Vascular Spasm in Cat Cerebral Cortex Following Ischemia

MICHAEL NOEL HART, M.D., MARTIN D. SOKOLL, M.D., LOYD R. DAVIES, and EDUARDO HENRIQUEZ, M.D.

SUMMARY The reaction of brain parenchymal vessels in areas of no-reflow following ischemia in cats was evaluated. A method was devised by which brain biopsies following ischemia were quickly frozen at −170°C; sections were cut and stained and vessels internal and external diameter measured. Vessels in the no-reflow areas had smaller internal and external diameters and thicker walls when compared to adjacent reflow areas as well as to normal control animals. By utilizing a 2-way analysis of variance in which reflow versus no-reflow vessel diameters were compared by region the differences were found to be statistically significant (p<0.05). The data raise the possibility that there may exist normal regional differences in the size of cerebral vessels.

FOLLOWING EXPERIMENTAL cerebral ischemia, the failure to perfuse random areas of the brain after circulation has been re-established is a well-known phenomenon variously referred to as no-flow or no-reflow state. The cause of no-reflow is generally ascribed to blockage of microcirculation, mainly because of ultra-structural studies which have shown swelling of glial foot processes, endothelial swelling and increased numbers of intraluminal flaps at the capillary level. Intraluminal clotting and stagnation of hematopoietic elements have not been ruled out as contributory causes, however. One of the major criticisms of the no-reflow observations is that they do not coincide with patterns of ischemic brain lesions in humans. However, we have observed perivascular patterns of necrosis in human cerebral cortices following ischemia combined with serum hyperosmolality. Upon re-examination of that material we concluded that the focal ischemic areas were associated with penetrating vessels large enough to contain significant amounts of smooth muscle in their walls. This observation raised the question of whether spasm of intraparenchymal cerebral vessels could occur and if it could be contributory to post-ischemic no-reflow in the
cerebral cortex. The present experiment is an attempt to elucidate the role of intraparenchymal arterial spasm in the experimentally ischemic cat brain.

**Materials and Methods**

Eighteen 2-6 kg cats were studied. Each was anesthetized with 30 mg/kg of intraperitoneal sodium pentobarbital. After anesthesia was established, the trachea was intubated and the cat was ventilated to maintain a \( \text{Paco}_2 \) of 35 ± 4 torr with a \( \text{Pao}_2 \) of 80–100 torr and a pH of 7.25–7.35. The left femoral vein and artery were cannulated for drug injection and arterial pressure recording. The right femoral artery was cannulated and a PE-50 catheter was inserted to the area of the aortic-arch for dye injection. Arterial pressure was continuously monitored with a Statham P23D6 transducer and recorded on a Beckman R-411 dynagraph. After each animal was prepared and stabilized, the head was placed in a stereotactic head holder and a pneumatic tourniquet was placed securely around the neck and inflated to 1200 mm Hg for a period of 30 minutes. During the ischemic period the skull was unroofed without damage to the dura or superficial vessels. This was followed in order by 1-5 minutes of re-circulation, intra-aortic injection of 10% fluorescein dye (0.25 mgm/K) and fifteen seconds of circulation to distribute the dye. Bilateral 1 × 1 × 0.4 cm biopsies were then taken either from the living animal or immediately after the animal was killed by intravenous injection of a saturated solution of KCl. The biopsies were immediately (10–20 sec) frozen in isopentane cooled to −170°C in liquid nitrogen. Several \( \mu \) thick sections at about 100\( \mu \) intervals were cut parallel to the surface from each biopsy specimen. Sections were mounted on glass slides, rapidly air dried and examined by fluorescent microscopy. Non-fluorescing (no-reflow) areas were circled and the sections then stained with an ATPase \(^9\) method to outline the vessels. Some additional sections were stained with H&E and trichrome. The no-reflow areas as well as adjacent perfused areas were photographed and enlarged 250 or 400 times. Intraluminal and external diameters of arteries 70\( \mu \) and larger (external diameter) were measured with a particle size analyzer. Approximately 170 vessels were measured in the no-reflow areas and 170 vessels from adjacent reflow (fluorescein stained) areas utilized as controls were also measured. Additional controls consisted of biopsies from 4 normal cats and from 4 cats which had been rendered hypoxic by ventilation with 95% nitrous oxide repetitively for 45 minutes total time. In both of these control groups the skull was unroofed and fluorescein dye was injected as in the test group. Approximately 120 vessels were measured from these groups. Positive controls consisted of thin slices of brain over lain with ergotamine and incubated for 1–2 hours at 37°C; mirror image slices of brain over lain with saline and incubated at 37°C for 1–2 hours served as controls for the ergotamine incubated sections. After incubation these sections were frozen at −170°C, sectioned, stained with ATPase, photographed and measured as were the test biopsies.

**Results**

Biopsies of all 18 ischemic cats contained areas devoid of fluorescein dye indicating absence of blood flow. These areas were usually 0.5–1.5 mm in diameter and more often involved the superficial cortex although they were also present in the deeper cortex and even in the white matter. None of the 8 control animals displayed significant no-reflow areas as evidenced by even distribution of dye throughout the cortex and white matter with the exception of one anoxic cat which showed two small cortical areas devoid of dye. No intraluminal clots were seen in any of the vessels. The normal and hypoxic cats were grouped together because measurements were similar in each.

Smaller vessel lumens and thicker walls from the no-reflow areas in contrast to adjacent reflow areas and non-ischemic control cats indicate that the no-reflow vessels are in a state of contraction (spasm) (table 1). The ergotamine incubated brain slices indicate both the fact that intraparenchymal vessels can undergo spasm (table 2) and that they can be frozen, sectioned, dried, stained and measured in that state of spasm.

The data were also approached by a 2-way analysis of variance using control versus no-reflow as well as regional differences (table 3). The regional values represent vessel measurements in one no-reflow area with adjacent control reflow areas as a set. Utilizing this method, there is a significant (\( p < 0.05 \)) difference between the internal diameters of control versus no-reflow areas (fig. 1). Although for external diameter and wall thickness the comparisons of no-reflow and control values showed a significant difference, the regional differences were also significant.

**Discussion**

Focal perfusion deficit following experimental brain ischemia has been demonstrated in several animal models. \(^5\) \(^6\) \(^7\) \(^8\) Ultrastructural changes in the areas of no-reflow in some of these models have shown fairly consistent swelling of the glial foot processes on the capillary basement membrane, often combined with endothelial swelling and increased numbers of intraluminal flaps with resultant narrowing of the capillary lumens. \(^9\) \(^10\) \(^11\) These findings have led investigators to believe that the cause of no-reflow lies at the level of the microcirculation. The possibilities of intraluminal clotting or increased platelet adhesiveness have had their advocates \(^6\) \(^7\) although other investigators have shown little or no beneficial effect of heparin use with ischemia. \(^10\) \(^11\)

The possibility that focal no-reflow results from occlusion of larger vessels is suggested by re-appraisal of some earlier experiments. First, Ginsberg and Myers \(^5\) have shown large

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Table 1: \( ~ \) Values of Measurements

<table>
<thead>
<tr>
<th></th>
<th>Intraluminal D</th>
<th>Ext D</th>
<th>Wall thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-reflow areas</td>
<td>51.8( \mu )</td>
<td>194.4( \mu )</td>
<td>66.5( \mu )</td>
</tr>
<tr>
<td>Adjacent reflow areas</td>
<td>96.3( \mu )</td>
<td>209.4( \mu )</td>
<td>56.8( \mu )</td>
</tr>
<tr>
<td>Normal and hypoxia control cats</td>
<td>74.4( \mu )</td>
<td>148.9( \mu )</td>
<td>37.5( \mu )</td>
</tr>
</tbody>
</table>

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Table 2: Ergotamine Controls

<table>
<thead>
<tr>
<th></th>
<th>Intraluminal D</th>
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<th>Wall thickness</th>
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</thead>
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<tr>
<td>Ergotamine incubated brain</td>
<td>21.8( \mu )</td>
<td>135.4( \mu )</td>
<td>56.6( \mu )</td>
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<tr>
<td>Ergotamine control</td>
<td>47.8( \mu )</td>
<td>144.3( \mu )</td>
<td>48.3( \mu )</td>
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</table>
TABLE 3 Two-way Analysis of Variance (Control versus No-Reflow and Region)

<table>
<thead>
<tr>
<th>Region</th>
<th>No reflow</th>
<th>Control</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>s.d.</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>A. Internal Diameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1</td>
<td>2.06</td>
<td>1.52</td>
<td>2.11</td>
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<tr>
<td>A-2</td>
<td>1.15</td>
<td>0.51</td>
<td>2.43</td>
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<tr>
<td>A-3</td>
<td>1.71</td>
<td>0.92</td>
<td>3.87</td>
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<tr>
<td>B-1</td>
<td>1.82</td>
<td>0.84</td>
<td>2.12</td>
</tr>
<tr>
<td>B-2</td>
<td>1.24</td>
<td>0.82</td>
<td>3.02</td>
</tr>
<tr>
<td>B-3</td>
<td>1.69</td>
<td>0.35</td>
<td>2.43</td>
</tr>
<tr>
<td>Total</td>
<td>1.56</td>
<td>0.90</td>
<td>2.59</td>
</tr>
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F p-value
Control vs. No-Reflow 22.005 0.0001
Region 1.747 0.130
Interaction 2.431 0.0397

B. External Diameter

<table>
<thead>
<tr>
<th>Region</th>
<th>No reflow</th>
<th>Control</th>
<th>Difference</th>
</tr>
</thead>
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<tr>
<td></td>
<td>$\bar{x}$</td>
<td>s.d.</td>
<td>$\bar{x}$</td>
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<td>A-1</td>
<td>5.16</td>
<td>1.24</td>
<td>4.80</td>
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<td>A-2</td>
<td>3.55</td>
<td>0.91</td>
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<td>A-3</td>
<td>3.94</td>
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<tr>
<td>B-1</td>
<td>4.44</td>
<td>1.32</td>
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<td>B-2</td>
<td>4.22</td>
<td>0.90</td>
<td>5.79</td>
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<tr>
<td>B-3</td>
<td>4.78</td>
<td>0.30</td>
<td>6.07</td>
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<tr>
<td>Total</td>
<td>4.37</td>
<td>1.21</td>
<td>5.03</td>
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F p-value
Control vs. No-Reflow 8.4702 0.0047
Region 3.5723 0.0055
Interaction 3.1846 0.0106

C. Vessel Wall Thickness

<table>
<thead>
<tr>
<th>Region</th>
<th>No reflow</th>
<th>Control</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>s.d.</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>A-1</td>
<td>1.55</td>
<td>0.22</td>
<td>1.35</td>
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<tr>
<td>A-2</td>
<td>1.20</td>
<td>0.22</td>
<td>1.19</td>
</tr>
<tr>
<td>A-3</td>
<td>1.11</td>
<td>0.27</td>
<td>1.25</td>
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<tr>
<td>B-1</td>
<td>1.31</td>
<td>0.26</td>
<td>0.83</td>
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<tr>
<td>B-2</td>
<td>1.84</td>
<td>0.14</td>
<td>1.38</td>
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<tr>
<td>B-3</td>
<td>1.54</td>
<td>0.04</td>
<td>1.82</td>
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<tr>
<td>Total</td>
<td>1.41</td>
<td>0.34</td>
<td>1.23</td>
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F p-value
Control vs. No Reflow 6.1527 0.0142
Region 16.6475 0.0001
Interaction 6.0471 0.0002

cortical areas of impaired reperfusion following total circulatory arrest in monkeys. By utilizing the technique of injecting a carbon black suspension just prior to termination of the experiment they show coronal brain sections having large cortical areas devoid of carbon black. Other investigators have reported similar findings. These observations appear more consistent with occlusion of larger, penetrating cortical vessels than with capillary occlusion, otherwise there has to be an explanation for simultaneous occlusion of many capillaries in one area with sparing of the capillaries in adjacent areas. Second, the perivascular lesions seen in humans following circulatory arrest combined with hyperosmolality are predominantly situated around penetrating cortical vessels and not associated with capillaries. The further possibility that larger vessel occlusion might be caused by spasm is suggested by Wade and associates who have demonstrated increased concentrations of potassium in rat brain interstitial fluid following ischemia and have postulated that the no-reflow state which they observed might possibly be due to vascular contraction since it is known that high potassium concentrations can cause contractions of vascular smooth muscle. Zervas et al. imply that vascular contraction might play a role in cerebral ischemia following their observation that dopamine was decreased in the brains of ischemic gerbils. In the present study, contraction (spasm) of the penetrating cortical vessels is indicated by smaller lumens and thicker walls in the no-reflow areas following ischemia. Proof that spasm can occur in these intraparenchymal vessels is supplied by the demonstration of spasm in the brain slices incubated with ergotamine, a drug chosen because of its known pharmacological effect of directly inducing smooth muscle contraction. This positive control experiment using ergotamine also shows that vessels can be "frozen" in their physiologic state and examined morphologically, a compromised analysis of form and function which cannot be achieved with any other conventional fixative to the best of our knowledge.

The rapid freezing technique results in excellent preservation of morphology of both meningeal and cortical vessels (fig. 2). Vessels in the areas of no-reflow which are presumed to be in spasm have thick, heavily stained walls (fig. 3) which rules against the possibility of edema causing the increased mural thickness.

It has been suggested that ergotamine has a greater direct contractile effect on venous than arterial smooth muscle. In the present model we cannot rule out the possibility that some of our measured vessels were veins. Although we excluded from measurement all vessels with thin walls, there remains the possibility that some veins could go into spasm, acquiring thicker walls and then appearing as arteries or arterioles on section. However, we do not feel that venous spasm occurred frequently because numerous veins in the no-reflow areas remained thin walled with large lumens.

The no-reflow areas decreased slightly in size as deeper sections were examined. Also, these areas did not neatly surround one penetrating vessel as was seen in human cases but usually encompassed two or more arterioles or arteries. Both of these observations raise the possibility of spasm in
the pia-arachnoid vessels prior to parenchymal penetration, a possibility which cannot be ruled in or out on the basis of this study because the pia-arachnoid vessels were sectioned at many different angles rendering them difficult to measure and because there is no proof that pia-arachnoid vessels observed overlying a no-reflow area actually penetrate into that same area rather than entering the brain at another site.

These brain biopsies were sectioned at intervals and consequently the same vessels were sometimes measured at 2 or even 3 locations. This was done because the no-reflow areas might likely be down-stream from the region of vessel spasm and because of the possibility that vessels might be in spasm segmentally as observed in rabbit basilar artery experimentally stretched. Segmental spasm in our experiment was suggested by the observation that some vessels became smaller and thicker in some sections only to be seen with larger lumens and thinner walls in subsequent deeper sections.

The measurements of the non-ischemic control vessels falling between no-reflow and reflow values might be explained by individual animal variation although the possibility exists that the ischemic cat brains displayed an
overall vascular dilatation with superimposed focal spasm. However, if that were true the hypoxic vessels might have displayed vessel calibers more similar to the ischemic vessel reflow areas than to the normal controls since it is known that systemic hypoxemia and systemic hypercapnea produce increased cerebral blood flow\(^ {19,20}\) presumably due to vascular dilatation, at least of the pial vessels. This underscores the lack of understanding of the relationships between pial and parenchymal vessels. Harper\(^ {21}\) has postulated that there is a reciprocal relationship between pial and parenchymal vessels; when the pial vessels dilate, the parenchymal vessels might constrict under certain circumstances and vice versa. It does not appear that overall vasodilatation and focal vascular spasm in the same vessels are mutually exclusive events either; focal spasm could conceivably be superimposed upon generalized cerebral and meningeal vasodilatation. Although our 4 hypoxic control cats showed no evidence of cerebral vasodilatation, they were inadequate for that type of study. After repeated hypoxia in the 4 control cats it became difficult to maintain adequate cardiac output. This produced a mild hypotensive ischemic superimposed upon the hypoxia, which probably accounts for the 2 small no-reflow areas seen in 1 of those brains.

The procedure of unroofing the skull might be expected to cause spasm of meningeal vessels accounting for focal no-reflow areas in the cortex; this did not likely occur in the present experiment however, because we did not observe no-reflow areas in the 8 control cats (with one exception mentioned previously) all of which underwent the skull unroofing procedure exactly as the test animals.

Our data which show differences in regional measurements suggest that there may be normal regional variations in vessel concentration, size and reactivity. By further defining size of reactive versus non-reactive vessels as well as vessel concentration in future studies we might possibly be able to predict those areas which would be prone to vascular spasm. Preliminary counts have shown comparable numbers of 70-300 \(\mu\) diameter vessels per unit area in both the no-reflow as well as the adjacent reflow areas. This finding suggests that vessel spasm is random in regard to vessel density. The spasm could be caused by hypoxia in combination with local accumulation of vasoactive metabolites, direct stimulation of vascular smooth muscle by intraluminal hematopoietic elements during stasis or by neurogenic stimulus perhaps mediated by venous engorgement. Emerson and Parker\(^ {22}\) have shown that increased cerebral venous pressure in the dog results in a corresponding drop in cerebral blood flow, presumably due to active vasoconstriction of precapillary vessels. Thus, it is not inconceivable that spasm might be initiated at the venous level. These possibilities also need further study.

We used the 30 minute neck choke technique in these animals because it produces definable no-reflow areas. An additional group of 9 cats was kept alive for 2 days to 2 weeks following 30 minutes of neck choke without skull unroofing. The brains of these cats were interesting in that they showed neuronal necrosis of hippocampal neurons and Purkinje cells, both of which are presumed to be selectively vulnerable to hypoxia. The cerebral cortices of these animals showed random ischemic damage, in some areas the necrosis appeared "pseudolaminar" while in other areas the necrosis was in patches which encompassed several small arteries. Many of the vessels in these patches of necrosis as well as the vessels in the "pseudolaminar" areas of necrosis showed reactive changes of endothelial proliferation. These observations support the conclusion that ischemic brain damage entails very complicated mechanics and several mechanisms might be operative. The technique of freezing and measuring vessels should be applied to brains rendered ischemic by means other than the choke method.

**Acknowledgment**

The authors wish to thank Mr. James C. Torner for statistical analysis.


**133Xenon Inhalation Method: Significance of Indicator Maldistribution for Distinguishing Brain Areas with Impaired Perfusion**

**An Index for Total Flow**

**Urs W. Blauenstein, M.D., James H. Halsey, Jr., M.D., Edwin M. Wilson, D.Sc., and Edward L. Wills, Ph.D.**

**SUMMARY** This paper introduces a new index for the assessment of regional cerebral blood flow. The index is proportional to total flow, and is obtained from the ratio of regional count rate to arterial blood flow. It accounts for brain tissue partly or totally deprived of its blood supply. Examples of clinical application are reported.

A good correlation with the findings of computer-assisted tomography has been found.

**THE CLINICAL APPLICATION** of the 133Xe inhalation method for the assessment of regional cerebral blood flow (rCBF) in cerebrovascular disease is still limited by methodological difficulties.

In addressing the problem of indicator maldistribution in ischemic brain regions 34 — commonly referred to as “look through” phenomenon or artifact 4 — 85Kr has been cited as a better alternative to 133Xe if the surface of the brain is directly accessible. 4 However, for non-invasive measurements of rCBF the converse appears to be true. The primary characteristic emission of 85Kr is predominantly β particle (99.6% β; 0.4% γ). Thus, measurements with 85Kr are either limited to a depth of 2.5 mm below the brain surface in the open skull, 5 4 or, when detecting the γ emission through the intact skull, require doses several times greater than with 133Xe. For whichever γ is detected, indicator maldistribution is equally possible whether using the inhalation or the injection method.

The localization and quantitation of impaired indicator delivery to brain regions partly or totally deprived of their blood supply would clearly be of clinical diagnostic value. They may be achieved by appropriate analysis of the indicator clearance curves which seem to contain the necessary information about indicator maldistribution. A parameter for total flow may offer a solution for this methodological problem. Our preliminary experience with such a parameter is reported here.

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**References**


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