmols per gram of dry brain. This was determined by measuring the osmolarity of the brain powder-water mixture and dividing this value by the grams of brain powder. The values in Column C were obtained by dividing Column B by Column A, and represent the apparent osmolarity of the reconstituted brain tissue.

Beneath each column is given the mean and the standard deviation as calculated from the standard formula

\[
S^2 = \frac{\sum X^2 - (\Sigma X)^2}{n} \quad \frac{n - 1}{n - 1}
\]

when \( S \) is the standard deviation; \( \sum X^2 \) is the sum of the square of the value; \( \Sigma X \) is the sum of the value, and \( n \) is the number of observations.

Results of the "t" test comparing the various results of the control and test groups

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
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<tbody>
<tr>
<td>Dog No.</td>
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<tr>
<td>---------</td>
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<tr>
<td>Normal</td>
</tr>
<tr>
<td>VIII 42 lb.</td>
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<tr>
<td>22.93</td>
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<tr>
<td>24.07</td>
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<td>IX</td>
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<tr>
<td>24.28</td>
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<tr>
<td>21.84</td>
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<tr>
<td>24.83</td>
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<tr>
<td>XI 37 lb.</td>
</tr>
<tr>
<td>23.88</td>
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<tr>
<td>24.93</td>
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<tr>
<td>25.62</td>
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<tr>
<td>XV 34 lb.</td>
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<tr>
<td>23.83</td>
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<tr>
<td>23.55</td>
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<tr>
<td>24.93</td>
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<tr>
<td>XVII</td>
</tr>
<tr>
<td>22.14</td>
</tr>
<tr>
<td>23.20</td>
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<tr>
<td>23.38</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>S.D.</td>
</tr>
<tr>
<td>Test</td>
</tr>
<tr>
<td>20.32</td>
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<tr>
<td>19.48</td>
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<tr>
<td>20.74</td>
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<tr>
<td>XIII</td>
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<tr>
<td>20.57</td>
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<tr>
<td>19.06</td>
</tr>
<tr>
<td>20.43</td>
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<tr>
<td>XIV 30 lb.</td>
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<td>19.93</td>
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<tr>
<td>Mean</td>
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<td>S.D.</td>
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</tbody>
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using

\[
t = \frac{X_1 - X_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}
\]
when \( X \) is the mean, \( S \) is the standard deviation and \( n \) is the number of observations, give a "t" for Column A of 10.6, Column B, 4.58 and Column C, 6.40. Using a standard table of degrees of freedom, all values are greater than \( t_{0.995} \) level, a high degree of significance.

The data were then recalculated by using the mean value of the four determinations of each dog. These data are summarized in Table 2. Reapplying the above formula, one finds the \( t \) for Column A is 11.31; for B, 3.36; and C, 8.58. Again a level beyond \( t_{0.995} \) was obtained.

The statistical analysis would indicate that the probability of having the difference found in these two groups occurring by chance alone is indeed remote, and one must assume a distinct difference in osmolarity-water content of the control and treated groups.

**DISCUSSION**

Several workers have devised techniques for evaluating cerebral edema. Earlier investigators of this problem realized that gross and microscopic observations of the brain were not accurate in making the diagnosis of cerebral edema. Indeed, such observations often were misleading. Small and Krehl, in a review of autopsy protocols, could find only a 16 per cent correlation of the gross findings of "edema" with microscopic studies manifested by an increase in the "interstitial" cells.

The usual technique for evaluation of edema in the traumatized brain has been the estimation of its water content, determined usually by heating to dry weight. There has been disagreement as to whether there is a detectable increase in water in the "swollen" brain. Eichelberger et al. felt that the total water content did not change after trauma. Pilcher also concluded from his experimental data that there was no significant increase of water in the traumatized brain. Alexander and Looney considered that cerebral "swelling" could not be expressed in terms of water content but was, rather, a "physiochemical association affecting water binding capacity of its colloid and lipid elements." White et al. however, showed that there is a distinct increase in water content up to 5.5 per cent. They felt Pilcher's experiment, as well as other investigations, were not accurate since the animal's head had been immobilized during the trauma and the brain, therefore, had not been damaged.

Other techniques for evaluation of cerebral "swelling" have been described. Greenfield, using autopsy material of patients with brain tumors, measured the volume of the skull and brain; he concluded that cerebral swelling (edema) was present when the difference in volume was less than 8 per cent. Hass and Taylor in 1953, described an experiment using cold injury to the brain to destroy a fixed volume per cent of brain and thus produce cerebral edema surrounding the destroyed brain. The L.D. was used as the experimental parameter. Hauser et al. in 1953, used psyllium seeds to induce swelling and measured the albumin content of the swollen brain after extraction by electrophoresis. They were of the opinion that the amount of albumin varied directly with the degree of swelling.

In our animals the normal value of the water content of cerebral tissue agrees well with that found by others.

Our data show clearly that in the brain injured by the technique used here, a definite
increase in water content and osmolarity was found. This increased osmolarity may well account for the greater water content; but other explanations, such as cessation of a hypothetical cellular water pump, must also be considered. Further experiments in which this technique is employed are still in progress. We hope to measure the parameters more precisely in order to determine how cerebral edema may be modified and how such modification takes place.

The second part of this study was undertaken to determine whether the corticosteroid, methylprednisolone sodium succinate (Solu-Medrol) would protect the dog brain from edema caused by trauma induced by the Edstrom technique. Cerebral swelling (edema) was measured by determination of tissue osmolarity as described. The evidence submitted above (Blinderman and Graf) showed that dog brain could be traumatized to produce edema and thereby show consistently higher osmolarity readings than those in the normal dog brain. This work was repeated (Fitzpatrick and Graf) in 6 dogs and confirmed.

MATERIAL AND METHODS

After cerebral injury produced by the Edstrom technique, 8 dogs, each weighing approximately 15 kg., were treated with Solu-Medrol.

The animals were anesthetized with Nembutal (60 mg./5 lb.) and intubated. An intravenous drip of 200 cc. normal saline or 5 percent glucose, to which 20 mg. of Solu-Medrol were added, was given in the femoral vein. Oxygen was administered for 1 or 2 min. every 2 hrs. The dogs required 1 cc. of Nembutal 2 or 3 times during the experiment to remain anesthetized.

After the common and external carotid arteries had been ligated bilaterally, 0.1 cc. of vegetable oil per kg. of body weight was injected into each internal carotid artery and flushed through with 3 cc. of normal saline. After the animals had been treated with Solu-Medrol from 2 to 9 hrs., they were sacrificed by an overdose of Nembutal. The brain, removed rapidly, was cut above the colliculi. Four sections, each the approximate size of a lima bean, were taken, weighed in 4 Dewar flasks, and frozen in liquid nitrogen. The sections then were placed in the Fisher Freeze-Dry apparatus where a vacuum of .001 mg. Hg was produced and maintained for 12 hrs. The specimens then were reweighed to determine the per cent of drying (dry weight over the wet weight). This averaged 22 per cent. Next, the brain sections were crushed to dust in a mortar and placed in 4 Erlenmeyer flasks. Fifty cc. of water were added to each flask, which was then centrifuged. The freezing-point depression was determined next on the Fiske osmometer expressing the osmolarity. By dividing the osmolarity per gm. of dry brain tissue by the percentage of drying, the osmolarity per gm. of wet brain tissue was obtained.

RESULTS OF EXPERIMENT

The previous findings (Blinderman and Graf), showing that the normal dog brain has a range of 3.9–5.5 mOsm./gm. with a mean of 4.8 and a standard deviation of 0.5, were duplicated. In the animals traumatized with intracarotid vegetable oil, the range became 6.3–7.9 mOsm./gm., mean 7.0 and standard deviation 0.6.

Of 8 dogs whose brains were traumatized (Edstrom method), and who had been treated with Solu-Medrol before trauma, cerebral edema failed to develop in 4; 2 showed cerebral edema; and 2 were judged as equivocal as determined by the osmolarity values.

Table 3 would indicate that the desired effect of prevention of increased osmolarity was obtained in those dogs receiving higher doses of Solu-Medrol. The 2 curves on the graph (Fig. 1) represent the probability curves for osmolarity of the normal dog brain (on the left) and the swollen brain (on the right). The abscissa shows the osmolarity from 4–8 mOsm./gm. of wet brain. The

![Fig. 1](image-url)

Fig. 1. Graph showing on left the normal distribution curve with a mean of 4.8, standard deviation of 0.5 and range in milliosmols of 3.9 to 5.5. On right is the distribution curve for the swollen brain. Here the mean is 7.0, standard deviation 0.6 and range is 6.3 to 7.9 milliosmols.
ordinate scale indicates the total dosage, per
dog, of Solu-Medrol used in the treated ani-
imals. The duration of treatment in hours is
shown by the small numeral beside the black
dots representing the respective dogs. A
normal range of osmolarity is noted in 4 ani-
mals that received high doses of Solu-Medrol;
in 2, the range was within that for the swol-
len brain; in the remaining 2, treated with a
low dose of the drug for a short period of
time, the range is in an intermediate position.

Table 3 shows the results obtained in the 3
sets of dogs used in the second part of the
study. Cerebral edema did not occur in 4 of
the animals treated. In all animals given
higher doses of Solu-Medrol (120–140 mg.)
edema was not produced. If one assumes that
the drug had no effect on cerebral edema,
then all the values of osmolarity would be in
the range 6.3–7.9 mOsm./gm. This, they are
not.

It is worth while to consider in our experi-
mental animals the dosage of Solu-Medrol in
relation to the recommended human dose.
Solu-Medrol has a half-life of 1.5 hrs. When a
dog is to be treated with a total dose of 120
mg. over a 6-hr. period, the dosage per hr.
would be 20 mg./hr. Our dogs weighed about
one-fifth of a normal-sized man, or about 13
kg. The animal dose would be comparable,
therefore, to 100 mg./hr. for the human.
Whether these dose values can be compared
as “effective dose values” because of species
differences, we do not know.

CONCLUSIONS

It has been shown that, in the brain dam-
aged by vegetable oil emboli, a significant
increase in the osmolarity of the brain tissue
and an increase of water content were found.
A technique is described for measuring these
parameters as a basis for study of the modifi-
cation of cerebral “swelling” or edema.

It is our belief that in those animals in
which cerebral edema occurred, the therapeu-
tic level of Solu-Medrol had not been
reached (Table 3, Dogs 10 and 11) and that a
higher dose should have been used to produce
the desired effect. The “critical” dose and
duration of treatment have not been de-
termined as yet. It is our opinion that when
smaller doses of the drug were employed, the
animal was not “protected” from develop-
ing cerebral edema.

Further studies in the experimental animal
are necessary to determine how long un-
treated cerebral edema, as produced by the
technique described, persists, before one can
evaluate Solu-Medrol in treatment of cere-
bral edema beyond a 9-hour period. We feel
that the method of study as outlined above
is a proper one, since it deals with known
causes of edema, i.e., osmolarity and ionic
strength change within the brain itself. Solu-
Medrol seems to exert its effect by moving
solutes in solution, and to strengthen the cell
and its membranes by its anti-inflammatory,
and “anti-upsetting” role.

The evidence of our study indicates that
the corticosteroid (Solu-Medrol), when given in adequate dosage, can prevent increased osmolarity (hence cerebral edema) in the traumatized dog brain.

REFERENCES