Experimental Brain Infarcts in Cats

I. Pathophysiological Observations

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SUMMARY In 48 cats the left middle cerebral artery was occluded under light barbiturate anesthesia using a transorbital approach. The animals were kept alive for 1, 2, and 4 hours after vascular occlusion. Regional cerebral blood flow was measured by the intracardiac microsphere injection technique before ischemia, 15 min after the onset of ischemia, and at the end of the experiments. The density of regional ischemia was correlated with EEG changes and with the electrolyte, water and metabolite content of the same tissue samples in which blood flow was assessed. In the territory of the occluded middle cerebral artery, cortical blood flow decreased from 41.4 ± 3.8 to 21.1 ± 4.0 ml/100 g/min (means ± SE), the actual flow rate depending on the individual efficacy of collateral blood supply. At flow rates below 10-15 ml/100 g/min, ischemia involved more than 50% of the middle cerebral artery territory, water and electrolyte homeostasis was severely disturbed and ischemic brain edema developed. Adenosine triphosphate decreased to about 60% of the control value at flow rates below 40 ml/100 g/min, but it remained at this level down to flow rates as low as 5 ml/100 g/min. EEG intensity — but not EEG frequency — decreased in parallel with blood flow, indicating that with increasing density of ischemia an increasing portion of the excitable neuropil was inhibited.

The development of ischemic brain edema determined the further progression of ischemia. When blood flow decreased below the threshold for water and ion disturbance, ischemia was progressive (critical ischemia), but an amelioration of flow occurred in animals in which flow remained above this level (non-critical ischemia). In the contralateral hemisphere the EEG, blood flow, water and electrolyte content did not change significantly during the initial few hours of ischemia. Diaschisis, in consequence, was not a prominent feature during the early phase of infarct development.

THE SUDDEN OCCLUSION of a major cerebral artery does not necessarily result in cerebral infarction. In cats, ligation of the middle cerebral artery (MCA) may be fully compensated for by collateral flow from the surrounding territories. In man, the number of clinically silent occlusions of major cerebral arteries is unknown but occasional angiographic findings suggest that even occlusion of the middle cerebral artery can be tolerated without permanent neurological damage.

It is obvious that the degree of neurological damage depends on the individual efficacy of collateral blood flow, and on the fact that ischemia has to be of sufficient severity before brain disturbances become apparent. Several laboratories have determined thresholds for functional, biochemical and morphological damage following experimental middle cerebral artery occlusion. It appeared that disturbances of electrophysiological signals such as the electroencephalogram, spontaneous cell discharges or evoked potentials occur at substantially higher flow rates than morphological alterations or disturbances of transmembrane ion homeostasis. Since the latter can be considered a sign of impeding cell death, prevention of irreversible damage requires the rise of blood flow above this critical value.

The threshold of ionic disturbances is approximately 10 ml/100 g/min. This value is surprisingly low when compared to the actual flow rates in the territory of the occluded artery which are very close to this level. It is, therefore, not unlikely that a relatively slight increase in collateral blood supply to the ischemic territory might raise blood flow above the critical threshold, and thus dramatically improve the functional outcome. Although the exploration of such a therapeutic approach is of clinical importance, few experimental data are available, mainly because of the difficulty of standardizing the ischemic impact. Since the size and degree of ischemia in the individual case are extremely variable, correlation of different parameters in different animals is almost impossible. For the study of the interrelationship between blood flow and tissue response, therefore, as many tissue parameters as possible should be assessed together with blood flow in the same animal, and, preferentially, even in the same tissue sample.

A convenient approach for this purpose is the radioactive microsphere injection technique for measuring cerebral blood flow. This method allows the direct correlation of the density of ischemia with
different biochemical and biophysical parameters because the radioactivity of microspheres on which the flow calculation is based, is measured in tissue specimens which are removed at the end of the experiments and can be subsequently processed for tissue constituents.

In the present investigation the effects of experimental occlusion of the middle cerebral artery have been studied in untreated animals in order to provide the basis for our therapeutic explorations.18, 14 A preliminary report of some of the data has been given before.1, 16

Material and Methods

The experiments were carried out in 48 adult cats weighing between 2 and 3.5 kg. The animals were anesthetized with a single intraperitoneal injection of 30 mg/kg pentobarbital (Nembutal), immobilized with gallamine triethiodide, and mechanically ventilated with air. Blood pressure and tidal carbon dioxide were continuously, and blood gases intermittently, monitored. The ventilation was adjusted to yield an apO₂ of more than 100 mm Hg, and an apCO₂ between 28 and 32 mm Hg.

Occlusion of the Middle Cerebral Artery (MCA)

The left MCA was permanently occluded using the transorbital approach of Hudgins and Garcia.18 with the modification for the cat, as described by O'Brien and Waltz.17 The left eye ball was removed and the optic foramen enlarged with a dental drill. Using an operating microscope, the dura was split and the MCA exposed at its origin from the internal carotid artery. The arachnoid membrane covering the MCA was split with fine needles, and the MCA was clamped close to the carotid artery with a Mayfield vessel clip. The vessel was additionally coagulated distally to the clamp in order to assure complete vascular occlusion.

At the end of the experiments, the extent of the ischemic territory was traced by injecting a suspension of carbon black or Evans blue into the left ventricle of the heart via the brachial or femoral artery. The correct position of the catheter was controlled by monitoring intracardiac pressure. Radioactive labelled microspheres with a diameter of 15 ± 5 micron were injected into the heart over a period of about 10 sec, and a reference blood sample was withdrawn during this time from the abdominal aorta at a calibrated speed of 3 ml/min. Flow measurements were made after exposure of the MCA (usually 15 min before ischemia), 15 min after MCA occlusion, and at the end of the experiments, which were terminated 1, 2 or 4 h after occlusion, respectively. The microspheres were labelled with 141-cerium, 113-tin, and 46-scandium, and radioactivity of blood and tissue samples (see below) was measured using a 3 channel gamma scintillation counter (Biogamma II, Beckman, Fullerton). In order to obtain statistically relevant data, the number of injected microspheres was calculated to yield a minimum of 400 spheres in the smallest tissue sample, which was about 150 mg. Cerebral blood flow of the cat is about 2-5% of cardiac output, and the weight of cat brain about 25 g. The number of microspheres injected into the heart, consequently, was approximately 2.0 million for each flow measurement, i.e., a number which does not cause major changes of general hemodynamics.

Regional cerebral blood flow was determined in the following way. At the end of the experiment, the brain was removed, tissue samples were dissected in a moist chamber from grey and white matter of various regions of the brain, and immediately weighed. Radioactivity of the microspheres was determined in the gamma scintillation counter and corrected for inter-channel spillover.14 Using the blood as a reference sample, regional blood flow (rCBF) was

\[
\text{rCBF} = \frac{R_t \times F_b \times 100}{W_t \times R_b} \quad (\text{ml}/100 \text{ g/min})
\]

where \(R_t\) is tissue radioactivity, \(W_t\) tissue weight, \(F_b\) the flow rate of blood sampling (ml/min) and \(R_b\) blood radioactivity.

Biochemical Investigations

Most of the brains of the present experimental series were processed for tissue osmolality and for the content of water, electrolyte and various metabolites.
TABLE 1. **Physiological Variables Before and After Transorbital Occlusion of the Middle Cerebral Artery**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>15 min</th>
<th>1 hour</th>
<th>2 hours</th>
<th>4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure</td>
<td>122 ± 9</td>
<td>118 ± 9</td>
<td>106 ± 11</td>
<td>122 ± 8</td>
<td>130 ± 6 mm Hg</td>
</tr>
<tr>
<td>Arterial Pco₂</td>
<td>122 ± 7</td>
<td>119 ± 12</td>
<td>115 ± 7</td>
<td>125 ± 10</td>
<td>121 ± 16 mm Hg</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.35 ± 0.01</td>
<td>7.37 ± 0.02</td>
<td>7.38 ± 0.01</td>
<td>7.36 ± 0.02</td>
<td>7.38 ± 0.03</td>
</tr>
<tr>
<td>Arterial Pco₂</td>
<td>30 ± 0.8</td>
<td>27 ± 1.0</td>
<td>27 ± 0.4</td>
<td>28 ± 1.2</td>
<td>27 ± 1.5 mm Hg</td>
</tr>
</tbody>
</table>

Values are means ± SE.

The sampling techniques and the analytical procedures are described in the following paper.¹⁴

**Results**

**General Physiological Effects**

Clamping of the middle cerebral artery did not have any major effects on the general state of the animals. Blood pressure remained in the normotensive range, and the acid-base state of the blood did not change significantly. Arterial blood gases also remained within normal limits (table 1). The animals were fully relaxed and maintained under artificial respiration which presumably stabilized the experimental situation. The absence of general physiological effects, therefore, may not be representative for changes occurring in stroke patients under clinical conditions.

**Infarct Size**

Size of the infarcts following middle cerebral artery occlusion was determined using the single dye passage¹⁸ for macroscopical demarcation of the ischemic territory. With this technique, the perfused region of the brain stained with the dye and the less, or unperfused regions, remained white (fig. 1). Photographs were taken of 4 coronal sections of the brain, and the size of the infarcts was determined planimetrically and expressed as percent of the volume of the ipsilateral hemisphere. According to this calculation, the mean extent of ischemia was 34 ± 3.4% (mean ± SE, range 18–62%) of the volume of the ipsilateral hemisphere. In the cat, the territory of the MCA covers approximately 60% of one hemisphere; the infarcts, consequently, affected between 30–100% of the MCA territory.

A correlation between duration of ischemia and infarct size did not exist. However, there was a certain relationship between the size of the infarct and the reduction in blood flow (fig. 2). In all animals in which the infarct covered more than 30% of the hemisphere (viz. 50% of the MCA territory) blood flow was below 18 ml/100 g/min, i.e. below the critical threshold for maintenance of ion homeostasis (see below). This indicates that collateral blood supply is not able to maintain blood flow above this threshold when more than 50% of the MCA territory is ischemic.

**Electroencephalogram**

A few seconds after MCA occlusion, the amplitude of the electroencephalogram over the affected hemisphere markedly decreased (fig. 3). Quantitative evaluation of EEG intensity by Fourier analysis revealed a decrease to about 40% of control (fig. 4).

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**Figure 1.** Regional cerebral ischemia following transorbital occlusion of the middle cerebral artery. The ischemic territory is recognized by its failure to stain with intrathecally injected carbon black (single dye passage).¹⁸

**Figure 2.** Relationship between cerebral blood flow and infarct size 1–4 hours following middle cerebral artery occlusion. Blood flow was measured in the cortex of the middle cerebral artery territory, and infarct size was determined by single dye passage (see fig. 1).
The EEG frequency index, in contrast, was little affected, indicating that the EEG flattened but did not slow down. Changes over the opposite non-ischemic hemisphere were minor: both EEG intensity and frequency slightly increased, presumably because of the gradual decay of anesthesia (fig. 3). The absence of contralateral EEG suppression demonstrates that electrophysiological diaschisis was not a prominent phenomenon during the acute phase of MCA occlusion.

**Regional Blood Flow and Thresholds of Ischemia**

Table 2 summarizes the mean values of regional cortical blood flow before and at different times after occlusion of the MCA. The decrease in flow was most

**FIGURE 3.** Recording of the EEG, endtidal CO₂, and arterial blood pressure (SAP) during occlusion of the left middle cerebral artery. Note the decrease in EEG amplitude over the affected hemisphere, and the absence of general cardio-respiratory effects.

**FIGURE 4.** Changes of EEG intensity and the EEG frequency index following middle cerebral artery occlusion. EEG was analysed by Fourier transform. Values are means ± SE (closed circles: significantly different from control, p < 0.05).
TABLE 2. Regional Cerebral Blood Flow Before and After Transorbital Occlusion of the Middle Cerebral Artery

<table>
<thead>
<tr>
<th></th>
<th>Ipsilateral hemisphere</th>
<th>Contralateral hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCA territory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IP = bilateral hemisphere</td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>41.4 ± 3.8</td>
<td>30.5 ± 3.7</td>
</tr>
<tr>
<td>white matter</td>
<td>32.9 ± 2.6</td>
<td>30.0 ± 1.7</td>
</tr>
<tr>
<td>Border zone</td>
<td>51.4 ± 4.8</td>
<td>48.5 ± 4.6</td>
</tr>
<tr>
<td>cortex</td>
<td>35.6 ± 2.3</td>
<td>46.4 ± 3.9</td>
</tr>
<tr>
<td>white matter</td>
<td>33.2 ± 3.4</td>
<td>42.4 ± 3.2</td>
</tr>
</tbody>
</table>

Values are mean ± SE of all experiments (critical and non-critical ischemia) ± SE (ml/100g/min). Statistical difference from control: *p < 0.05, **p < 0.01.

pronounced in the territory of the MCA, but there was also a significant decrease in the border zone of the middle cerebral artery territory, and in the territory of the posterior cerebral artery. As has been demonstrated above, the individual blood flow rate depended on the size of the infarct and, therefore, varied from animal to animal. We have correlated blood flow with various physiological and biochemical parameters, in order to determine the thresholds of ischemic brain damage (fig. 5).

![Graphs showing water content, Na/K ratio, EEG intensity, and ATP levels](http://stroke.ahajournals.org/)

**Figure 5. Relationship between blood flow (abscissa) and brain water, the ratio of sodium/potassium content (Na/K), adenosine triphosphate (ATP) and EEG intensity (ordinate) 1–4 hours following middle cerebral artery occlusion. Measurements were performed in the cortex of the middle cerebral artery territory. Pre-ischemic control values of EEG intensity and ATP are indicated by the bars (mean ± se).**
Correlation of EEG intensity with cortical blood flow in the middle cerebral artery territory revealed a gradual decrease over the whole range of flow values. A definite threshold for the beginning of EEG changes could not be established because of the great scatter of the individual values (fig. 5). It was of interest to note, however, that even in those animals in which clamping of the MCA was fully compensated for by collateral blood flow, i.e. in which the flow values were in the normal range, a distinct decrease in EEG amplitude in comparison to the pre-ischemic situation was present.

This was in contrast to the changes of cortical water and electrolyte content which were definitely threshold-dependent. Down to a flow rate of 10–15 ml/100 g/min changes were insignificant; below this value, however, there was a steep increase in both water content and the ratio of sodium/potassium content of the cerebral cortex, indicating the development of brain edema and electrolyte disturbances (fig. 5). The degree of brain edema depended on the duration of survival, as described in more detail in the following paper.¹⁴

The threshold for the initiation of brain edema was distinctly higher than that for the breakdown of energy metabolism. Both adenosine triphosphate and creatine phosphate decreased to 50–70% with relatively mild ischemia, but these concentrations remained at this level when flow rates were as low as 5 ml/100 g/min (fig. 5).

The presence of a clearly defined threshold for the induction of electrolyte disturbances and brain edema allowed the distinction between 2 groups of animals. In animals in which the sodium/potassium ratio increased by more than 2 standard deviations, blood flow 15 min after MCA occlusion decreased to 9.4 ± 1.5 ml/100 g/min, and it subsequently further deteriorated (fig. 6). Since progression of ischemia precludes functional recovery, a reduction below this threshold was referred to as "critical ischemia." In the animals in which the sodium/potassium ratio remained normal after MCA occlusion, blood flow

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**Figure 6.** Regional cerebral blood flow following occlusion of the left middle cerebral artery in animals with critical ischemia. Measurements were performed in the grey matter of the middle cerebral artery territory, the marginal zone and the territory of the posterior cerebral artery. Values are means ± se (closed symbols: significantly different from control, p < 0.05).
was 39.1 ± 2.8 ml/100 g/min after 15 min but it returned to normal after one h (fig. 7). Later, it again decreased to about 60% of the control value but it remained above the critical threshold for the induction of brain edema during the whole observation period. Ischemia in these animals, therefore, was referred to as "non-critical." In non-critical ischemia the percent decrease in flow in the territory of the ipsilateral posterior cerebral artery was more pronounced than in the middle cerebral artery territory, indicating the occurrence of an intracerebral steal phenomenon.

The pre-ischemic control value in the non-critical group was higher than in animals with critical reduction in blood flow, which suggests that the density of ischemia, viz. the efficacy of collateral blood supply, greatly depends on the pre-ischemic hemodynamics.

Blood flow in the opposite hemisphere was little affected. In both critical and non-critical ischemia a transient decrease was followed by a slight overshoot above normal, but after 4 hours significant differences were absent. There was, in consequence, no evidence of hemodynamic diaschisis during the early period of cerebral infarction.

Discussion

Transorbital occlusion of the middle cerebral artery is a well-established technique which is generally considered to be one of the least traumatic methods for...
the production of focal cerebral ischemia. This technique has been applied in monkeys, dogs and cats under both acute and chronic conditions, and it has allowed the detailed description of functional and morphological sequelae of brain infarct. An inherent problem with this technique is the great variability of the density of ischemia which depends on the actual location of the vessel clip and on the individual efficacy of collateral vascularization. It is therefore necessary to precisely measure blood flow in the ischemic brain, in order to obtain meaningful results whenever a correlation with the functional or biochemical changes is attempted.

In many of the earlier investigations clearance techniques have been used for this purpose. The usual intra-arterial xenon injection technique has the great disadvantage that the spatial resolution with extracranial detectors is relatively poor, particularly in small animals. Furthermore, the insufficient saturation of the ischemic territory with the indicator results in the so-called look-through phenomenon in which the density of ischemia is underestimated because the detector is activated mainly by the non-ischemic surrounding tissue. Hydrogen clearance techniques with implanted electrodes give regional results, but the number of regions which can be investigated is limited by the number of implanted electrodes. Probably the best available technique for 3-dimensional evaluation of blood flow is the C-antipyrine autoradiography. The major disadvantage of this technique is the fact that only one measurement can be performed. It is, therefore, not possible to establish the kinetics of ischemic changes.

In the present investigation, the intracardiac microsphere injection technique was used to measure blood flow, as a compromise between regional and temporal resolution. When microspheres are labeled with different nuclides, at least 3 and possibly up to 6 flow measurements can be obtained in the same animal. At the end of the experiment, regions of interest of the brain can be dissected, and blood flow can be measured in samples as small as 100 to 150 mg, which is adequate for a precise differentiation between ischemic and non-ischemic regions. Finally, tissue constituents such as brain water, brain electrolytes, or — after freezing the tissue in situ — metabolites can be measured in the same sample, which is also processed for blood flow, thus allowing an exact correlation among these parameters.

There are, however, also methodological problems which have to be considered when the results are evaluated. The microsphere technique gives reliable results from a statistical standpoint only when at least 400 spheres are contained in one tissue sample. This may pose a problem when small tissue samples are taken, because the total number of microspheres injected into the arterial circulation has to be increased accordingly. In the present series of experiments, 3 charges of about 2 million microspheres each, were injected into the left ventricle of the heart which is probably near the upper limit of what can be tolerated without major hemodynamic disturbances. However, an adequate number of microspheres accumulates in the tissue samples only at normal flow rates. When blood flow decreases, the number of trapped microspheres decreases accordingly, and a statistical error is introduced unless the sample size is increased.

Another problem is a change in the water content of the tissue. Since blood flow is generally referred to as wet weight, correction of the flow rate should be made when changes of water content occurred during the course of the experiment. In the present investigation, such a correction was not made in order to facilitate comparison with the results obtained by clearance techniques. From a methodological standpoint, however, it would be more appropriate to refer to blood flow as dry and not wet weight, when microspheres or other volume-related flow measurements are performed.

These technical limitations of the microsphere method are minor in comparison to the advantage of measuring blood flow and tissue constituents in the same brain sample. This is reflected by the fact that it was possible to unequivocally establish a threshold of blood flow at which a clear distinction can be made between progressing (critical) and non-progressing (non-critical) ischemia. Under light barbiturate anesthesia this threshold is between 10 and 15 ml/100 g/min, and it is identified as the threshold for the development of ischemic brain edema. Recent unpublished measurements indicate that the same threshold also applies to other forms of anesthesia, in particular nitrous oxide and halothane.

The relationship between edema and progression of stroke has been realized before. Ischemic water uptake is mainly intracellular, and since swelling affects preferentially perivascular astrocytic processes, a compression of the microcirculation occurs leading to increase in vascular resistance. In the absence of cell swelling, on the other hand, vascular resistance remains low because tissue acidosis develops, and improvement of collateral blood supply tends to increase blood flow in the ischemic brain. It is therefore not surprising that the threshold of edema observed in the present series of experiments is identical with that of morphological damage reported before. The present findings in the cat brain corroborate earlier results by Symon and colleagues in the monkey brain following MCA occlusion. These authors observed a threshold of 8 to 11 ml/100 g/min for the release of extracellular potassium, a threshold of 15 to 20 ml/100 g/min for the decrease of specific gravity of brain tissue (which is an indicator of water content), and a threshold of 6 to 9 ml/100 g/min for the increase in cortical impedance as an indicator of the development of cell hydrops. It has been stressed by these authors, that there are small but significant changes between the thresholds for the disturbance of water and ion homeostasis. In our series, these differences were not evident although, in contrast to these investigations, determinations of water content, electrolyte content and blood flow were made in the same tissue sample; there was no strict correlation.
between the water and electrolyte changes. This could be explained either by different mechanisms of ion and water disturbance, as suggested by Symon et al., or by different kinetics of the equilibration of the ionic and osmotic gradients developing during ischemic brain edema.

In contrast to brain edema, there was no definite flow threshold for the development of EEG changes. EEG power gradually decreased over the whole range of flow rates, indicating that with the increase in density of ischemia an increasing portion of the excitable neuropil is inhibited. The rather unexpected finding of the absence of EEG slowing may be explained by the fact that during the early development of ischemia, suppression rather than modulation of the EEG generator is prominent. It has been observed during tumor development that there is a correlation between vasogenic edema and EEG slowing. Since following middle cerebral artery occlusion vasogenic edema does not develop earlier than after 4 to 6 hours, the absence of EEG slowing may be explained by the absence of vasogenic edema during the early phases of ischemia.

Delayed post-ischemic phenomena may also be responsible for the diastolic impairment of hemodynamic and metabolic functions, as described by several authors before. In the present investigation the phenomenon of diachisis was not observed. Neither blood flow nor EEG activity changed significantly during the initial 4 hours of ischemia. This suggests that diachisis associated with regional ischemia is not a purely functional phenomenon in the sense of von Monakow but must be related to some kind of maturating process which seems to develop slowly in the course of the ischemic process.

A distinct relationship between density of ischemia and energy-rich compounds did not exist. There was a significant decrease in ATP content at relatively high flow rates which were far above the threshold for disturbances of ion homeostasis, i.e. disturbances of the ion exchange pumps. On the other hand, even at flow rates distinctly below this threshold, substantial amounts of ATP and creatine phosphate were present. This is in agreement with earlier reports, and indicates that changes in ion concentration gradients are not solely dependent on the state of energy metabolites. The relatively high ATP level over a broad range of flow rates may also be due to the fact that with increasing density of ischemia the gradual depression of EEG activity leads to decreasing energy requirements (cortical shutdown). Energy production, therefore, might be lower than can be concluded from the high levels of energy-rich phosphates.

This does not preclude, however, that the critical phenomenon for the development of an infarct following middle cerebral artery occlusion is the development of brain edema and not energy failure.

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