Reperfusion of cerebral vessels is impaired following total cerebral ischemia of more than five minutes; this is possibly the initial factor responsible for neurological sequela. Failure of cellular ion transport mechanisms during ischemia was thought to lead to swelling of endothelial cells and perivascular glia and this, in turn, was thought to be the primary cause of the impairment of recirculation. A method of quantitating this circulatory impairment in rabbits was devised after infusing carbon black into the ischemic cerebral vasculature in a standardized way. The amount of circulatory impairment was shown to be unaffected by heparin, inversely related to the infusion pressure of the carbon black, and greatly reduced by acute hemodilution with saline. This latter observation does not support the theory that cellular swelling is the major cause of the postischemic vascular impairment, but rather implicates changes in the blood itself, possibly erythrocyte aggregation which is responsible for increased blood viscosity in low flow and no flow states.

Additional Key Words: 
- cerebrovascular impairment
- cerebral ischemia
- circulatory impairment
- erythrocyte aggregation

Introduction

Perfusion of cerebral vessels following total cerebral ischemia has been shown to be impaired and this has been proposed as a factor limiting brain recovery. Impaired cerebral reperfusion was demonstrated in rabbits after cerebral circulatory arrest of greater than five minutes by the infusion of a suspension of carbon black and subsequent inspection of coronal sections of the brain. With ischemia of five minutes or less, the brain was stained an even black as virtually every vessel had been perfused with the ink. When ischemia was greater than five minutes some areas appeared a discrete, contrasting white because the vessels within did not fill with ink. Light and electron microscopy studies of these areas revealed swelling of perivascular glia and endothelial cells with bleb formation and lumen obstruction.

Two interrelated events were postulated to account for this vascular impairment: (1) decrease in vascular lumina, and (2) increased blood viscosity. A proposed fall in ATP consequent to the ischemia was thought to have led to failure of active transport processes, resulting in movement of salts and water from plasma into perivascular cells. This would account for swelling of both endothelial...
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cells and perivascular glia and would cause local hemoconcentration and increased blood viscosity. The possibility remains that local vascular changes seen by electron microscopy may have occurred after the intended experimental period of ischemia because of failure to infuse the involved areas adequately with fixative (glutaraldehyde).

The present studies were undertaken to examine further the etiology of the impairment of postischemic cerebral perfusion, with particular attention to the role of possible changes in the blood itself. Reperfusion of brains, after blood flow had been interrupted completely for varying intervals, was studied using carbon black as an indicator. The effect of increasing the pressure of the perfusing fluid to overcome the pressure of stagnant blood was examined, as was the effect of hepatic and preischemic dilution of the blood with saline.

Methods

New Zealand white rabbits, 1.5 to 2.5 kg, of either sex were used. After intravenous pentothal anesthesia (30 mg/kg) a tracheostomy was performed. A small animal pump respirator (Harvard Apparatus Company, Model 607) was used to provide ventilation and maintain P_{CO_2} and P_{O_2} within the normal range. After sternotomy, the pericardium was opened and the aorta cross-clamped at its base for the desired period of time. The descending aorta and the ears were then cross-clamped and the arms ligated at the axilla to minimize flow to these regions during postischemic infusion. The jugular veins were divided just above the clavicles to minimize intracerebral venous pressure. Two hundred millimeters of saline warmed to 37°C were administered intravenously 15 minutes prior to aortic clamping. The amounts removed or administered were adjusted to maintain mean arterial pressure at the pre-exchange level. The usual exchange period was approximately 20 minutes. Ischemia for 15 minutes followed by carbon black infusion with the reservoir level at 70 cm were then performed as before.

In an anticoagulated series animals were given sodium heparin U.S.P., 500 units/kg, intravenously 15 minutes prior to aortic clamping. The duration of subsequent ischemia was 15 minutes and the reservoir level set at 70 cm.

Animals were rejected if the carbon black infusion catheter could not be passed from the ventricle into the base of the ascending aorta, if inadequate cross-clamping of the descending aorta was detected, or if there was an obvious direct leak of the ink from the heart or the aorta into the chest.

Coronal sections of the brain were made at the sites indicated by Cantu: (1) midfrontal lobe, (2) anterior optic chiasm, (3) anterior to the mammillary bodies, (4) posterior to the mammillary bodies, (5) superior colliculus, and (6) midpons. Brains were read as unknowns by one person, the following regions being evaluated for the percentage of the cut surface represented by white areas: the cortex and basal ganglia of the surfaces of sections 1, 2, and 3; the thalamus of section 4; and the brainstem of sections 5 and 6.

These regions of brain were scored by a modification of Chalkley's method for the quantitative, morphological analysis of tissues. Parallel lines 2 mm apart were placed at right angles to each other and a grid of points made at the sites of intersection of the lines. This grid was then transposed to a piece of transparent plastic,
which was placed over the surface of each brain section, and inspected with a dissecting microscope under low-power magnification. The number of points lying in the white areas and the number lying in the black areas were counted. For each region the ratio of points in white areas to the total points counted was determined and expressed as a percentage which represented the relative surface area of each region that was white. The ranges of total points counted were 100 to 110 for cortex, 40 to 50 for basal ganglia, 20 to 30 for thalamus, and 30 to 40 for brainstem.

Five groups of animals were studied to investigate the following variables: duration of ischemia, infusion pressure of carbon black, acute hemodilution with saline and anticoagulation.

Group 1: 4.5 minutes' ischemia, reservoir at 28 cm above the heart (two animals), 40 cm (two animals), and 110 cm (two animals).

Group 2: 15 minutes' ischemia, reservoir at 70 cm (six animals), 110 cm (eight animals) and 170 cm (seven animals).

Group 3: 30 minutes' ischemia, reservoir at 170 cm (six animals).

Group 4: Hemodilution, 15 minutes' ischemia, reservoir at 70 cm, HCT 21 to 32 (five animals); HCT 4 to 13 (six animals).

Group 5: Heparin, 15 minutes' ischemia, reservoir at 70 cm (six animals).

Results

Animals with 4.5 minutes of ischemia failed to demonstrate impaired cerebral reperfusion even with perfusion pressures as low as 28 cm of water, all brains in Group 1 being completely and evenly black (table 1).

There was significant impairment of perfusion after 15 minutes of ischemia (Group 2). This impairment was reduced as the pressure of carbon black infusion was increased (table 1). The reduction was statistically significant in thalamus and brainstem (p < 0.05) when comparing an infusion pressure of 70 cm with one of 170 cm. The difference was not statistically significant in basal ganglia and cortex. In fact, the amount of white area in cortex after 15 minutes' ischemia was not statistically different from zero at any level of infusion pressure. This sparing of cortex and increased susceptibility of brainstem was noted by Cantu and Ames.8

Thirty minutes of ischemia (Group 3) produced greater amounts of white areas than 15 minutes of ischemia at comparable infusion pressures. These differences (table 1) were statistically significant in all areas of the brain.

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of ischemia, minutes</th>
<th>Nbr. of animals</th>
<th>Mean percentage of white area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>2</td>
<td>Cortex: 28, 0, 0, 0</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>2</td>
<td>Cortex: 2, 1, 0</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>3</td>
<td>Cortex: 30, 6, 0</td>
</tr>
<tr>
<td>4</td>
<td>Hemodilution, 4-13</td>
<td>15</td>
<td>Cortex: 15, 6, 0</td>
</tr>
<tr>
<td>5</td>
<td>Heparin, 4-13</td>
<td>15</td>
<td>Cortex: 30, 6, 0</td>
</tr>
</tbody>
</table>

*Not different from zero with statistical significance.

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studied (p < 0.025 for brainstem, p < 0.001 for thalamus, p < 0.005 for basal ganglia, and p < 0.005 for cortex).

Acute hemodilution with saline (Group 4) resulted in a great improvement in reperfusion following 15 minutes of ischemia. White areas were seen in only one (an unexplained "sport") of six animals in the severely hemodiluted group and in none of the five moderately hemodiluted animals. Combining the results on all acutely hemodiluted animals, as shown in table 1, and comparing them with the nonhemodiluted animals with the same duration of ischemia (15 minutes) and the same carbon black infusion pressure (reservoir 70 cm above the heart), there was significant reduction of white areas in basal ganglia (p < 0.05), thalamus (p < 0.005), and brainstem (p < 0.001). The cortex did not show statistically significant amounts of white areas with or without hemodilution.

The impairment of cerebral reperfusion after 15 minutes of ischemia was unaffected by anticoagulation (Group 5). There was no significant difference between the heparinized animals and the comparable untreated animals in Group 2 (carbon black infusion pressure of 70 cm).

Discussion

Heparin produced no improvement in postischemic cerebral reperfusion in our experimental system (see table 1). The activity of heparin is reduced at low pH, but even if capillary pH fell as low as 6.6 toward the end of ischemia, heparin would still be expected to prolong clotting times quite significantly and to have a beneficial effect on reperfusion if clots were an important factor. Furthermore, the protection afforded by raising perfusion pressure would be difficult to explain if fibrin clots were a cause of vessel obstruction and these were not found in studies with the electron microscope.

The reduction we observed in postischemic cerebral circulatory impairment when we increased reperfusion pressure has been noted previously both clinically and experimentally. The fact that we observed quantitatively less circulatory impairment than was seen in previous experiments is probably accounted for by the fact that we used a large aortic catheter (intravenous tubing) for carbon black infusion, whereas in the experiments cited needles of narrower diameter were used which would have resulted in a lower effective perfusion pressure within the intracerebral arteries. The observation that the amount of circulatory impairment revealed is an inverse function of the infusion pressure does not help to distinguish between narrowed lumina and alterations in the blood itself as a cause of the circulatory defect.

The importance of viscosity is strongly suggested by the marked improvement of postischemic cerebral circulation in animals pretreated by acute hemodilution with saline. Neely and Youmans demonstrated unusual survival of dogs following cerebral ischemia caused by increasing the intracranial pressure until the vessels were emptied of blood. Improved postischemic cerebral circulation has also been noted to follow the infusion of hyperosmolar agents. This response has been attributed to a reduction of endothelial and perivascular glial swelling and its role has not been excluded in the studies cited. Clinical reports that hyperosmolar agents improve cerebral blood flow before reducing intracranial pressure add further support to the hypothesis that blood viscosity plays a significant role in producing the postischemic changes.

Blood is a non-Newtonian fluid because viscosity increases in low flow and no flow states due to red cell aggregation. This aggregation has been shown by Merrill and coworkers to be a function of the third power of the hematocrit and the square of the fibrinogen concentration. Aggregation has also been shown in model systems to continue to increase with time over a period of many minutes because the blood sediments into the dependent portions of the vascular network to produce localized increases in hematocrit. Thus it is reasonable to assume that red cell aggregation during stasis may be an important factor in the problem of reestablishing cerebral blood flow following ischemia. This would explain the beneficial effect of acute hemodilution which reduces both red cell and fibrinogen concentration. In addition, the observation that the impairment of cerebral reperfusion appears in discrete, macroscopic regions, rather than diffusely, may be accounted for if increased blood...
viscosity due to stasis is a major contributing factor. The resistance in different regions of the vascular system will vary, depending on the length, diameter and arborization of their arteries, capillaries and veins. During reperfusion flow would occur first in regions of lowest resistance. The fall in viscosity consequent to flow would lower the resistance of these regions even more, and they would act as shunts, reducing the pressure imposed on adjacent, higher resistance regions which would subsequently be revealed as macroscopic areas of impaired recirculation.

In our experimental system, the lowest infusion pressure studied was 28 cm of water, which probably made it insensitive to the earliest changes in blood viscosity. Impairment of reperfusion was first demonstrated only after stasis of longer than four and a half minutes and increased significantly when the period of stasis was extended from 15 to 30 minutes. Although these observations may be fully accounted for by the inverse relationship between flow and viscosity, additional factors (such as decreased red cell deformability, progressive hemoconcentration, or luminal narrowing due to cell swelling or spasm) might also be operative. The beneficial results obtained in the present studies, when blood was diluted with saline, cannot be explained easily by a reduction in swelling of perivascular cells. On the other hand, alterations in viscosity alone could not explain why impairment of circulation following stasis appears earlier in brain than in other organs. A greater overall resistance of cerebral vessels may make them more sensitive to changes of blood viscosity, or one or more of the other factors mentioned may have increased importance in brain.

Acknowledgment
The authors are indebted to Mrs. Elizabeth Kovendy for her technical help and to Frances B. Nesbett for her assistance in statistical analysis of the data.

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*(Stroke)*. 1972;3:538-542
doi: 10.1161/01.STR.3.5.538

*(Stroke)* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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