Flow and Neuronal Density in Tissue Surrounding Chronic Infarction


SUMMARY In 6 cats, cerebral infarction was produced by transorbital occlusion of the left middle cerebral artery (MCA). Five animals developed typical cortical infarcts. Eight weeks later, cerebral blood flow (CBF) was determined by \(^{14}\)C-iodoantipyrine autoradiography and the number of intact neurons was counted histologically. Two non-operated cats served as controls. Cortical blood flow in the infarcted hemisphere was reduced by 24.6–74.4% when compared to the flow in the contralateral cortex and in controls. Averaged white matter flow was decreased by 39.1%. Regional cortical flow was gradually reduced from parasagittal regions towards the infarct. In the surrounding of the infarct, cortical perfusion was decreased to 24.8 ± 9.7 ml/100 g/min, i.e. 19.7% of contralateral flow. Although the infarcts were sharply demarcated macroscopically, the number of cortical neurons decreased gradually from the midline to the peri-infarct zone. A significant linear correlation was found between absolute CBF-values and the number of neurons in areas of the infarcted hemisphere. The homolateral gyrus lateralis had normal neuronal density but flow was reduced by 20%.

These findings suggest that the blood flow reduction in tissue surrounding chronic infarcts is due to neuronal cell loss and to functional inactivation caused by damage of afferent fibers.

EXPERIMENTALLY PRODUCED CEREBRAL ISCHEMIA results in neurological deficits related to the brain regions affected by the acute flow disturbance.1

Reversibility of neurological deficits and extension of the persisting brain damage is dependent on the collateral flow,2 by which transiently impaired perfusion may be improved and tissue necrosis avoided. The resulting infarcts appear as morphologically well-demarcated necrotic areas with surrounding tissue of preserved structure.

In the acute and subacute stage of focal ischemia, disturbed blood flow has been observed in the center and in the surrounding of an ischemic lesion.3,4 A circumscribed hypoperfused region has been described.

---

From the Max-Planck-Institut für Hirnforschung, Köln-Merheim, Ostmerheimer Strasse 200, 5 Köln 91, Federal Republic of Germany, and *Universitätsklinik für Neurochirurgie Auenbruggerplatz A-8036 Graz, Austria.

Address correspondence to: G. Mies, M.D., Max-Planck-Institut für Hirnforschung, Ostmerheimer Strasse 200, 5 Köln 91, Federal Republic of Germany.

Received May 28, 1982; revision accepted August 10, 1982.
as the cause and consequence of the flow impairment, but hypo- as well as occasional hyperperfused areas have also been seen outside the region of ischemic lesion. However, in chronic states following occlusion of the middle cerebral artery (MCA) in baboons, disturbed blood flow, decreased CO$_2$-reactivity and impaired autoregulation was observed in macroscopically intact brain tissue surrounding infarcted areas. This flow decrease could lead to an ongoing pathogenetic factor resulting in further tissue damage, although such flow changes could also be caused by slight morphological damage due to the primary disturbance of blood supply or could be a consequence of functional inactivation in the vicinity of ischemic infarcts. An attempt to answer this question was made by measuring regional flow in chronic animals after MCA-occlusion and by relating the flow values to the number of neurons in the tissue surrounding chronic infarcts.

Material and Methods
Experiments were performed in 8 cats of both sexes weighing 1.9 to 3.4 kg. Two animals served as controls. The cats were anesthetized with an intraperitoneal dose of 30 mg/kg pentobarbital (Nembutal). After fixing the head the left middle cerebral artery (MCA) was exposed via the transorbital approach. Using an arterial clip (Aukland No 1) the MCA was occluded. Thereafter, hammered muscle taken from the left quadriceps muscle was placed into the orbit and fixed with tissue glue (Histacyl). Then the orbit was filled with foam-gel (Gelastyp-S) soaked with antiseptic fluid (Betasodona). Thereafter, the eyelids were sutured. Animals were allowed to recover and neurological examination was performed repeatedly. During the first three postoperative days penicillin was injected intra-muscularly.

After 8 weeks the animals were anesthetized again with 30 mg/kg pentobarbital given intraperitoneally. PVC-catheters were placed into both femoral arteries and veins. The animals could breathe spontaneously. Body temperature was kept constant at 37°C by means of a heat lamp. The head was fixed into a stereotaxic frame and the skull exposed. The physiological parameters measured in all animals met the following criteria: PaO$_2$ 117 ± 8 mm Hg, PaCO$_2$ 36 ± 3 mm Hg, mean arterial pressure 106 ± 8 mm Hg.

Cerebral blood flow was determined according to the method of SAKURADA et al. Two hundred and fifty $^{14}$C-iodoantipyrine dissolved in Ringer-solution was applied intravenously for one minute by means of a ramp infusion. At the same time arterial blood samples were collected at 6 sec intervals. At the end of infusion, 10 ml of saturated potassium chloride solution were injected intravenously as a bolus. Thereafter, animals were decapitated to stop blood circulation completely. After removal of the skull bone and dura, the brain was lifted at its frontal parts to dissect brain stem and connecting nerves. The cerebellum was separated by incision. Then the brain was carefully removed from the remaining skull avoiding pressure on the cortex. The cerebellum was cut coronally through the center of the infarct which could be identified by the retracted tissue. The frontal part was moved into 10% formaline solution and remained there for a week. After embedding it in paraffin routine histology was performed. The occipital part was immersed in liquid methylbutane chilled to −70°C for 5 minutes and stored in a freezer at −70°C until it was sectioned the next day.

Sections of 20 μm thickness were cut on a cryostat (SLEE, Mainz) at −20°C for autoradiography and dried immediately on a heating plate at 60°C to prevent diffusion of the tracer. The next brain section cut at 10 μm was used for histological examination in order to obtain almost identical localization for blood flow estimation and histological examination of the infarct and its surroundings.

Tissue samples were taken from different brain regions to determine the $^{14}$C-iodoantipyrine concentration per gram wet weight and compared to the neighbouring autoradiograms (autocalibration). These tissue probes were digested in 0.3 ml hyamine-hydroxide, dried at 60°C and suspended in 1 ml distilled water. Thereafter, radioactivity was measured with a scintillation counter. Autoradiography was performed by exposing 20 μm thick brain sections to a Kodak-NMB film for 4 weeks. The $^{14}$C-concentrations of the different tissue samples were plotted against the optical density of corresponding brain areas read from the neighbouring autoradiograms. The concentrations of $^{14}$C-iodoantipyrine in the arterial blood samples was measured with a Beckman scintillation counter. Local blood flow was calculated according to SAKURADA et al. using a tissue/blood partition coefficient for $^{14}$C-iodoantipyrine of 0.8.

The 10 μm thick cryostat sections were fixated for histological examination in an ascending alcohol chain and stained. Neurons were counted in 0.2 x 0.2 mm areas of individual gyr in corresponding regions of the infarcted and the non-infarcted hemisphere. Additionally, the extent of the ischemic lesion was obtained using 7 μm thick sections from the formaline-fixated and paraplast-imbedded frontal parts of the brain.

Results
1. Neuropathological observations
a) Gross morphological changes
The typical pattern of chronic infarction after MCA-occlusion in five cats is shown in figure 1. The loss of brain volume in the left hemisphere is partly due to reduction in or absence of central white matter accompanied by necrosis of cortical brain regions. The extension of cortical infarcts is listed in table 1. The ecosylvian gyrus is almost completely necrotic in all animals while the gyrus suprasylvicus anterior, lobus piriformis and caudate nucleus were affected to a varying degree. The cortical defects showed up sharply against the surrounding tissue and were filled with a spongy mass. The atrophy of brain tissue in the left hemisphere caused an enlargement of the lateral ventricle. In cat no 6 chronic MCA-occlusion only caused infarc-
tion of the basal ganglia. Therefore, this animal was not included in the evaluation.

b) Histological findings

The infarcted cortical areas exhibited a typical picture of neuronal and glial cell death. The degradation process in the infarcted zones had not been completed as demonstrated by the presence of macrophages near the infarct borders. In the white matter necrosis of axons, myelin sheaths and glial cells was evident.

Towards the infarcted border zone the molecular layer was frequently preserved as a thin isolated zone covering the infarct cysts. Bridges of surviving tissue and its vessels reached into the territory of the infaracts. Pial membranes and vessels appeared to be normal.

A broad zone of brain tissue which surrounded the infarcts was of spongy appearance. In these peri-infarct regions the average number of histologically intact neurons was \( 20.4 \pm 10.7 \) cells/0.04 mm\(^2\). With increasing distance, i.e. in the gyrus suprasylvicus anterior, the number of neurons was still significantly reduced, reaching a value of \( 57.2 \pm 9.8 \) cells/0.04 mm\(^2\) as compared to the average neuronal number of the contralateral gyrus with \( 74.3 \pm 8.4 \) cells/0.04 mm\(^2\). In the gyrus lateralis the number of neurons was normal. Representative histological findings as described above are shown in figure 2.

### Table 1

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Gyrus suprasylvicus anterior</th>
<th>Gyrus ectosylvicus anterior</th>
<th>Lobus piriformis</th>
<th>Nucleus caudatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \dagger )</td>
<td>( $ )</td>
<td>( \dagger )</td>
<td>( \dagger )</td>
</tr>
<tr>
<td>2</td>
<td>( \dagger )</td>
<td>( $ )</td>
<td>( \dagger )</td>
<td>( \dagger )</td>
</tr>
<tr>
<td>3</td>
<td>( \dagger )</td>
<td>( $ )</td>
<td>( \dagger )</td>
<td>( $ )</td>
</tr>
<tr>
<td>4</td>
<td>( \dagger )</td>
<td>( $ )</td>
<td>( $ )</td>
<td>( \dagger )</td>
</tr>
<tr>
<td>5</td>
<td>( \dagger )</td>
<td>( $ )</td>
<td>( \dagger )</td>
<td>( \dagger )</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>( \dagger )</td>
</tr>
</tbody>
</table>

\( \dagger \) = very low grade; \( \dagger \) = low grade; \( \dagger \) = middle grade; \( \$ \) = high grade.

2. Cerebral blood flow

Cerebral blood flow in various brain regions in control animals is listed in table 2. Average blood flow was \( 112.3 \pm 6.1 \) ml/100 g/min for the cortex and \( 26.6 \pm 5.8 \) ml/100 g/min for the white matter. The flow values are slightly increased due to moderate hypercapnia during the experimental procedure. In figure 3a an autoradiogram of a control animal is shown.

In the infarcted average blood flow in grey matter of the non-infarcted hemispheres was \( 114.6 \pm 6.9 \) ml/
blood flow on the number of histologically intact neurons $(y = 1.03 + 0.922 x, r = 0.8235, n = 42, p < 0.001)$. The relationship between flow and neurons in the controls and non-infarcted hemispheres is included in figure 4. As evident from the figure, areas of the infarcted hemisphere with normal cell counts (gyrus lateralis) have decreased flow values.

**Discussion**

The data presented here demonstrate that, in contrast to the well defined macroscopical demarcation of chronic infarcts, rCBF values do not line up sharply on the border between infarcted and healthy tissue; on the contrary, rCBF values fall off gradually from high values in areas not affected by MCA-occlusion to low values within the infarcted tissue. The decreased flow in peri-infarct areas has been already shown punctually using a few measuring probes indicating no sudden reduction of blood flow towards the chronic infarcted territory. This is in contrast to the perfusion pattern as measured during acute focal ischemia where the infarcts are demarcated by hyperemic borderzones while in the center of the infarction blood flow is drastically reduced.

**TABLE 2** Blood Flow and Neuronal Numbers Expressed as Mean ± Standard Deviation in Experimental Animals and in the Controls Summarized from All Data. Significant Differences were Calculated with Student's t Test.

<table>
<thead>
<tr>
<th></th>
<th>Experimental animals (n = 5)</th>
<th>Control animals (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infarcted hemisphere</td>
<td>Non-infarcted hemisphere</td>
</tr>
<tr>
<td>CBF ml/100 g/min</td>
<td>Neuronal numbers per 0.04 mm$^2$</td>
<td>CBF ml/100 g/min</td>
</tr>
<tr>
<td>Gyrus lateralis</td>
<td>81.8 ± 15.8 &amp; $^*$</td>
<td>81.2 ± 11.4 &amp; $^*$</td>
</tr>
<tr>
<td></td>
<td>n = 44</td>
<td>n = 26</td>
</tr>
<tr>
<td>Gyrus suprasylvicus</td>
<td>67.6 ± 16.4 &amp; $^*$</td>
<td>57.2 ± 9.8 &amp; $^*$</td>
</tr>
<tr>
<td>anterior</td>
<td>n = 44</td>
<td>n = 26</td>
</tr>
<tr>
<td>Gyrus ectosylvicus</td>
<td>43.6 ± 20.4 &amp; $^*$</td>
<td>36.0 ± 9.3 &amp; $^*$</td>
</tr>
<tr>
<td>anterior (intact</td>
<td>n = 18</td>
<td>n = 9</td>
</tr>
<tr>
<td>regions)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gyrus ectosylvicus</td>
<td>24.8 ± 9.7 &amp; $^*$</td>
<td>20.4 ± 10.7 &amp; $^*$</td>
</tr>
<tr>
<td>anterior (peri-infarct zone)</td>
<td>n = 26</td>
<td>n = 13</td>
</tr>
<tr>
<td>Infarcted zone</td>
<td>20.1 ± 3.8</td>
<td>n = 11</td>
</tr>
<tr>
<td>White matter</td>
<td>16.2 ± 5.5 &amp; $^*$</td>
<td>23.5 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>n = 21</td>
<td>n = 22</td>
</tr>
</tbody>
</table>

*$^p < 0.05$: comparison with control values.

$^*p < 0.05$: comparison with non-infarcted hemisphere.

$^*p < 0.001$: comparison with control values.

$^*p < 0.001$: comparison with non-infarcted hemisphere.
flow and neuronal loss in the cortex is the most important finding in this investigation. The effect of changes in brain tissue consistency due to shrinkage or atrophy on the blood flow results were minimized by means of autocalibration. With this procedure a direct measure of flow per unit wet weight was obtained thus excluding the influence of changes in tissue water content in different brain regions. Cryostat sections per se represent the spatial distribution of tissue in relation to the actual wet weight. For the use of histological evaluation these sections have to be fixated and stained which may lead to minimal shrinkage of the tissue. This means, neuronal counts per mm$^2$ will be slightly overestimated. Another problem which arises is compression of brain tissue during removal from the skull which could result in additional damage to the histological appearance of cells.

Histological methods do not permit a decision about the functional state of a neuron. However, the highly significant linear correlation between reduced blood flow and decreased number of neurons within the hemisphere housing the infarction indicates an adaptation of perfusion to the metabolic demand of the surviving nerve cells. This assumption is not supported by the observation of a 20% flow reduction in the gyrus lateralis where the number of cells was as high as in the contralateral hemisphere. The flow reduction in this region is probably caused by functional inactivation as a consequence of ischemic damage to the white matter (fig. 1). By white matter necrosis afferent fibers are interrupted and the functional input to the cortex is decreased. Additionally, an ischemic infarction involving primarily the white matter damages efferent fibers leading to retrograde degenerations and selective cell necrosis in the cortex. The decreased number of histologically intact nerve cells in the gyrus suprasylvius and gyrus ectosylvicus can be caused by retrograde degeneration due to white matter infarction and by transient impairment of blood supply severe and long enough to cause selective ischemic cell necrosis.\(^\text{9}\)

While a strong correlation exists between flow values and number of neurons, an additional effect of functional inactivation on the flow values cannot be ruled out, and both mechanisms may synergistically reduce perfusion in peri-infarct areas.

There are no indications that at this stage of chronic infarction brain swelling or compression against the

---

**FIGURE 3.** A. Autoradiogram from cerebral blood flow measurement with $^{14}$C-iodoantipyrine in a control animal. B. Cerebral blood flow in chronic infarction (8 weeks). Note the gradual decline of flow towards the infarct. Values are expressed as ml/100 g/min.

---

**FIGURE 4.** Correlation of cortical blood flow and number of neurons measured in the same area. The regression line includes the gyrus lateralis of the infarcted hemisphere ($\circ$) with $y = 1.09 + 0.922 x$ ($r = 0.8235$, $p < 0.001$). For comparison, the flow/neuron relationship from the opposite hemisphere is added ($\bullet$).
skull plays an important role. The infarcted hemispheres show substantial loss of brain parenchyma with concomitant enlargement of the lateral ventricles. Shrinkage or atrophy of the ischemic hemisphere has been seen in chronic infarction before.6 In acute cerebral ischemia, neuronal function and morphological integrity of cells are related to two thresholds of blood flow: electrical failure of cerebral cortex occurs if flow drops below 15 to 20 ml/100 g/min; below 10 ml/100 g/min active ionic transport ceases and intracellular potassium is liberated. The small flow range between the two thresholds where neurons are supposed to remain structurally intact but functionally inactive and has been called the ischemia "penumbra" of cerebral circulation.10 In four cats regional CBF values as measured in the peri-infarcted regions were well above the threshold of electrical failure ranging from 20–30 ml/100 g/min with concomitant neuronal loss. Only in one cat flow values between 12–15 ml/100 g/min were obtained around infarcted tissues but in these regions the number of neurons were reduced accordingly. The remaining perfusion in infarcted tissue reflects circulation in vessel shunts and in trabecules of connective tissue.1 The moderate hypercapnia present in our experiments is of negligible influence because CO2-reactivity in the infarcted territory and in the peri-infarct zones was found to be suppressed.7

As demonstrated by the results presented here it is unlikely that flow is primarily decreased in peri-infarct areas and that this flow reduction is the cause for functional and morphological damage of nerve cells. One would expect that under such conditions the number of cells remains close to normal with gradually decreased flow values and then drops sharply at a flow value in the range of the penumbra. However, in the peri-infarct areas with critical flow values of 20–30 ml/100 g/min or below, "silent" neurons may exist which are inactive but have preserved structural metabolism. Due to the heterogeneity of regional CBF during and after ischemic events8,12 pericellular flow may sometimes fall below the threshold critical for cell survival in the course of events following an ischemic attack, irreversibly damaging those cells. The existence of "silent" cells which regain their normal function after improvement of flow can explain recovery of neurological defects observed in some patients with chronic ischemic infarcts shortly after extra-intracranial arterial bypass surgery.14-16 However, restoration of neuronal function can only be achieved when the nerve cells and their afferent and efferent connections are not irreversibly damaged: by flow increases in regions supplied at penumbra values nervous function was not always restored although a nearly physiological ion distribution was reestablished.7,18 The influence of brain swelling and consecutive increase of intracranial pressure cannot be ruled out.19

The time course of peri-infarct neuronal damage in relation to rCBF changes after an ischemic attack is of utmost importance for therapeutic measures to preserve brain tissue. Whether any treatment, conserva-

References
Flow and neuronal density in tissue surrounding chronic infarction.
G Mies, L M Auer, G Ebhardt, H Traupe and W D Heiss

*Stroke*. 1983;14:22-27
doi: 10.1161/01.STR.14.1.22

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/14/1/22

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/