SUMMARY The effect of pentobarbital and hypothermia on the development of ischemic brain edema was studied in 23 rhesus monkeys undergoing transorbital middle cerebral artery occlusion. Fifteen additional animals served as unclipped controls.

Regional cortical cerebral blood flow (rCBF), arteriovenous oxygen content difference (AVDO₂), and regional cortical metabolic rate of O₂ (rCMRO₂) were measured hourly until sacrifice 11 hours postocclusion, at which time ischemic cerebral edema was measured.

In 8 animals no treatment followed the occlusion, and these developed significant edema.

In 7 animals pentobarbital 14 mg/kg was administered intravenously 30 min after occlusion and 7 mg/kg every 2 hours thereafter. In this group ischemic brain edema was negligible.

In 8 animals, hypothermia to 25.9 ± 0.5°C was started 30 min after occlusion and maintained until sacrifice; ischemic brain edema was not significantly altered from untreated-clipped animals.

On the basis that both pentobarbital and hypothermia produced similar changes in rCBF, AVDO₂, and rCMRO₂, but only pentobarbital prevented edema, it is postulated that the mode of action of barbiturates in preventing ischemic brain edema is not entirely related to their known effect on blood flow and metabolism.

Ischemic Brain Edema: Comparative Effects of Barbiturates and Hypothermia

FREDERICK A. SIMEONE, M.D., GLENN FRAZER, B.S., AND PABLO LAWNER, M.D.

SYSTEMIC BARBITURATE administration has been reported to have a "protective" effect on the brain in cerebral ischemia and hypoxia.1-3 When measured in terms of infarct size and neurological deficit, different animal models have consistently demonstrated at least temporary improvement when barbiturates were administered around the time of a major cerebral artery occlusion.4-11

The mechanism of barbiturate action in cerebral ischemia is unknown, although many investigators express the feeling that the reduced metabolic requirements of the brain after barbiturate administration improves neuronal survival in ischemic tissue. Hypothermia has been advocated for acute cerebral ischemia and hypoxia with the same rationale.12-14

More recently, barbiturates have been shown to inhibit the formation of cerebral edema produced by cryogenic injury.15,16 The superimposed development of edema on the ischemic brain plays a significant role in the severity and final outcome of the ischemic process. These studies were designed to compare the effect of pentobarbital and hypothermia on the development of ischemic brain edema. After the middle cerebral artery was occluded, brain blood flow and metabolism were reduced to equal levels in both barbiturate and hypothermia studies.

Materials and Methods

The experimental series consisted of data from 38 rhesus monkeys; 15 served as unaltered controls and 23 underwent transorbital middle cerebral artery occlusion. The latter group was divided into 3 groups according to postocclusion treatment: 8 were untreated, 8 subjected to hypothermia, and 7 given pentobarbital.

Anesthesia was induced with phencyclidine HCl 1
mg/kg i.m. and atropine sulfate 0.1 mg/kg i.m. Each animal was also given a prophylactic dose of penicillin and streptomycin. The animals were paralyzed with gallamine triethiodide 1 mg/kg i.v. with supplemental doses as needed, intubated and passively ventilated with a Harvard animal respirator on 70% N2O-30% O2. Respiratory rate and tidal volume were adjusted to achieve a 5% end tidal CO2. Before surgery, subcutaneous lidocaine HCl was used on all incision sites. Catheters were placed in the femoral artery and vein to measure arterial blood pressure, to collect blood gases, and to administer drugs and fluids. A catheter was placed into the lingual artery and passed retrograde into the common carotid artery for the injection of Kr86 dissolved in saline. A fourth catheter was placed into the internal jugular vein for the return of cortical venous blood. Through the transorbital approach of Hudgins and Garcia,17 the right middle cerebral artery was exposed and, with the aid of the Zeiss operating microscope, occluded at its origin with a miniature Mayfield clip. This operative procedure averaged 2 hours in duration. Occlusion was confirmed by a second observer at the time of clipping and at autopsy.

Head surgery was then done as previously described.18 A cortical parietal vein of relatively constant distribution was exposed near the midline and cannulated just before its entrance into the sagittal sinus, after full heparinization of the animal. The craniectomy was extended laterally and the dura reflected to allow the measurement of rCBF by Kr85 washout. The cortical vein catheter was connected to a drop-rate meter and the blood returned via the jugular vein. This permitted monitoring patency of the shunt system and gave a convenient point to sample the cortical venous blood. This procedure required an additional 3 hours.

Arterial blood gases were measured hourly with an IL 113 blood gas analyzer and maintained within physiologic limits by respirator adjustments and intravenous sodium bicarbonate as needed; pH 7.37 ± 0.0008, PO2 100 ± 3 torr, and PCO2 38.0 ± 0.5 torr in all groups. Arterial and venous hemoglobin percent O2 saturations were measured with an IL 182 co-oximeter. (All numbers expressed as mean ± SE).

Arterial blood pressure was monitored with a Sandborn pressure transducer and recorded on a Sandborn 350 physiograph along with the end-tidal CO2 and heart rate derived from the blood pressure waveform. Mean arterial blood pressure was maintained at 110 ± 3.3 torr by the use of a tilt table and i.v. normal saline. There were no significant differences between the treatment groups (p > 0.2). Rectal and cortical temperatures were monitored by appropriate thermistors, via a Yellow Springs instrument meter, and recorded hourly. A circulating water heating pad was used to maintain body temperature at 36.5 ± 0.5°C, except in the hypothermia group.

rCBF was measured hourly starting 6 hours after occlusion by the Kr86 washout technique, using a bolus injection of 2 mCi of isotope. The blood flow was calculated by the height/area method.19 Simultaneous arterial and venous blood samples were drawn for determinations of AVDO2, which were corrected for dissolved oxygen content. The venous blood was collected under mineral oil and allowed to flow into the sampling tube under normal venous pressure. All determinations were done in duplicate and averaged. rCMRO2 was calculated as the product of rCBF and AVDO2.

Eleven hours after occlusion, the animals were sacrificed with an intravenous dose of saturated KCl and the brain removed. The brain was immediately rinsed to remove any external blood and dabbed dry. It was then sectioned at the cerebral peduncles and the cerebrum divided through the midline. The sections were weighed and placed in a vacuum oven at 60°C and 0.5 atmosphere for 48 hours.

The procedure from sacrifice until the oven took no longer than 20 min. From the wet and dry weights, the percent water content of each hemisphere and the brainstem-cerebellum was calculated. The difference in water contents between the 2 hemispheres in each animal was used as a measure of edema.20

Animals in the untreated group received no therapy. The pentobarbital group of animals received an initial dose of 14 mg/kg of pentobarbital intravenously 30 min after occlusion and 7 mg/kg every 2 hours thereafter until sacrifice.4 Serum barbiturate levels ranged between 1.7 and 4.5 mg%. Animals in the hypothermia treated group were packed in ice bags starting at 30 min postocclusion and cooled over no greater than a 90 min period to a body temperature of 25.9 ± 0.5°C. Cortical temperature averaged 1.7°C warmer than body temperature throughout the procedure once cooling had begun. These animals were maintained at the lower temperature until sacrifice. Blood gas and pH determinations on this group were made at 27°C during the hypothermic interval.

Non-parametric statistical tests of significance were chosen because some of the sample sizes were unavoidably small and the distributions did not conform adequately to the assumptions of the parametric analyses. The Kruskal-Wallis Anova test21 was conducted over all 4 groups, for each of the 4 dependent variables. If statistical significances were detected, Mann-Whitney U tests were conducted on each of the 6 combinations of groups.

Results

In all groups, the rCBF, AVDO2, and rCMRO2 values were stable over the period of time studied. The water content of the unclipped hemispheres (76.68 ± 0.33) was not significantly different among treatment groups and the controls (p > 0.05). Similarly, water contents of all brainstem-cerebelli were the same (75.15 ± 0.27) (table 1).

The average of mean arterial blood pressures for each animal was compared to edema (percent water content difference between the 2 hemispheres), using
regression analysis and the t-test for independence. No correlation was found between blood pressure and edema within groups or in the pooled data ($r = 0.2$, $p > 0.2$).

A summary of all rCBF, AVDO$_2$, rCMRO$_2$, and edema data is included in table 2.

Controls
Each of the control values represents the mean of 48 determinations on 15 animals. The anesthetic technique and the method for measuring the above parameters were the same for all groups.

Untreated
Transorbital occlusion of the middle cerebral artery produced a drop in rCBF of 40.9% ($p < 0.001$), a rise in AVDO$_2$ of 13.7% ($p > 0.05$), and a drop in rCMRO$_2$ of 29.3% ($p > 0.05$) as compared to controls. The increase in water content of the clipped hemisphere, compared to the contralateral unclipped, was $0.68 \pm 0.24$.

When edema was compared with rCBF prior to sacrifice, this group showed a significant negative correlation ($r = -0.881$, $p < 0.01$), over a large range of rCBF and edema values (fig. 1A).

Pentobarbital
When pentobarbital-treated animals were compared to untreated animals, there was a drop in rCBF of 20.2% ($p > 0.05$), an increase in AVDO$_2$ of 7.2% ($p > 0.05$), and a decrease in rCMRO$_2$ of 15.7% ($p > 0.05$).

Pentobarbital treatment did decrease edema significantly in the clipped hemisphere to $0.01\% \pm 0.03$ of the contralateral unclipped hemisphere; this represented a 98.5% ($p < 0.01$) edema reduction over the untreated animals. When edema was compared to rCBF prior to sacrifice of each animal in this group, there was no correlation ($r = 0.001$, $p > 0.2$), but after pentobarbital administration there was a tight aggregation of rCBF values with essentially no edema (fig. 1B).

Hypothermia
With hypothermia to $25.9 \pm 1.3^\circ C$ there was a drop in rCBF of 24.9% ($p > 0.05$), a decrease in AVDO$_2$ of 3.5% ($p > 0.05$), and a drop in rCMRO$_2$ of 21.5% ($p > 0.05$). There was no significant difference in rCBF, AVDO$_2$, and rCMRO$_2$ between hypothermia and pentobarbital treated animals ($p > 0.05$ for all comparisons).

Hypothermia produced a decrease in edema in the clipped hemisphere to $0.27 \pm 0.06$ of the contralateral unclipped hemisphere. This represented a non-statistically significant edema reduction of $60.3\%$ ($p > 0.05$) over the untreated group. However, this was a significant increase in edema over the pentobarbital treated group ($p < 0.01$). Comparison of edema with rCBF for this group shows no correlation ($r = -0.223$ $p > 0.2$) (fig. 1C).

Discussion
These data indicated that the effects of hypothermia and pentobarbital on rCBF, AVDO$_2$, and rCMRO$_2$ were similar. Despite this, barbiturates abolished acute ischemic edema, whereas hypothermia reduced it by only a statistically insignificant amount.

Several investigators have shown that barbiturates

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Table 1 Percent Water Contents of Clipped Hemisphere, Control Hemisphere and Brainstem-Cerebellum for Each Preparation

<table>
<thead>
<tr>
<th>Group</th>
<th>Clipped</th>
<th>Control</th>
<th>Brainstem-Cerebellum</th>
<th>Difference between hemispheres (Edema)</th>
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<tr>
<td>Controls:</td>
<td>76.92</td>
<td>75.23</td>
<td>74.52</td>
<td>1.69</td>
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<tr>
<td>77.35</td>
<td>76.67</td>
<td>74.87</td>
<td>75.79</td>
<td>0.88</td>
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<tr>
<td>77.78</td>
<td>77.50</td>
<td>75.79</td>
<td>76.20</td>
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</tr>
<tr>
<td>77.85</td>
<td>77.79</td>
<td>76.78</td>
<td>77.20</td>
<td>0.06</td>
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<tr>
<td>77.85</td>
<td>77.14</td>
<td>75.76</td>
<td>76.70</td>
<td>0.71</td>
</tr>
<tr>
<td>76.30</td>
<td>75.97</td>
<td>74.42</td>
<td>75.80</td>
<td>0.33</td>
</tr>
<tr>
<td>76.35</td>
<td>76.37</td>
<td>75.42</td>
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<tr>
<td>77.76</td>
<td>70.05</td>
<td>74.01</td>
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<td>1.71</td>
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Pentobarbital

<table>
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<tr>
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<th>X = SE</th>
<th>CI = SE</th>
<th>CI = SE</th>
<th>CI = SE</th>
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<td>Control</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>83.11</td>
<td>3.22</td>
<td>6.87</td>
<td>0.28</td>
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<tr>
<td>Untreated</td>
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</tr>
<tr>
<td>8</td>
<td>49.13</td>
<td>3.46</td>
<td>7.81</td>
<td>0.46</td>
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<tr>
<td>7</td>
<td>39.23</td>
<td>0.89</td>
<td>8.37</td>
<td>0.38</td>
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<tr>
<td>Hypothermia</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>36.92</td>
<td>1.41</td>
<td>7.54</td>
<td>0.40</td>
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</table>

Table 2 Data Summary

<table>
<thead>
<tr>
<th>Group</th>
<th>rCBF (ml/100 gm/min)</th>
<th>AVDO$_2$ (ml O$_2$/100 gm/min)</th>
<th>rCMRO$_2$ (ml O$_2$/100 gm/min)</th>
<th>% Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>X = SE</td>
<td>X = SE</td>
<td>X = SE</td>
<td></td>
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<tr>
<td>15</td>
<td>83.11 ± 3.22</td>
<td>6.87 ± 0.28</td>
<td>5.60 ± 0.27</td>
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<tr>
<td>Untreated</td>
<td>49.13 ± 3.46</td>
<td>7.81 ± 0.46</td>
<td>3.96 ± 0.67</td>
<td>0.68 ± 0.24</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>39.23 ± 0.89</td>
<td>8.37 ± 0.38</td>
<td>3.34 ± 0.18</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>36.92 ± 1.41</td>
<td>7.54 ± 0.40</td>
<td>3.11 ± 0.31</td>
<td>0.27 ± 0.06</td>
</tr>
</tbody>
</table>
BARBITURATES, HYPOTHERMIA IN ISCHEMIA/Simeone et al.

DECREASE CMRO2 up to 50–60%, and that additional
doses do not produce further reduction. Michenfelder
noted that at this point the EEG became isoelectric.22
This is presumptive evidence that the barbiturates
inhibit neuronal conduction, but do not significantly
affect the metabolic machinery necessary to maintain
 cellular integrity. In our preparation, barbiturates
produced a drop in rCMRO2 of only 15.7% after transorbital middle cerebral artery occlusion. The
occlusion itself, however, produced a reduction in
rCMRO2 of 29.3% for a total reduction of 40.4% over
unclipped controls. This reduction is similar to the
maximal metabolic depressant action of barbiturates
independent of vascular occlusion. The small dif-
ference is explained by the fact that this barbiturate
dose represents the dose needed to induce only a mod-
erate level of anesthesia as indicated by the EEG.4
Hence, the data suggested that the barbiturate effect
reached the theoretical maximum independent of the
level of brain metabolism at the time of administra-
tion.

The effect of hypothermia on CMRO2 is not con-
fined to the prevention of neuronal transmission. As
temperature drops all cellular metabolic processes are
diminished. Hypothermia does not affect the energy
balance of the brain as judged by the concentrations of
phosphocreatine, ATP, ADP plus AMP; however,
temperature reduction had a profound effect on lact-
tate and pyruvate concentrations.23 This suggested
that in hypothermia energy production diminished at
a rate commensurate with reduction in energy utiliza-
tion.

Both barbiturates and hypothermia reduced
cerebral blood flow. Middle cerebral artery occlusion
produced an initial reduction in rCBF of 40.9%. To
this was added a 20.2% reduction by pentobarbital and
a comparable 24.9% drop by hypothermia. Alteration
of cerebral blood flow cannot be explained on the basis
of change in mean arterial blood pressure because of
careful maintenance of steady pressure by a tilt table.
The mechanism of flow reduction may be secondary to
an increase in cerebrovascular resistance. Vasoco-
striction secondary to pentobarbital may be
limited to the normal brain, and this could, perhaps,
improve flow by shunting blood to focal ischemic
regions ("reverse steal"). Figure 1B shows a phe-
nomenon which was demonstrated consistently in all
pentobarbital treated animals. The "normal"
variability of flow seen in the untreated and in the con-
trol animals was minimized and serial blood flow
values clustered in a narrow range after barbiturates.
This was observed, but to a much lesser extent, in the
hypothermia treated group. Thus, barbiturates
seemed to have the effect of stabilizing blood flow in
ischemic brain.24

Although both hypothermia and pentobarbital
modified rCBF, ADVO2, and rCMRO2 to the same
degree, in barbiturate-treated animals edema was
reduced by 98.5% over unclipped controls. The reduc-
tion of 60.3% produced by hypothermia was consistent
among all animals in this group, but in terms of sam-
ple size this was not statistically significant. The actual
edema produced by middle cerebral artery occlusion
in untreated animals appeared to be very small
(0.68 ± 0.24%). This is easily understood since the in-
farct size produced by middle cerebral artery occlu-
sion in the monkey is quite variable, but usually small.
Michenfelder, Milde, and Sundt4 found that the in-
farct size with this technique varied from zero to 100%
of a hemisphere with a mean of 18.7%. Consequently,
we must assume that the edema in the ischemic
hemisphere is restricted to a relatively small percent-
age of this total volume.

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**Figure 1.** Percent edema compared to mean rCBF (ml/100 gm/min) prior to sacrifice. Each point represents one animal. A: untreated, B: pentobarbital, C: hypothermia.

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Our results complement the work of others and suggest that ischemic brain edema can be reduced or delayed effectively by barbiturates, but not by hypothermia.25

Acknowledgment

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