BRAIN EDEMA is an important pathological state to investigate as it interferes with cerebral circulation. The condition is thought to comprise two types of edema: cytotoxic and vasogenic. The effect of brain edema is complicated not only because of the resulting mass effect but also due to its effect on cerebral metabolism. Until recently, most experiments on brain edema in association with brain ischemia have been carried out employing morphological and biochemical methods; however, these experimental methods have not provided a complete understanding of water characteristics in the brain. The introduction of nuclear magnetic resonance (NMR) techniques in the field of biophysics as a non-invasive method should provide important information. Using NMR, the state of water in the tissue can be evaluated by measuring relaxation times. We have already applied these techniques successfully to the problems of brain edema using cold injury, TET (triethyl-tin) intoxication, and brain tumor models in rats experimentally.

In the present study, we measured proton relaxation times in experimental cerebral ischemia induced in Mongolian gerbils in order to investigate the state of water in the ischemic brain. The effect of glycerol on the ischemic brain was also examined by measuring changes in relaxation times and water content. From these results, it might be said that ischemic brain edema includes both the cytotoxic and vasogenic types of brain edema. Glycerol was demonstrated to affect brain ischemia by decreasing the water content and by shortening the slow component of T2. By analysis of the relaxation times and water content, we examined the pathophysiological characteristics of water molecules in ischemic brain tissue.

Materials and Methods

1) The Model of Ischemic Brain Edema

One hundred and fifty Mongolian gerbils (Meriones unguiculatus) weighing about 80g each were used. After ether anesthesia, right cerebral hemispheric ischemia was induced by ligating the right carotid artery under a surgical microscope. Sixty percent of the animals showed rolling seizures or severe suppression of motor activity immediately after the operation, and they were judged to be suitable for further experiments. Gerbils were divided into three groups: normal...
control, untreated ischemic, and glycerol treated ischemic groups. In the treated group, four or five animals were decapitated at 1, 3, 5, and 7 hours after ligation. In the treated group, 1 g/kg of 10% w/v glycerol was administered intravenously 30 minutes before decapitation at 7 hours after ligation. When the duration of the carotid ligation exceeded 7 hours, the mortality rate markedly increased due to cardiorespiratory problems; therefore, experiments were limited to the initial 7 hours.

2) Preparation
Immediately after decapitation, brain samples were removed in a humid chamber from the temporoparietal regions of the cerebral hemisphere. Brain tissue was wrapped in a plastic sheet and placed in a NMR tube, 10 cm in diameter. A roll of paper, moistened with 0.8 ml of deuterium oxide, was placed in the bottom of the tube to prevent further drying and also to function as an internal lock for the NMR measurement. Measurement of proton relaxation times was made within several minutes after decapitation. Immediately after NMR measurement, the materials were weighed in a humid chamber and then they were weighed again after dehydration at 100°C for three days. Water content was expressed as a percentage of initial wet weight.

3) Method of NMR Measurement
A pulse Fourier-Transform NMR spectrometer (JEOL PFT-100, Tokyo, Japan) was used at a 1H resonant frequency of 100 MHz. The measurements were made with a single scan at room temperature (24°C). The longitudinal relaxation time (T1) was measured by the inversion recovery method. Repeated measurements of magnetization decay were performed 14 times at various values up to two seconds. The value of T1 was calculated from the slope of the plot of log[(M-Mz)/2M] versus τ, where M is the signal amplitude at zero time and Mz is the signal amplitude at time τ. The transverse relaxation time (T2) was measured by the Carr-Purcell method of pulse sequences modified by Meiboom-Gill. The T2 value was estimated by graphic analysis of 80 plotted points of echo signals up to 800 msec. When a semilogarithmic plot of the magnetization decay versus time gave a straight line, T2 was regarded as consisting of a single component. If this semilogarithmic plot did not coincide with a straight line, T2 was regarded as consisting of at least two components. The value of these relaxation times (T1, T2) was calculated using a method reported previously.

Results
1) Longitudinal Relaxation Time (T1) and Water Content
The time course of changes in T1 and water content in the case of cerebral ischemia is shown in figure 1. The mean value and standard deviation of T1 in the normal cerebrum was 1107 ± 63.1 msec. Following ligation, T1 on the ipsilateral side began to increase from 1147 ± 45 msec at 1 hour after ligation to 1210 ± 39.9 msec at 7 hours. However, T1 on the contralateral side showed very little increase. The water content in the cerebrum of the ligation side increased from a normal value of 79.2% to 82.1% at 7 hours after ligation, but increase in the water content on the contralateral side was minimal. The change in water content was parallel to that of T1.

2) Transverse Relaxation Time (T2) and Water Content
The mean value and standard deviation of T2 in the normal cerebrum was 76.2 ± 2.2 msec, consisting of a single component. The time course of T2 and water content following ischemia are shown in figure 2. T2 on the ipsilateral side to the ligation separated into one slow component and one fast one at an early stage of ischemia. The slow component began to lengthen 1 hour after ligation and reached a maximum value of 117.7 ± 6.1 msec at 7 hours. However, on the contralateral side, separation of T2 was observed only 5 and 7 hours after ligation. The relaxation time value of the slow component on the contralateral side was shorter than that on the ligation side.

3) Effect of Glycerol
Relaxation times (T1, T2) and water content (H2O) of the cerebrum on the ligation side were compared...
FIGURE 2. Serial changes of proton transverse relaxation time (T2) and water content in brain ischemia. T2 on the ligated side hemisphere separates into two components even one hour after the ligation and the slow component increase gradually along the time course after the ligation, shown by continuous line. The separation of T2 on the contralateral side is observed only 5 and 7 hours after the ligation, shown by dotted line.

between the group treated with glycerol and the untreated group (table 1) at 7 hours after ligation, when the slow component of T2 value reached its maximum measured value. It was found that the longitudinal relaxation time (T1) in the treated group was almost the same as that of the untreated group. The transverse relaxation time (T2) was shorter in the treated group than in the untreated group. Water content in the hemisphere of the ligation side was less in the treated group than in the untreated group.

Discussion
Brain edema is generally classified into two types: cytotoxic and vasogenic. In the clinical field, it is important to investigate the pathophysiological state of edema occurring in various brain diseases. Recently, many experiments have been performed to clarify the pathophysiological state of ischemic brain edema. The content of macromolecules in edema fluid influences proton relaxation time, especially T2. Using this characteristic, the cytotoxic and vasogenic types of brain edema in rats were discerned by quantitative analysis of relaxation time (T2) and water content in a previous report. We applied a similar quantitative analysis in the present study in order to elucidate the problems of ischemic brain edema, and report these new results in this paper.

The values of the relaxation times and water content of the normal control gerbil were not the same as those of the rat. This is due to the difference in the species and the samples used in each experiment. The sample in this experiment contains both gray and white matter. However, the measurements were performed on gray and white matter separately in the previous experiment. We have reported that the important point for differentiating the type of edema is not the absolute value but the relative change in relaxation times and water content. Therefore, we believe that the difference in species and samples is not a problem in this comparative discussion.

The relaxation rate R, reciprocal of the relaxation time, versus water content in the ischemic brain and the edematous brain are plotted together on figures 3 and 4. The relationship between the longitudinal relaxation rate (R1) versus water content is shown in figure 3. As all points lie on one straight line for both ischemia and edema types, the change in R1 reflects only the change of water content. However, the relationship between the transverse relaxation rate (R2) and water

**TABLE 1** Effect of Glycerol on Brain Ischemia

<table>
<thead>
<tr>
<th></th>
<th>No Treatment</th>
<th>Treated with Glycerol</th>
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<tbody>
<tr>
<td>T1</td>
<td>1210 ± 39.9</td>
<td>1222 ± 50.3</td>
</tr>
<tr>
<td>T2</td>
<td>117.7 ± 6.1</td>
<td>109.0 ± 8.3*</td>
</tr>
<tr>
<td>H2O</td>
<td>82.1 ± 0.4</td>
<td>81.1 ± 0.6†</td>
</tr>
</tbody>
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(n = 4–5).  
* p < 0.1.  
† p < 0.005.  
T1 = Longitudinal relaxation time (msec); T2 = Transverse relaxation time (msec); H2O = Water content (%).
content in the ischemic and edematous brain differed from that of R1, as is illustrated in figure 4. From this figure, important conclusions can be drawn. Ischemic brain edema can be distinguished from other forms of brain edema by the slope of the line obtained from the plots of R2 versus water content. The value of T2 in vasogenic brain edema is shorter than that of cytotoxic brain edema when the water content has the same value; furthermore, the slope of the line obtained from the relationship between R2 and water content in vasogenic brain edema is biphasic and less than that in cytotoxic brain edema by the slope of the line obtained from the relationship between R2 and water content in vasogenic brain edema. From plotting R2 versus water content, two different slopes would have been demonstrated. Although ischemic brain edema is assumed to comprise two types of brain edema (cytotoxic type in its early stage, vasogenic in the later stage), we could not detect the turning point from the cytotoxic to the vasogenic type by NMR data. If two types were clearly separated, two different slopes would have been demonstrated. The possibility remains that the turning point of the two types of brain edema may occur within one hour after the ligation, a time not included in this study. From this analysis of the slope, it is concluded that ischemic brain edema is not identical with TET-induced brain edema, but that it nevertheless contains some degree of the vasogenic factor even at an early stage of the insult. That is, edema fluid of ischemic brain edema contains some level of macromolecules which affect the value of T2. The measurement and the interpretation of proton relaxation times are important for understanding the state of water molecules in the ischemic brain.

The therapeutic effect of agents such as glycerol can also be demonstrated by measuring the proton relaxation times and water content. Glycerol decreases the water volume in edema fluid due to its osmotic effect, though it may not produce a parallel reduction of macromolecules contained in edema fluid. The relative content of macromolecules in the edema fluid may have increased; this is expected to result in a shortening of T2 far greater than that expected by the total removal of all the edema fluid components. T1 did not change after the administration of glycerol. The reason for this possibly relates to the high magnetic field we used causing T1 variation to be small. Therefore, the shortening of the T1 value resulting from glycerol administration might not be detectable.

The results obtained here, through NMR techniques, provide important information on the nature of edema, and these results may be useful in the future clinical application of NMR techniques.

References

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