Isovolemic hemodilution in experimental focal cerebral ischemia

Part 2: Effects on regional cerebral blood flow and size of infarction

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Seventy-six splenectomized dogs were entered in a study of the value and effects of isovolemic hemodilution. Of these, seven were not included in the analysis because of technical errors. Of the remaining 69 dogs, 35 were treated with hemodilution; 28 were subjected to a 6-hour period of temporary occlusion of the distal internal carotid artery and the proximal middle cerebral artery, and seven underwent a sham operation only, with arterial manipulation but no occlusion. The other 34 dogs were not subjected to hemodilution; 26 of these underwent temporary arterial occlusion and eight had a sham operation only. In each group the animals were about equally divided into 1) an acute protocol with regional cerebral blood flow measurements by a radioactive microsphere technique and sacrifice at the end of the acute experiment, and 2) a chronic protocol with survival for 1 week to permit daily neurological assessment and final histopathological examination but without blood flow measurements. The general experimental protocol, the hemodynamic and rheological measurements, and the changes in intracranial pressure are described in Part 1 of this report.

In the animals with arterial occlusion, blood flow decreased significantly in the territory of the ischemic middle cerebral artery. This decrease was partially reversed by hemodilution in the animals so treated. When the changes in blood flow before and after hemodilution in treated animals are compared with the changes at equivalent times in animals without hemodilution, the increases in flow in the gray matter of the ischemic hemisphere brought about by hemodilution are statistically significant. The neurological condition of the animals in the chronic protocol (sacrificed 1 week after occlusion) with hemodilution, as evaluated by daily neurological assessment, was significantly better than that of the control animals.

In the animals sacrificed acutely (8 hours after arterial occlusion), the volume of infarction as estimated by the tetrazolium chloride histochemical method was 7.36% of the total hemispheric volume in the control animals and 1.09% in the hemodiluted animals, showing a statistically significant difference (p < 0.005). In the chronic animals these values were 9.84% and 1.26%, respectively (p < 0.005), as calculated by fluorescein staining. By histopathological examination the volume of infarction in the chronic animals was calculated as 10.92% in the control animals and 1.20% in the hemodiluted animals (p < 0.005). There was good correlation between the size of infarction and the decrease in hematocrit and viscosity, and excellent correlation between the size of infarction estimated by fluorescein and that determined by histopathological examination in each animal in the chronic group.

**Key Words** • hemodilution • viscosity • cerebral ischemia • cerebral infarction • cerebral blood flow • dog

In Part 1 of this study, it was shown that, in contrast to hypervolemic hemodilution, isovolemic hemodilution reduced viscosity without resulting in cardiac overloading and without exacerbating the increase in intracranial pressure (ICP) seen after a focal ischemic insult. The present study reports the effects of this form of therapy on regional cerebral blood flow (rCBF) and its efficacy in protecting the brain from the consequences of ischemia.

**Materials and Methods**

**Study Groups**

Figure 1 outlines the general experimental protocol. Seventy-six splenectomized dogs of either sex, each
Isovolemic hemodilution in cerebral ischemia, Part 2

FIG. 1. Flow chart illustrating the general experimental protocol. The number of animals in each subgroup studied is indicated. Animals in the acute group had blood flow studies and were sacrificed a few hours after insult. Their brains were infused with tetrazolium hydrochloride for determination of infarct size. Animals in the chronic group did not have blood flow studies and were kept for 1 week for daily neurological assessment. They were sacrificed at the end of the week for histopathological examination.

weighing between 12 and 20 kg, were used for this study. National Institutes of Health standards were met or exceeded in the preparation, surgical and anesthetic techniques, and housing of the animals. Seven dogs were excluded as a result of technical failure. Of the 69 remaining dogs, 35 underwent rCBF studies and were sacrificed about 8 hours after arterial occlusion. Their brains were studied to determine the gross size of infarction at the acute stage of the experiment. In one of the animals (assigned to the group without hemodilution) the blood flow data were unsatisfactory due to a technical error. Thirty-four dogs were treated in the same manner except that they did not undergo rCBF measurement. One animal in the group that was assigned to receive hemodilution died as a result of disconnection from the respirator at the conclusion of the acute study. The remaining animals were kept alive for 1 week for daily neurological assessment and final detailed histopathological examination. Within each group approximately half of the animals underwent hemodilution (“hemodilution group”) and half did not (“control group”). A few animals in each group underwent identical procedures but without arterial occlusion (“sham-operated control group”). The rationale for separating the dogs into acute and chronic groups is given in Part 1 of this study.50

The preparation of the animals, the physiological and hemorheological measurements obtained, and the technique for arterial occlusion were described in detail in Part 1.50

Regional Cerebral Blood Flow Measurement

Before craniotomy, the animals were placed in the lateral position with the left side up. Thoracotomy was performed via the fifth intercostal space to insert a No. 16 catheter into the left atrium for injection of microspheres. The deflated left lung was then well inflated with an Ambu bag, and the chest wall was closed in a watertight fashion. Another long No. 16 catheter was inserted in the left femoral artery and advanced to the aortic arch in order to collect reference blood samples during the injection of microspheres. By repeating arterial blood gas measurement and continuous end-tidal pCO2 monitoring, arterial pCO2 was adjusted to a range of 38 to 42 torr before each measurement of rCBF.

Measurement of rCBF was carried out by the use of radiolabeled microspheres.15 The microspheres were 15 ± 3 μm in size and were provided at a density of 1.3 gm/ml.* Immediately before each measurement a dose of 40 to 50 mCi microspheres was diluted, mixed with 10 ml saline, and injected over 15 seconds into the left atrial catheter which was flushed with 10 ml heparinized saline. At the time of injection, reference blood samples were withdrawn simultaneously from the aortic catheter by a Harvard infusion/withdrawal pump at a rate of 5 ml/min for 3 minutes.† Microspheres with four different radionuclides for four different measurements were used in the present study. The first measurement of rCBF was performed with microspheres labeled with tin-113 before the dura was opened. The second measurement of rCBF was performed with microspheres labeled with tin-113 before the dura was opened. The second measurement was performed with ruthenium-103-labeled microspheres 30 minutes after the occlusion of intracranial arteries. Immediately after this second rCBF measurement, isovolemic hemodilution was performed in the group of animals assigned to receive hemodilu-

* Microspheres were supplied by New England Nuclear, Boston, Massachusetts.
† Harvard infusion/withdrawal pump manufactured by Harvard Apparatus, South Natick, Massachusetts.
tion. The third measurement of rCBF was performed with niobium-95-labeled microspheres injected at 3 hours after arterial occlusion or approximately 2 hours after completion of hemodilution. The last measurement of rCBF was performed with scandium-46-labeled microspheres injected 30 minutes after reflow, or 61/2 hours after arterial occlusion. In sham-operated animals the last three measurements were performed at the corresponding time sequence after sham operation.

After the brains were prepared for measurement of infarct size, as described below, brain slices obtained 1 cm anterior and 1 cm posterior to the optic chiasma were fixed in 10% formalin solution for 2 days to facilitate dissection of different regions. By referring to a standard anatomical atlas, brain tissue samples were taken from both hemispheres and separated into the following areas: cortex of the anterior cerebral artery (ACA) territory, white matter of the ACA territory, cortex of the middle cerebral artery (MCA) territory, white matter of the MCA territory, basal ganglia, and thalamus. Efforts were made to obtain samples from the same area and of the same size from each hemisphere. These tissue samples as well as reference blood samples were weighed, and their radioactivity was measured in an LKB gamma counter. These measurements and the known reference blood sample withdrawal rate can be used to calculate rCBF in each specific brain tissue sample by a standard method. The rCBF was calculated as the average flow in samples from the same areas of two consecutive slices. The average rCBF at each location for each group was determined and the means and standard error of the means were obtained. The differences in average rCBF before and 2 hours after hemodilution (that is, between the second and third measurements of rCBF in the different regions) were tested by the two-tailed paired Student t-test.

Measurement of Infarct Size in Acute Study

After the last rCBF measurement, a ventral midline incision was made in the neck of each acute study group animals to place catheters in both common carotid arteries. The brain was then perfused with 2000 ml normal saline at 37°C, followed by 800 ml 2% 2,3,5 triphenyl-2H-tetrazolium chloride (TTC), § infused over a 30-minute period. This TTC solution was buffered with phosphate saline solution to a pH of 7.4. The perfusion pressure was maintained at approximately 10 mm Hg above the monitored mean systemic arterial pressure to prevent artificial disruption of tight capillary junctions or migration of injected microspheres. The animal died soon after the commencement of TTC infusion and the brain was carefully removed. The brain was sliced coronally into 1-cm slices starting at the optic chiasm. The slices were incubated in a 2% TTC solution in a 37°C oven for 30 minutes. The area of each slice not stained by TTC was measured in the same manner described below for the chronic group. The unstained area was assumed to be “infarced” for the purposes of this study. The total volume of infarction was calculated and expressed as a percentage of the total hemispheric volume, which was calculated by the water displacement method. Differences in mean infarction volume between the animals with and those without hemodilution were tested by the unpaired Student t-test.

Neurological Assessment in Chronic Study

After reperfusion, closure of the craniotomy wound, and measurement of the last set of physiological parameters, the dogs in the chronic study group were placed overnight in an animal recovery cage with a heating blanket. They were then housed in an animal farm for a week. Prophylactic antibiotics were given for 3 days. Daily neurological assessment of the animals in both hemodilution and non-hemodilution groups was performed by a neurologist and a neurosurgeon separately, according to a modification of the criteria of Crowell and Olsson:

Grade I: normal, no neurological deficit
Grade II: mild hemiparesis, occasionally circles toward operated side, stands without assistance
Grade III: moderate hemiparesis, circles toward operated side, stands only with assistance, no impairment of consciousness
Grade IV: severe hemiparesis with decreased level of consciousness, cannot stand
Grade V: dead.

Histopathological Examination in Chronic Study

At the end of 1 week, the animals in the chronic study were anesthesized again with intravenous thiamytal sodium (15 mg/kg), and 15 ml of 10% sodium fluorescein was given intravenously. Thirty minutes after the injection of sodium fluorescein the animals were sacrificed with an overdose of thiamytal sodium and the brains were removed. The brains were sliced coronally into 1-cm slices, using the chiasm as a reference point, and then examined under ultraviolet light with a wavelength of 366 μm. Areas stained with fluorescein, which roughly corresponded to the infarcted areas, were traced on a transparent sheet with 1-mm grids. The volume of infarction in a single slice was calculated from the average of the infarction areas seen on both sides of the slice multiplied by its thickness. Then total infarction volume was calculated from the sum of each slice and expressed as a percentage of total hemispheric volume in the same manner as in the acute animals. Paraffin-embedded sections of all the brain slices from each animal were examined under light microscopy. The extent of infarction was outlined on photographs of these slices magnified with a constant ratio. The actual sizes of these areas were then com-

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‡ Gamma counter, Model 1282, manufactured by LKB Instruments, Gaithersburg, Maryland.
§ Triprenyl-2H-tetrazolium chloride supplied by Polysciene, Inc., Warrington, Pennsylvania.
Regional cerebral blood flow in animals subjected to 6 hours occlusion of the left ICA and MCA*  

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Measurement Site</th>
<th>Baseline</th>
<th>30 Min Postocclusion</th>
<th>3 Hrs Postocclusion</th>
<th>30 Min Postreflow</th>
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<tbody>
<tr>
<td>Hemodilution Group (14 animals)</td>
<td>right</td>
<td>ACA gray matter</td>
<td>36.2 ± 3.9</td>
<td>33.4 ± 3.2 (92.3%)</td>
<td>35.0 ± 2.8 (96.6%)</td>
</tr>
<tr>
<td></td>
<td>ACA white matter</td>
<td>30.8 ± 4.0</td>
<td>25.9 ± 3.3 (84.0%)</td>
<td>28.2 ± 2.9 (91.6%)</td>
<td>29.7 ± 4.4 (96.4%)</td>
</tr>
<tr>
<td></td>
<td>MCA gray matter</td>
<td>38.9 ± 4.8</td>
<td>36.0 ± 3.1 (92.5%)</td>
<td>38.6 ± 2.6 (99.2%)</td>
<td>37.7 ± 3.5 (96.9%)</td>
</tr>
<tr>
<td></td>
<td>MCA white matter</td>
<td>30.2 ± 3.9</td>
<td>28.6 ± 3.5 (94.7%)</td>
<td>29.8 ± 2.7 (98.7%)</td>
<td>26.7 ± 3.3 (88.4%)</td>
</tr>
<tr>
<td></td>
<td>basal ganglia (MCA)</td>
<td>56.8 ± 7.1</td>
<td>48.5 ± 4.6 (85.3%)</td>
<td>62.6 ± 6.8 (100.0%)</td>
<td>55.8 ± 7.2 (98.2%)</td>
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<tr>
<td></td>
<td>thalamus</td>
<td>39.4 ± 4.9</td>
<td>37.5 ± 3.0 (95.2%)</td>
<td>43.1 ± 4.4 (109.4%)</td>
<td>38.3 ± 6.0 (97.2%)</td>
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<tr>
<td></td>
<td>left</td>
<td>ACA gray matter</td>
<td>40.1 ± 3.8</td>
<td>35.4 ± 3.0 (88.3%)</td>
<td>36.7 ± 4.0 (91.5%)</td>
</tr>
<tr>
<td></td>
<td>ACA white matter</td>
<td>30.8 ± 4.0</td>
<td>25.9 ± 3.3 (84.0%)</td>
<td>28.2 ± 2.9 (91.6%)</td>
<td>29.7 ± 4.4 (96.4%)</td>
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<tr>
<td></td>
<td>MCA gray matter</td>
<td>39.3 ± 5.1</td>
<td>30.3 ± 3.4 (77.0%)</td>
<td>37.1 ± 3.1 (94.4%)</td>
<td>36.9 ± 2.9 (93.9%)</td>
</tr>
<tr>
<td></td>
<td>MCA white matter</td>
<td>29.4 ± 4.8</td>
<td>21.8 ± 3.2 (74.1%)</td>
<td>24.6 ± 2.9 (83.7%)</td>
<td>26.6 ± 3.5 (90.5%)</td>
</tr>
<tr>
<td></td>
<td>basal ganglia (MCA)</td>
<td>60.7 ± 6.3</td>
<td>39.7 ± 3.8 (65.4%)</td>
<td>51.7 ± 5.7 (85.2%)</td>
<td>56.4 ± 5.4 (92.9%)</td>
</tr>
<tr>
<td></td>
<td>thalamus</td>
<td>40.2 ± 3.4</td>
<td>34.3 ± 3.2 (85.3%)</td>
<td>41.4 ± 4.4 (102.2%)</td>
<td>41.0 ± 4.5 (102.0%)</td>
</tr>
<tr>
<td>Non-Hemodilution Group (13 animals)</td>
<td>right</td>
<td>ACA gray matter</td>
<td>32.6 ± 3.5</td>
<td>34.0 ± 2.9 (104.3%)</td>
<td>32.0 ± 3.3 (98.2%)</td>
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<tr>
<td></td>
<td>ACA white matter</td>
<td>25.1 ± 2.4</td>
<td>25.6 ± 2.3 (102.0%)</td>
<td>26.6 ± 3.2 (106.0%)</td>
<td>25.8 ± 4.0 (102.8%)</td>
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<tr>
<td></td>
<td>MCA gray matter</td>
<td>37.3 ± 4.1</td>
<td>35.3 ± 3.7 (94.6%)</td>
<td>33.8 ± 4.0 (90.6%)</td>
<td>29.6 ± 6.0 (79.4%)</td>
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<tr>
<td></td>
<td>MCA white matter</td>
<td>28.9 ± 2.0</td>
<td>27.0 ± 3.3 (93.4%)</td>
<td>27.0 ± 3.2 (95.8%)</td>
<td>23.4 ± 3.2 (81.0%)</td>
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<tr>
<td></td>
<td>basal ganglia (MCA)</td>
<td>54.4 ± 6.5</td>
<td>52.1 ± 7.6 (95.8%)</td>
<td>53.8 ± 8.8 (98.9%)</td>
<td>61.2 ± 11.6 (112.5%)</td>
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<tr>
<td></td>
<td>thalamus</td>
<td>37.3 ± 2.0</td>
<td>35.1 ± 3.0 (94.1%)</td>
<td>35.7 ± 5.0 (101.7%)</td>
<td>32.1 ± 4.3 (86.1%)</td>
</tr>
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<td>left</td>
<td>ACA gray matter</td>
<td>34.1 ± 4.4</td>
<td>31.6 ± 3.3 (92.7%)</td>
<td>28.3 ± 2.8 (83.0%)</td>
</tr>
<tr>
<td></td>
<td>ACA white matter</td>
<td>29.4 ± 2.6</td>
<td>26.8 ± 1.2 (91.2%)</td>
<td>29.9 ± 3.3 (101.7%)</td>
<td>25.9 ± 4.0 (88.1%)</td>
</tr>
<tr>
<td></td>
<td>MCA gray matter</td>
<td>38.1 ± 6.0</td>
<td>28.7 ± 3.8 (75.3%)</td>
<td>24.8 ± 3.7 (65.1%)</td>
<td>48.9 ± 9.6 (128.3%)</td>
</tr>
<tr>
<td></td>
<td>MCA white matter</td>
<td>26.4 ± 2.7</td>
<td>20.1 ± 2.7 (76.1%)</td>
<td>18.6 ± 2.8 (70.4%)</td>
<td>38.4 ± 9.8 (145.5%)</td>
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<tr>
<td></td>
<td>basal ganglia (MCA)</td>
<td>56.8 ± 5.7</td>
<td>38.9 ± 6.0 (68.5%)</td>
<td>34.8 ± 5.4 (61.2%)</td>
<td>58.4 ± 5.2 (103.5%)</td>
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<tr>
<td></td>
<td>thalamus</td>
<td>39.6 ± 3.3</td>
<td>32.1 ± 4.3 (81.1%)</td>
<td>25.5 ± 2.3 (79.4%)</td>
<td>40.0 ± 3.6 (101.0%)</td>
</tr>
</tbody>
</table>

* Regional cerebral blood flows are expressed as ml/100 mg/min and are given as means ± standard error of the means. Figures in parentheses indicate the percentage of the mean of its baseline value. ICA = internal carotid artery; MCA = middle cerebral artery; ACA = arterial cerebral artery.

computed with the aid of a Bioquant Hipad Digitizer interfaced with an IBM personal computer. The infarction volume was calculated and expressed as a percentage of the volume of the entire hemisphere. The average infarction volume for each group was calculated, and the means and standard error of the means were obtained. The difference between the groups with and without hemodilution was compared by the unpaired Student t-test. The correlation between infarction size and the final viscosity or hematocrit was tested by analysis of covariance. The correlation between the infarction size measured by the fluorescein stain method and by histopathological examination was also tested by covariance analysis.

**Results**

**Regional Cerebral Blood Flow**

Table 1 shows the average rCBF at different sites and times: at baseline, 30 minutes after arterial occlusion, 3 hours after arterial occlusion (2 hours after hemodilution in the treated animals), and 30 minutes after reperfusion. To summarize, in the hemodilution group animals, rCBF in the right hemisphere decreased slightly to between 85% and 95% of control values after arterial occlusion and this decrease was reversed by hemodilution. In the left hemisphere the decrease in rCBF after occlusion of the left internal carotid artery (ICA) or MCA was more pronounced (65% to 88% of control value). As expected, this decrease was more severe in the MCA territory (65.4% to 74.1%), particularly in the basal ganglia (65.4%). With hemodilution, the decrease in rCBF in the left hemisphere was substantially but not totally reversed (rCBF ranged from 83.7% to 102.2% of baseline value). Regional cerebral blood flow after reperfusion varied considerably both in the right and the left hemisphere but, in general, it tended to return toward baseline value.

In the non-hemodilated (control) animals the decrease in rCBF with arterial occlusion was similar to that observed in the hemodiluted animals; however, in the hemodiluted animals this decrease was substantially reversed with hemodilution and in the control animals the rCBF at 3 hours postocclusion actually showed a further decrease. Reperfusion partially but inconsistently reversed this decrease.

Table 2 is an attempt to summarize and compare pertinent portions of these data in animals with arterial occlusion. Three sites in the MCA territory (gray matter, white matter, and basal ganglia) and two different times of measurement (30 minutes after arterial occlusion and 30 minutes postreperfusion) were compared. The data showed that in the hemodiluted animals the decrease in rCBF was more pronounced (65% to 88% of control value) and this decrease was reversed by hemodilution.

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TABLE 2
Regional cerebral blood flow (ml/100 gm/min) in the MCA territory in animals with arterial occlusion*

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Measurement Site</th>
<th>Before Hemodilution†</th>
<th>After Hemodilution‡</th>
<th>Change (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodilution Group (14 animals)</td>
<td>right gray matter</td>
<td>36.0 ± 3.1</td>
<td>38.6 ± 2.6</td>
<td>↑ 7.2</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>white matter</td>
<td>28.6 ± 3.5</td>
<td>29.8 ± 2.7</td>
<td>↑ 4.2</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>basal ganglia</td>
<td>48.5 ± 4.6</td>
<td>62.0 ± 6.8</td>
<td>↑ 27.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>left gray matter</td>
<td>30.3 ± 3.4</td>
<td>37.7 ± 3.1</td>
<td>↑ 22.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>white matter</td>
<td>21.8 ± 3.2</td>
<td>24.6 ± 2.9</td>
<td>↑ 12.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>basal ganglia</td>
<td>39.7 ± 3.8</td>
<td>51.7 ± 5.7</td>
<td>↑ 30.2</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Non-Hemodilution Group (13 animals)</td>
<td>right gray matter</td>
<td>35.3 ± 3.7</td>
<td>33.8 ± 4.0</td>
<td>↓ 4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>white matter</td>
<td>27.0 ± 3.3</td>
<td>27.7 ± 3.2</td>
<td>↑ 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>basal ganglia</td>
<td>52.1 ± 7.6</td>
<td>53.8 ± 8.8</td>
<td>↑ 3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>left gray matter</td>
<td>28.7 ± 3.8</td>
<td>24.8 ± 3.7</td>
<td>↓ 13.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>white matter</td>
<td>20.1 ± 2.7</td>
<td>18.6 ± 2.8</td>
<td>↓ 11.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>basal ganglia</td>
<td>38.9 ± 6.0</td>
<td>34.8 ± 5.4</td>
<td>↓ 10.6</td>
<td></td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. MCA = middle cerebral artery; NS = not significant. The left hemisphere was ischemic (occlusion of the internal carotid artery and the MCA).
† Values obtained 30 minutes after arterial occlusion but before hemodilution in the animals to be hemodiluted.
‡ Values obtained 3 hours after arterial occlusion (2 hours after hemodilution in the hemodiluted animals).

Table 3 shows the rCBF changes in the sham-operated animals in an analogous manner as in Table 2 where these changes were depicted for the animals subjected to arterial occlusion. Hemodilution in the sham-operated animals (arterial manipulation but no occlusion) resulted in a substantial but statistically insignificant increase in rCBF in the basal ganglia in both hemispheres. In the control (non-hemodiluted) animals the rCBF tended to decrease slightly with time.

Neurological Assessment

Based on the scale set out above, the average neurological grade for the control (arterial occlusion but no hemodilution), hemodiluted (hemodilution after arterial occlusion), and sham-operated (no arterial occlusion with or without hemodilution) animals is depicted in Fig. 4. In control animals the average neurological grade remained relatively stable throughout the experiment. In the hemodiluted animals, the grade increased slightly after hemodilution but returned to baseline levels within the subsequent 4 hours. In contrast, the grade in the sham-operated animals showed a significant decrease immediately after the sham manipulation and continued to decrease throughout the experiment. This decrease was significant at all time points compared to the baseline grade. The average grade in the sham-operated animals was significantly lower than in the control group at all time points except immediately after the sham manipulation (p < 0.05).
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FIG. 2. Histograms comparing percentage-change in the regional cerebral blood flow (rCBF) from the value 30 minutes after arterial occlusion (before hemodilution) to the value obtained 3 hours after occlusion (or 2 hours after hemodilution in hemodiluted animals). Black blocks = hemodilution group; shaded blocks = non-hemodilution group. The left hemisphere was ischemic due to occlusion of the middle cerebral and internal carotid arteries.

grade was Grade IV (hemiplegic and drowsy) on the 1st day after occlusion, Grade III on Days 2 and 3, and Grade II (mild hemiparesis) thereafter. In treated (hemodiluted) animals the average grade was Grade II for the first 3 days and Grade I (normal) thereafter. Sham-operated animals remained neurologically normal throughout the study.

Infarct Volume

The average infarct volume, expressed as a percentage of the total hemispheric volume for the acute animals (as determined by lack of staining with TTC) and for the chronic animals (as determined independently by fluorescein staining and by histopathological examination) is depicted in Table 4. In the acute group (sacrificed about 8 hours after arterial occlusion) the average volume of infarction was 7.36% ± 1.32% in the control group versus 1.09% ± 0.28% in the hemodiluted groups (p < 0.005). There was essentially no infarction in the sham-operated animals.

In the chronic group (sacrificed 1 week after arterial occlusion) the volume of infarction was 9.84% ± 3.29% by fluorescein stain and 10.92% ± 5.44% by histopathological examination in the control group. In the hemodiluted animals these values were 1.26% ± 0.23% and 1.20% ± 0.42%, respectively. The differences between hemodiluted and control animals were statistically significant (p < 0.005) by both methods of assessment. Again, there was no significant infarction in the sham-operated animals. There was a very high correlation (r = 0.58, p < 0.005) between the volume of infarction in each brain as determined independently by fluorescein staining or by histopathological examination, performed in a blinded fashion.

Covariance analysis showed a good correlation between hematocrit and infarct volume (r = 0.46, p < 0.001) and between viscosity and infarct volume (r = 0.42, p < 0.001) in animals with arterial occlusion. The relationship between rCBF and infarct volume in the acute group was very complex but, in general, animals with the lower rCBF in the MCA territory after arterial occlusion tended to have the largest infarcts. There was no correlation between rCBF after reperfusion and infarct size.

Discussion

Our results indicate that isovolemic hemodilution augments rCBF in ischemic regions of the brain. This effect was particularly pronounced in the basal ganglia, which could be explained in part by the fact that the basal ganglia suffered the greatest decrease in rCBF after arterial occlusion. This is not surprising since there is

FIG. 3. Histograms showing post-hemodilution regional cerebral blood flow (rCBF) (or rCBF measured at equivalent times in control animals) at different sites of the middle cerebral artery territory. These rCBF's are normalized as percentage of the baseline value. Values are expressed as mean ± standard error of the mean. Black blocks = hemodilution group; shaded blocks = non-hemodilution group. Asterisks: p < 0.05.

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FIG. 4. Average daily neurological grade according to the scale of Crowell and Olsson. See text for definition of grades. Data are given for the control (arterial occlusion but no hemodilution), hemodilution (arterial occlusion and hemodilution), and sham-operated (no arterial occlusion with or without hemodilution) groups of animals.
less potential for leptomeningeal collateral circulation in the basal ganglia. In addition, the greatest decrease in flow might be expected in the areas of highest preoclusion flow, which have the greatest local perfused capillary density. Samples from the basal ganglia in our study showed by far the highest baseline rCBF (Table 1). However, since hemodilution augmented rCBF not only in the ischemic hemisphere but also in the contralateral hemisphere and even in sham-operated animals, other factors must be operative in the observed heterogeneous response to hemodilution. It may be that, just as the high capillary density microvasculature of the basal ganglia is most vulnerable to ischemia, it can also be most responsive to hemorheological manipulations (such as hemodilution) that appear to exert disproportionate influence at the microcirculatory level.

The increases in rCBF observed with isovolemic hemodilution were not as large as those observed in similar experiments using hypervolemic hemodilution. This is probably due to the fact that, in addition to achieving the same rheological benefits related to a reduction of viscosity, hypervolemic hemodilution significantly augments cardiac output. The latter effect does not occur with isovolemic hemodilution, as shown clearly in Part 1 of our study. There is considerable controversy as to the relative contributions of reduced viscosity and increased cardiac output to the increased rCBF observed with hypervolemic hemodilution. Some investigators believe that the reduction in viscosity is primarily responsible for this effect, whereas others consider that the increase in cardiac output is the primary factor involved. In a study by Wood, et al., in which volume expansion was achieved with autologous blood, cardiac output was augmented (as would be expected from volume expansion) but rCBF did not increase; this indicates that the rheological effects achieved with hemodilution, but not with volume expansion alone, are important in achieving the observed increase in rCBF. Our findings indicate that a reduction in viscosity alone, as achieved with isovolemic hemodilution, without an increase in cardiac output or systemic blood pressure, results in increased rCBF in ischemic brain. This increase, however, does not seem to be as marked as when the cardiac output is also increased by volume expansion.

The main goal of our study was to determine whether isovolemic hemodilution was efficacious in protecting the brain during a temporary focal ischemic insult. Our results in dogs clearly indicate that this form of therapy improves outcome as measured by neurological condition over a 1-week period of observation and by a final pathological estimation of the size of infarction. Although a very significant reduction in the estimated size of the infarct was noted in the acute animals, this would not have been a definitive result in view of the difficulty in determining whether brain tissue that appears damaged during the first few hours after ischemia will in fact be nonviable. The "marker," TTC, used to estimate the size of infarction in the acute animals is taken up by viable mitochondria, and it is believed that tissue not stained with this dye, even in the acute stage, contains little or no viable mitochondria and will eventually become grossly infarcted. Still, rather than depending on this indirect method, the experiment was designed so that about half of the animals would be kept alive for 1 week for clinical observation and final histopathological examination. The findings in the latter chronic group confirmed our findings in the acute group. The degree of brain protection achieved with isovolemic hemodilution is similar to that achieved by Wood, et al., using hypervolemic hemodilution.

Clinically, there have been several studies linking hematocrit (and indirectly blood viscosity) with frequency of and outcome after stroke and with cerebral blood flow (CBF). Patients with polycythemia vera or leukemia carry a higher risk of transient ischemic attack. In the Framingham study, the incidence of infarction among males with hemoglobin con-
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centration exceeding 15 gm% and females with hemoglobin concentration exceeding 14 gm% was twice that found in the normal population. In an autopsy study, Tohgi, et al., reported that the incidence of cerebral infarction was higher in patients with a hematocrit greater than 46% (or 41% in elderly patients). In another study, Thomas, et al., found that patients with higher hematocrit values had lower CBF and venesection in these patients could increase CBF by a mean of 50%. In contradistinction, the hematocrit did not emerge as an important prognostic factor in a recent large study of patients with occlusive cerebrovascular disease.

These observations led not only to some of the experimental studies already referred to here but also to some clinical studies of hemodilution as therapy for cerebral infarction. The earlier studies used mostly hypervolemic hemodilution by volume expansion with colloid solutions; beneficial effects were noted in some and not in others. More recently, there have been some trials of isovolemic hemodilution. In a brief report of 11 patients with stroke, Wood, et al., found an acute increase in rCBF and improvement in the electroencephalogram after venesection and replacement with serum albumin. In a recent Scandinavian trial, significant improvement in outcome was noted in stroke patients treated by venesection and colloid infusion; however, when this trial was enlarged to include several centers these results were not sustained. The latter trial has been criticized because hemodilution was very slowly achieved over a 3- to 5-day period, the hematocrit was lowered only an average of 37%, and patients were entered in the study relatively late after the onset of stroke (up to 48 hours; only 28% of the patients were entered within 12 hours of the onset of stroke). It may be that, if this therapy is to be effective, hemodilution should be completed during the first few hours after the onset of ischemia. In fact, in a study of 20 stroke patients where hemodilution by venesection and plasma administration was achieved within 72 hours of the onset of symptoms, a substantial increase in rCBF and an improvement in evoked potentials and clinical condition was noted in most patients.

Our study was designed to optimize the chances of demonstrating a benefit for isovolemic hemodilution should such benefit exist. To this effect, a relatively mild ischemic insult was used (6 hours of temporary ICA and MCA occlusion) which, in our control animals, resulted in an average infarct of only 10% of the hemispheric volume. In addition, the animals were hemodiluted rapidly after the onset of ischemia (hemodilution was completed by 1 hour after arterial occlusion) and to a relatively profound degree (hematocrit 30% to 32%). It is obviously impossible to reproduce our experimental paradigm in most clinical settings where patients with more severe strokes may be seen only several hours after the onset of symptoms and where it may not be prudent to reduce the hematocrit so rapidly to such low levels. Therefore, it remains to be seen whether in experiments that more closely resemble the clinical situation (more severe strokes treated later with slower and less profound hemodilution) this form of therapy will still be beneficial.

Conclusion

Our study has demonstrated that, in this model of mild temporary focal cerebral ischemia, isovolemic hemodilution accomplished rapidly after the onset of ischemia results in an increase in rCBF in ischemic brain, a better neurological outcome, and a decrease in the severity of infarction. These benefits were achieved without an increase in systemic blood pressure or cardiac output, and appeared to have been related to the decrease in viscosity achieved by lowering the hematocrit. In contradistinction to hypervolemic hemodilution, there was no increase in ICP that could be attributed to the therapy per se. These results suggest that further clinical trials are in order in which isovolemic hemodilution by venesection and colloid replacement is achieved promptly after the ischemic insult. This form of therapy would be most appropriate for patients with acute stroke who are at risk for intracranial hypertension and for elderly patients or patients with compromised cardiovascular status who may not tolerate well the effects of volume expansion. Younger, healthy patients, such as most patients with symptomatic vasospasm, who do not yet have frank cerebral infarction or evidence of increased ICP, are probably best treated with hypervolemic hemodilution to achieve not only the rheological effects of hemodilution but also the increase in cardiac output that accompanies volume expansion. Even in these cases, however, hemodilution can be achieved faster and will last longer if some blood is removed by phlebotomy at the same time that total blood volume is expanded by colloid infusions in excess of the amount of blood removed.

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