The Measurement of Cerebral Infarction Edema With Sodium 22

BY BRUCE BRUNSON, M.D., JAMES T. ROBERTSON, M.D., HOWARD MORGAN, M.D., AND BEN I. FRIEDMAN, M.D.

Abstract: Sodium 22 was used to determine quantitatively cerebral infarction edema in cats after occlusion of the middle cerebral artery. A comparison was made of the sodium 22 edema determination with the wet weight-dry weight method of edema determination. There was a high degree of correlation between the two methods. The sodium 22 method of measuring cerebral infarction edema was less time consuming and easier than the wet weight-dry weight method, and served as a reliable means of quantitatively comparing the edema content in the hemispheres of animals with stroke.

Additional Key Words wet weight-dry weight method radioisotopes

Introduction
The edema of a cerebral infarction not only increases the size of the infarcted brain but is generally conceded as the cause of decreasing levels of consciousness and death due to brain stem compression. The edema fluid is derived from plasma and is thought to accumulate due to an interference with the blood-brain barrier. The edema appears to be the cause of the "no-flow" phenomena seen after restoration of cerebral flow and unquestionably interferes with collateral flow on the cerebral surface as the evolution of an infarction is observed.

The best model for producing a cerebral infarction with direct clinical correlation was introduced by Sundt and Waltz. This extracerebral approach to the middle cerebral artery with subsequent clip occlusion has received extensive utilization and study in this laboratory.

The problems of measuring infarction size and the degree of cerebral edema produced by the infarction is well known. The only unquestioned method of quantifying cerebral edema has been the time-consuming wet weight-dry weight method. Radioisotopes have been used in the study of cerebral edema, but none has been applied as a simple means of quantifying the degree of edema with cerebral infarction. Radioactive sodium 22 has recently received use in the measurement of edema produced by laser trauma in rats, and this method has been applied in our laboratory to assess the edema produced by the infarction of middle cerebral artery occlusion.

Experimental Method
The experimental animal was the adult cat, unselected as to sex, ranging in weight from 1.5 to 5.5 kg. Anesthesia was induced using intraperitoneal sodium pentobarbital and maintained at an adequate level with intravenous injections as needed.

The middle cerebral artery was exposed through the retro-orbital, extradural approach. The scalp incision and all dissection was done with sharp instruments. Hemostasis was maintained with the bipolar electrocautery. With the aid of the Zeiss binocular operating microscope and a small pneumatic drill, the orbital ridge was resected, the orbital contents swept anteriorly, and the sphenoid wing resected to the optic nerve. No retraction of cerebral tissues was necessary. The dura overlying the optic nerve was then opened and the arachnoid surrounding the middle cerebral artery was dissected away sharply. The middle cerebral artery was occluded with a miniature Mayfield clip, and the wound was closed in a layer fashion.

All the animals received a neurological examination preoperatively and at 24 and 48 hours postoperatively. This included respiratory rate, corneal reflex, pupillary reflex, toe pinch (forelimb and hindlimb), righting reflex, placing reflex (with and without vision), deep tendon reflex, and head shake. Daily weights, hematocrits, and serum sodium determinations were used to evaluate the hydration of the animals. They received no foods or fluids per mouth after the onset of the experiment. Parenteral fluids were given intraperitoneally immediately postoperatively and at 24 hours.
Twenty-four hours postoperatively, each animal was injected with 25 μC of radioactive sodium 22* intraperitoneally. Prior to sacrifice at 48 hours, the animals were again sedated and blood was drawn for serum sodium determination by flame photometry and for analysis in an automatic gamma well counter. The brains were likewise removed, sectioned between the midbrain and the pons, divided in half along the midline, weighed and counted. The brains were then fixed in 10% buffered formalin solution and sectioned for microscopic examination, except those brains used for wet weight-dry weight determinations.

In this study, 22 cats were used and divided into five groups:

- **Group 1: Control.** Three cats without anesthesia or operative procedure received a 24-hour maintenance dose of 5% dextrose in sodium lactated Ringer's solution intraperitoneally (10 ml per kilogram). This was repeated at 24 hours.

- **Group 2: Control.** Three cats were anesthetized, but did not undergo the operative procedure. They received a maintenance dose of 5% dextrose in lactated Ringer's at the time of anesthesia and at 24 hours postoperatively.

- **Group 3: Control.** Two cats were anesthetized and had a sham operation which included all but clipping the right middle cerebral artery. These animals also were given a maintenance dose of 5% dextrose in lactated Ringer's solution postoperatively and at 24 hours.

- **Group 4: Four cats were anesthetized and had clipping of the right middle cerebral artery. They, too, were given a maintenance dose of 5% dextrose in lactated Ringer's solution postoperatively and at 24 hours.

- **Group 5: Ten cats, numbers 1 through 10, were used to compare the sodium 22 method of evaluation of cerebral edema to the wet weight-dry weight method of cerebral edema determination. These cats had clipping of the right middle cerebral artery as previously described. Hydration was maintained with 5% dextrose in lactated Ringer's solution, 10 ml per kilogram of body weight immediately postoperatively and at 24 hours postoperatively.

In groups 1 through 4 edema was quantitatively measured with the use of sodium 22. The specific activity of sodium in the serum (SA_{s}) was calculated by dividing the counts per minute per milliliter of serum by the chemical concentration of sodium in micro-equivalents per milliliter. Since the sodium is contained in the extracellular fluid, the sodium concentration of the brain is assumed to be equivalent to that of serum. The specific activity of the sodium in the serum equals the specific activity of the sodium in the brain after 24 hours of equilibration. The concentration of the sodium in the brain is determined by the counts per minute per gram of fresh brain divided by the specific activity of sodium in the serum. The sodium space of the brain is actually the tissue-blood ratio and is defined as the micro-equivalents of sodium per gram of brain divided by the micro-equivalents of sodium per milliliter of serum and this is expressed as milliliters of serum per gram of brain.

The determination is summarized as follows:

\[
\text{Specific activity of the Na in serum (SA_{s})} = \frac{\text{CPM/ml of serum}}{\text{μEq/ml of Na}}
\]

\[
\text{Na concentration of brain is} = \frac{\text{CPM/gm of brain}}{\text{μEq of Na/gm of brain}}
\]

\[
\text{Na space of brain (i.e., tissue-blood ratio)} = \frac{\text{μEq of Na/gm of brain}}{\text{μEq of Na/ml of serum}} = \text{ml of serum/gm of brain}
\]

\[
\text{Total amount of serum in each hemisphere (ml)} = \frac{\text{tissue-blood ratio}}{\text{(ml serum/gm of brain)}} \times \text{wet weight of each hemisphere (grams)} = \text{ml of serum.}
\]

\[
\text{Total } ^{22}\text{Na edema (ml of serum)} = \text{total amount of serum in infarcted hemisphere (ml of serum)} - \text{total amount of serum in noninfarcted hemisphere (ml of serum)} = \text{ml of serum.}
\]

In group 5 tissue-blood ratios of both hemispheres and the following calculations were made to correlate wet weight-dry weight determinations with the sodium 22 determinations. The wet weight-dry weight determination of edema was done after radioactive counting. The hemispheres were placed in an oven at approximately 60°C for 96 hours until a constant weight was reached. The amount of water in each hemisphere was determined by subtracting the dry weight from the wet weight of each hemisphere. The amount of edema in the infarcted hemisphere based on the wet weight-dry weight method was calculated by subtracting the amount of water in the noninfarcted hemisphere from the amount of water in the infarcted hemisphere.

The tissue-blood ratio serves as a means of comparing the edema content of different animal brains of different weights, since the tissue-blood ratio is in...
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units of milliliters of serum per gram of brain. To compare the sodium 22 method with the wet weight-dry weight method, the total amount of sodium 22 edema in the infarcted hemispheres of Group 5 animals was calculated. This was done by multiplying the tissue-blood ratio by the wet weight of each hemisphere to determine the total amount of serum in each hemisphere. The difference between the serum content of the infarcted and noninfarcted hemispheres represents the total calculated edema in the infarcted hemisphere by the sodium 22 method. The summary of these last calculations is:

Results
All cats included in this study who had clipping of the middle cerebral artery had an infarct demonstrated clinically by reduction in respiratory rate, decreased corneal reflex, and contralateral hemiparesis with circling toward the side of the lesion on attempting to walk. When suspended freely by the back of the neck, the animals adducted the paretic upper extremity. There was decreased response to toe pinch, greater in the forelimb than in the hindlimb, decrease in head shake, absent or marked abnormality in the righting reflex, and absent placing response, even with vision. Grossly, the infarcted hemisphere was softened and was of increased weight as compared with the noninfarcted hemisphere. On microscopic section, there was evidence of infarction in the distribution of the middle cerebral artery.

A paired "T" test was used to compare the left and right hemisphere in Groups 1, 2, and 3 with a resulting "T" of zero, indicating no difference between these hemispheres. The tissue-blood ratio and the percent wet weight of each hemisphere was essentially the same, indicating that neither the anesthetic nor the operative procedure, with exception of clipping of the middle cerebral artery, had any effect on these measurements (tables 1 and 2).

A random "T" test showed that the noninfarced hemispheres in Group 4 were not statistically different from the control hemispheres. The infarcted hemispheres, however, had a marked increase in their tissue-blood ratio, indicating that the edema was chiefly localized to the infarcted hemisphere. A paired "T" test was used to compare the infarcted and noninfarcted hemispheres in Group 4. The resulting "T" was 2.527 with a "P" of less than 0.05, indicating a significant difference between the infarcted and noninfarcted hemispheres comparing the tissue-blood ratios. This correlates well with the wet weights of the brains which also showed an increase in the infarcted hemisphere in a relative manner.

In the fifth group of animals comparing the two methods of quantitative determination of edema (table 3), the edema based on the sodium 22 method appears greater than the edema fluid determined by the wet weight-dry weight method. However, the relationship between the determinations approaches linearity when plotted on a graph (fig. 1). Fitting an equation to this relationship is simple. Using the method of least squares, a regression line is determined and the equation is: $E_w = 0.063 + 0.5195E_n$ where $E_w$ is the wet weight-dry weight edema and $E_n$ is the sodium 22 edema. The correlation coefficient is 0.9801 and the "P" is less than 0.001, indicating a high degree of correlation between the two methods of determining cerebral edema.

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue-blood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
</tr>
<tr>
<td>1</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>0.55 ± 0.07*</td>
</tr>
</tbody>
</table>

*Infarcted hemisphere.

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>% of total brain weight (wet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>51*</td>
</tr>
</tbody>
</table>

*Infarcted hemisphere.

**FIGURE 1**

The relationship of edema determinations—the wet weight-dry weight method versus the sodium 22 method.
TABLE 3

Comparison of Net Weight-Dry-Weight and Na$^{22}$ Methods of Cerebral Infarction Edema Measurement

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Wet weight (grams)</th>
<th>Dry weight (grams)</th>
<th>Tissue blood ratio (ml serum/gm brain)</th>
<th>$^{22}$Na edema* (ml serum/gm brain)</th>
<th>Wet wt-Dry wt edema (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10.9</td>
<td>9.3</td>
<td>2.1</td>
<td>2.1</td>
<td>0.648</td>
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<tr>
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<td>10.9</td>
<td>8.8</td>
<td>2.0</td>
<td>2.1</td>
<td>0.712</td>
</tr>
<tr>
<td>3</td>
<td>12.1</td>
<td>10.6</td>
<td>2.8</td>
<td>2.7</td>
<td>0.595</td>
</tr>
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<td>4</td>
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<td>9.4</td>
<td>2.2</td>
<td>2.2</td>
<td>0.661</td>
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<td>10.7</td>
<td>2.4</td>
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<td>0.596</td>
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<td>10.2</td>
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<td>0.471</td>
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<td>2.0</td>
<td>0.477</td>
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<td>9.3</td>
<td>2.3</td>
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<td>0.691</td>
</tr>
</tbody>
</table>

*Calculated by multiplying the tissue-blood ratio of each hemisphere by the wet weight of the hemisphere to determine the total serum content. The difference between the calculated serum content of the two hemispheres represents the edema as determined by the $^{22}$Na method.

+Calculated by subtracting the dry weight from the wet weight in each hemisphere to determine the water content. The difference between the water content of the two hemispheres represents the edema as determined by the wet weight-dry weight method.

The explanation for the difference in these two determinations of edema is probably related to the phenomenon of sodium being more concentrated in certain areas of the infarct than in other areas and that the sodium concentration in the infarct is greater than that in a normal brain. This disparity in sodium concentration was described by Bakay\cite{10} in traumatic cerebral edema in which the sodium in the edematous brain was more concentrated near the traumatic lesion.

Summary

The sodium 22 method of assaying laser trauma edema in the rat is applicable to measuring cerebral infarction edema in the cat. The long half-life of sodium 22 and its ready availability make it an attractive radioisotope for laboratory investigation. The sodium 22 method is faster and easier to perform than wet weight-dry weight determinations of cerebral edema. The tissue-blood ratio serves as a reliable means of quantitatively comparing infarction edema in the cerebral hemispheres of animals with stroke. This model can now be used to evaluate the use of drugs and parenteral fluid therapy in vogue in the treatment of cerebral infarction edema.

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Dr. Harry S. Robinson, Associate Professor, Chief of Biostatistics, and Director of the Arthritis Research Program at the University of Tennessee Medical Units, Memphis, Tennessee, was beneficial in the statistical preparation of this paper.

References

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