Brain pH, Cerebral Blood Flow, and NADH Fluorescence During Severe Incomplete Global Ischemia in Rabbits

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Background and Purpose: The aim of this experiment was to study the serial changes in brain intracellular pH, cerebral blood flow, and the oxidation/reduction level of intramitochondrial nicotinamide adenine dinucleotide fluorescence across the cortical surface during severe incomplete global ischemia.

Methods: Reduced nicotinamide adenine dinucleotide fluorescence and brain intracellular pH using the pH-sensitive indicator umbelliferone were measured with in vivo panoramic fluorescence imaging of the cortical surface. Cerebral blood flow was measured with the clearance of both umbelliferone and xenon-133. Fifteen minutes of severe incomplete global ischemia was produced by temporary occlusion of the innominate, left carotid, and subclavian arteries in five fasted New Zealand White rabbits.

Results: Baseline brain intracellular pH was homogeneous over the exposed cortex, measuring 7.00±0.02, while cerebral blood flow was 48.0±2.6 ml/100 g/min. During 15 minutes of ischemia, cerebral blood flow measured 6.3±1.8 ml/100 g/min and brain pH declined to 6.61±0.02 (p<0.005); in addition, there were acidic foci with pH measuring 6.40±0.10. During reperfusion, there was an initial normalization of brain intracellular pH without an alkaline shift followed by a recurrent cortical acidosis of pH 6.88±0.06. There was a heterogeneous pattern of fluorescence that increased significantly following 60 minutes of reperfusion, coinciding with a postischemic hyperperfusion. The hyperperfusion was a uniform reduction in cerebral blood flow over the brain's surface, with reductions of 42.5% and 44.2% at 30 and 45 minutes, respectively.

Conclusions: During incomplete global ischemia there is a heterogeneous pattern of brain intracellular pH and reduced nicotinamide adenine dinucleotide changes that do not correlate with changes in cortical blood flow. The acidic foci that were approximately 0.2 pH units more acidic than the surrounding cortex may be the result of continued glucose delivery under anaerobic conditions. The degree of reduced nicotinamide adenine dinucleotide fluorescence suggests that the cortex is most vulnerable to metabolic failure after 60 minutes of reperfusion following severe incomplete global ischemia. The heterogeneous pattern of brain intracellular pH and reduced nicotinamide adenine dinucleotide changes suggest that there may be a selective vulnerability of cortical tissue to an ischemic challenge. (Stroke 1993;24:435–443)

Key Words • brain intracellular pH • cerebral blood flow • cerebral ischemia • rabbits

The metabolic and blood flow changes that occur during both complete global and focal cerebral ischemia have been well characterized. From a metabolic perspective, some of the features common to both include depletion of adenosine triphosphate as oxygen availability is exhausted, lactic acidosis, arrest of protein synthesis, and a release of arachidonic and free fatty acids.1–5 One of the potential differences between the two is the degree of brain acidosis based on the supposition that during incomplete ischemia glucose delivery under anaerobic conditions may increase lactic acidosis.6–8

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The differences in blood flow between global and focal cerebral ischemia center on the fact that by definition during focal ischemia there is the potential for collateral blood flow. Although this residual blood flow may lead to reversibility of ischemic injury, it may have the opposite effect of increasing lactic acidosis. A second difference between the two is the pattern of blood flow changes following flow restoration. In most models of complete global ischemia, there is hyperperfusion followed by a period of hyperperfusion.9–12 Alternatively, in focal ischemia the blood flow changes during reperfusion, such as the absence of postischemic hyperperfusion,13–15 are variable.

Clinically, complete global ischemia occurs during cardiac arrest followed by a complete arrest of cerebral blood flow (CBF). The more common focal ischemia occurs during intracranial or extracranial vascular embolism or thrombosis. The least studied situation, incomplete global ischemia, occurs during cardiac arrhythmias such as...
as ventricular fibrillation or severe hypotensive episodes that might transpire during septic shock. The goal of this experiment was to characterize some of the metabolic and blood flow changes that occur across the brain’s surface during incomplete global ischemia by using in vivo fluorescent panoramic imaging.16 Recently it has been shown that during global ischemia there is a variable pattern of brain intracellular pH (pHi) changes, supporting the concepts of brain pH compartmentalization and differential pH responses to an ischemic insult.17 It had been previously demonstrated that during incomplete global ischemia there is a heterogeneous pattern of reduced nicotinamide adenine dinucleotide (NADH) fluorescence that occurs in a columnar pattern and has been attributed to microcirculatory failure.18 Accordingly, one specific aim of this experiment was to use the spatial resolution of the umbrelliférence imaging system to test the hypothesis that the potential heterogeneous changes in cortical brain pH and NADH fluorescence during incomplete global ischemia would correlate with focal changes in cortical blood flow.

Materials and Methods

Animal Preparation

Five overnight-fasted New Zealand White rabbits weighing 3.5–4.5 kg were induced, operated, and studied under 4.0%, 2.5%, and 1.5% halothane anesthesia, respectively. A tracheostomy was performed, and the animals were placed on a respirator (Harvard Apparatus, South Natick, Mass.). The animals were given 0.15 mg/kg pancuronium bromide (Organon Inc., West Orange, N.J.) to abolish respiratory efforts. The animals were kept normoxic and normocarbic during the surgical procedure and throughout the experimental procedure with supplemental CO2 and O2.

Catheters were inserted into the right femoral artery and vein for monitoring blood pressure, sampling arterial blood gases, and administering drugs. A PE-50 catheter was inserted into the right lingual artery so that the catheter tip was located at the origin of the external carotid artery for the retrograde delivery of the indicator umbrelliférence and of xenon-133 into the internal carotid artery.

The arch of the aorta was exposed through the anterior mediastinum after excision of the left first rib to gain access to the innominate, left carotid, and left subclavian arteries in preparation for vessel occlusion. The skin, subcutaneous tissue, and muscle were excised over the right supraorbital ridge and parietal area. A craniectomy was performed using a high-speed air drill (Hall Surgical, Division of Zimmer, Santa Barbara, Calif.) with the aid of an operating microscope (Olympus, Tokyo, Japan). The majority of the frontal and parietal cortex was exposed for imaging. The dura was removed and carefully cauterized at the margins of the craniectomy then covered with thermoplastic film (Saran Wrap) to prevent surface oxygenation and to keep the brain moist. Blood loss for the surgical preparation did not exceed 5 ml.

Following surgical preparation, the rabbit was removed from the operating table and placed on an intravital-type microscope stand. The microscope was focused on an area centered about the suprasylvian gyrus with 1.5 cm2 of cortex imaged for pH, CBF, and NAD/NADH measurements. Arterial blood pressure was measured by a strain gauge (Statham, Oxnard, Calif.) attached to the femoral artery catheter and recorded on a polygraph (model 78, Grass Instrument Co., Quincy, Mass.). The animals were kept normothermic by the use of a heating blanket (K-Pad, Gorman-Rupp, Bellville, Ohio), and body temperature was monitored with a rectal digital thermometer. Pco2, Paco2, and arterial pH were measured using a blood gas analyzer (model PHM-73, London Radiometer, Copenhagen, Denmark).

After control values were measured, severe incomplete global cerebral ischemia was produced by occlusion of the innominate, left common carotid, and left subclavian arteries at the origin of the vessels from the aortic arch using aneurysm clips. With vessel occlusion, severe elevations in mean arterial blood pressure (MAP) were controlled by withdrawing arterial blood into a heparinized syringe. After 15 minutes the clips were removed and the previously withdrawn arterial blood was reinfused at a rate that maintained MAP at control values. No pressor agents were administered during the 3-hour reperfusion period.

In Vivo Video Fluorescent Instrumentation

The instrumentation was designed to perform serial panoramic imaging of cortical brain pH, and focal cortical blood flow with umbrelliférence fluorescence.16 The optical characteristics were such that the majority of the entire hemisphere could be studied simultaneously through a large craniectomy. The system described briefly below consisted of a honeycomb optical bench onto which was mounted a light source, an intravital-type microscope, an animal stage, and a camera assembly.

Illumination for the excitation of umbrelliférence was provided by a modular system consisting of a 500-W xenon arc light source powered by a DC-regulated power supply and a filter changer used for selecting filters of the appropriate wavelength for excitation under computer control. The interference filters selected for excitation were 340±5 and 370±5 nm. A stereoscopic zoom microscope body (model SMZ-10, Nikon, Inc., Melville, N.Y.) with a Pleo-type illuminator for the excitation was attached to a vertical stand mounted on an optical bench. This microscope has a working distance of 50 mm with a field of view of 15 mm. The animal platform and head micromanipulator were mounted on the optical bench below and lateral to the microscope unit. An SMZ-10 trinocular body (Nikon) consisting of a beam-splitting arrangement whereby either 100% of the image was forwarded to the observer for visual inspection or to an image-intensified camera was attached to the top of the microscope body. The image-intensified camera used a single-stage microchannel plate intensifier with relay optics for coupling to a charge-coupled device chip camera. An SX-70 Land camera (Polaroid Corp., Cambridge, Mass.) on the top of the trinocular body was used for photographing from either the left or right eyepiece. Between the closed-circuit television adapter attached to the trinocular body tube and the camera assembly a 450±5 nm interference filter was inserted. This was the selected wavelength for recording the umbrelliférence fluorescent emission.

The fluorescent video image was processed by an ITI-150 series image analyzer and a Personal Iris 4D/35
The computer was interfaced to the image analyzer via a VME bus. The processed image was displayed on a color video monitor (model PVM-1271Q, Sony, Tokyo, Japan). Processed images were displayed along with the calibration bars in a 100 x 100 mm format on a color video printer (model CP100U, Mitsubishi, Piscataway, N.J.). Custom software (G.W. Hannaway and Assoc., Boulder, Colo.) was written to acquire, process, display, and store images on magnetic tape. Paired images, one for each excited wavelength, were acquired at 5-second intervals for 180 seconds. Acquired images were then corrected for background NADH fluorescence prior to processing. NADH fluorescence images were stored for later analysis of mitochondrial function. Images were acquired using 16-frame averaging to increase the signal-to-noise ratio. The reduction of pixel noise by averaging over n frames was reduced by a factor of \( \sqrt{n} \). The images from the 340 nm excitation were processed to compute regional cerebral blood flow measured using umbelliferone fluorescence (rCBF-umbelliferone) using the 1-minute initial slope term. The rCBF-umbelliferone image was then displayed and stored on tape for final analysis. For the processing of the pHi image, paired images from the 340 and 370 nm excitations were ratioed and the resultant pHi image was then displayed and stored on tape for final analysis.

**Characteristics of Umbelliferone**

Umbelliferone is the generic term for 7-hydroxy-coumarin. It is nontoxic, fat-soluble, and freely diffusable across the blood–brain barrier as an uncharged molecule. The pKₐ of the indicator is 7.5 with an overall measured range of pH 5.2–8.0, the fluorescence varying linearly from pH 6.6 to 8.0. Umbelliferone was prepared for injection by dissolving 0.2 g of indicator in 200 ml of 5% glucose-saline at 90°C for 30 minutes. The solution was then passed through a 0.22-μm mesh filter prior to injection. The volume of injectate was 1.5 ml in this study.

This pH-sensitive indicator has two fluorophors: anion and isosbestic. The anionic and isosbestic forms are excited at 370 and 340 nm, respectively, and have a common emission at 450 nm. The fluorescence of the anion varies directly with pH, while fluorescence of the isosbestic form varies directly only with indicator concentration. Therefore, it is possible to create a nomogram from the ratio of 340 nm and 370 nm excitation to determine brain pH. Artifacts relating to proteins, cations, phosphorylation, and oxygen in a fat-soluble environment have been previously discussed in detail. The indicator concentration normally found in vivo is in the range of 10⁻⁷ to 10⁻⁶ M, which is in the linear range of indicator concentration versus fluorescence intensity, similar to that of 4-methylumbelliferone. This effect allows the ratio technique to correct for changes in indicator concentration. The excitation of the dye was kept below 800 μW/cm² to reduce the effects of photobleaching. We set a ratio versus fluorescence curve based on six different pH buffer values and calculate a fitted n°-degree polynomial curve to set up the calibration bar for the video fluorometer display.

Because umbelliferone is an uncharged molecule below the pKₐ of 7.5, it rapidly equilibrates across cell membranes and is distributed through the cytoplasm. The term “pHi” is accepted and widely used throughout the literature. However, pH has different ionic gradients within the various cellular compartments and therefore is considered nonhomogeneous. Siesjö et al. proposed the term “equivalent pH” to denote a homogeneous compartment that has an HCO₃⁻ concentration and a PaCO₂ equal to the corresponding mean tissue values. In vivo calibration by the use of weak acids or bases cannot be justified in this study in that it would compromise the integrity of the preparation. In a previous study in which addition of either HCl or NaOH to the systemic circulation in cats had corresponding effects on the brain extracellular pH, brain pH was not altered as measured by umbelliferone. In this study, we are not mapping the pH distribution; therefore, the measurements can reflect an “equivalent pH.”

Umbelliferone has been shown to be a reliable indicator of CBF. Anderson et al. have analyzed the different techniques of measuring CBF. They found that a distinction can be made between CBF as measured by radiolabeled compounds and rCBF or focal CBF as measured by umbelliferone. By definition rCBF as measured by radiolabeled compounds is that in areas that contain major vessels as well as capillaries and arterioles. The rCBF or focal CBF as measured by umbelliferone is defined as that in areas that are relatively avascular and contain only capillaries. The imaging system allows the measurement of rCBF by setting the number of pixels to cover specified areas.

**Statistical Analysis**

Because of anatomic variation of the microvasculature from animal to animal, single points along an x,y coordinate cannot be averaged frame by frame from different animals at the same time. Therefore, regional pHi, rCBF-umbelliferone, and NADH fluorescence were measured in areas devoid of major vessels. Measurements were made over these relatively avascular areas by averaging 61,000 pixels (1,235,250 μm²), and the mean and standard error were tabulated. Statistical significance was determined by Student’s t test of paired and unpaired data.

**Xenon-133 Regional Cerebral Blood Flow Measurements**

Xenon-133 rCBF was measured using a cadmium telluride detector (RMD, Watertown, Mass.) system. The detector, which was a 2 mm² solid-state device housed in a collimator, had a measurement volume of 0.50 mm³. The window discriminator was set at 76 keV and 200 keV to minimize Compton scatter. The resultant counts were recorded on a strip chart recorder. The 1-minute initial slope index was used to calculate rCBF. The partition coefficient (λ) used for xenon-133 was 0.63.

**Results**

**Systemic Parameters and Video Acquisition**

There were no significant differences between rabbits studied in the measurements of PaCO₂, PaO₂, MABP, body temperature, glucose and lactate concentrations, and hematocrit (Table 1). However, there was a significant (p<0.05) decrease in arterial pH during the first 75 minutes after restoration of blood flow due to buildup of lactate in the tissues during ischemia. This acidosis normalized over the remainder of the study.
TABLE 1. Systemic Measurements in Rabbits

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PCO₂ (torr)</th>
<th>pH</th>
<th>PO₂ (torr)</th>
<th>MABP (mm Hg)</th>
<th>Temperature (°C)</th>
<th>Glucose (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>−15</td>
<td>40.7±1.9</td>
<td>7.29±0.01</td>
<td>351.6±31.9</td>
<td>84.6±2.0</td>
<td>39.3±0.33</td>
<td>218.6±19.9</td>
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<td>0</td>
<td>40.3±1.6</td>
<td>7.32±0.02</td>
<td>346.8±12.2</td>
<td>85.6±2.6</td>
<td>39.3±0.35</td>
<td>193.4±17.8</td>
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<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>40.0±3.6</td>
<td>7.21±0.06</td>
<td>303.2±47.2</td>
<td>89.4±1.7</td>
<td>38.9±0.26</td>
<td>287.6±75.1</td>
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<td>Reflow</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>40.4±1.5</td>
<td>7.17±0.04*</td>
<td>334.0±50.4</td>
<td>89.4±4.7</td>
<td>38.7±0.18</td>
<td>233.2±34.8</td>
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<td>30</td>
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<td>7.18±0.03*</td>
<td>325.0±39.9</td>
<td>83.0±4.1</td>
<td>38.7±0.20</td>
<td>257.6±36.6</td>
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<tr>
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<td>7.18±0.04*</td>
<td>317.0±29.2</td>
<td>77.4±3.7</td>
<td>38.7±0.19</td>
<td>250.0±46.8</td>
</tr>
<tr>
<td>60</td>
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<td>7.17±0.05*</td>
<td>298.6±32.4</td>
<td>78.4±3.2</td>
<td>38.7±0.19</td>
<td>254.4±46.0</td>
</tr>
<tr>
<td>75</td>
<td>44.3±1.9</td>
<td>7.19±0.05*</td>
<td>332.2±24.0</td>
<td>74.6±5.6</td>
<td>38.7±0.18</td>
<td>253.2±45.9</td>
</tr>
<tr>
<td>90</td>
<td>40.6±1.2</td>
<td>7.22±0.04</td>
<td>339.0±21.5</td>
<td>76.8±4.3</td>
<td>38.7±0.21</td>
<td>254.4±41.1</td>
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<td>120</td>
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<td>7.21±0.04</td>
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<td>77.0±4.0</td>
<td>38.5±0.15</td>
<td>246.6±34.2</td>
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<td>150</td>
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<td>7.22±0.04</td>
<td>307.0±40.2</td>
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<td>38.5±0.15</td>
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<td>7.23±0.04</td>
<td>263.0±45.8</td>
<td>75.4±3.8</td>
<td>38.4±0.21</td>
<td>237.6±25.8</td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure. Values are mean±SEM. *p<0.05 different from preischemic control values.

(Table 1). Depicted in Figure 1 are video pictures of a typical experiment.

Brain pHₐ

Preocclusion and ischemia. Baseline brain pHₐ was uniform over the entire exposed cortex, measuring 7.00±0.02. After 15 minutes of severe incomplete global ischemia, pHₐ fell significantly (p<0.005) to 6.61±0.02 (Figure 2), with small patchy areas of acidotic foci. These acidotic foci had a pHₐ of 6.40±0.10.

Postocclusion. Brain pHₐ showed a delayed normalization, with an initial value of 6.85±0.06 at 15 minutes, still significantly different from the preischemic control value (p<0.025). At 30 minutes pHₐ rose to 6.92±0.04 but then slowly declined to 6.88±0.06 after 3 hours of reperfusion (p<0.05, Figure 2).

Regional Cerebral Blood Flow by Umbelliferone

Preocclusion and ischemia. Baseline rCBF-umbelliferone measured 48.0±2.6 ml/100 g/min. Fifteen minutes after occlusion, rCBF-umbelliferone fell significantly (p<0.005) to 6.3±1.8 ml/100 g/min (Figure 3).

Postocclusion. Fifteen minutes after blood flow restoration, rCBF-umbelliferone increased to preischemic control levels without significant hyperemia. At the 30- and 45-minute intervals rCBF-umbelliferone declined to 27.6±4.6 (p<0.01) and 26.8±5.5 (p<0.025) ml/100 g/min, respectively, reflecting postischemic hypoperfusion. This hypoperfusion was uniform across the brain's surface. Although rCBF-umbelliferone then remained relatively constant (p<0.05) for the next 75 minutes, there was a trend toward a decline in rCBF-umbelliferone at 3 hours (Figure 3).

Regional Cerebral Blood Flow by Xenon-133

Preocclusion and ischemia. Baseline 133Xe-rCBF was 46.5±4.9 ml/100 g/min. Fifteen minutes after occlusion, 133Xe-rCBF fell significantly (p<0.005) to 9.9±2.6 ml/100 g/min (Figure 4).

Postocclusion. Fifteen minutes after blood flow restoration, 133Xe-rCBF rose to control levels without hyperemia. At the 30- and 45-minute intervals 133Xe-rCBF declined to 40.8±4.4 (difference not significant) and 28.2±3.9 (p<0.005) ml/100 g/min, respectively, then remained relatively constant (p<0.005) for the next 75 minutes (Figure 4).

NADH Fluorescence

Preocclusion and ischemia. The control NADH fluorescence gray scale level was 15.5±1.45 and increased to 46.6±3.5 at 20 minutes after death, an increase of 300%. Fifteen minutes after occlusion NADH fluorescence increased by 150±11.0% (p<0.01, Figure 5). NADH fluorescence showed a heterogeneous distribution over the cortical surface.

Postocclusion. Fifteen minutes after blood flow restoration, NADH fluorescence demonstrated an apparent undershoot by decreasing to 82±10% of control (difference not significant). During the next 60 minutes NADH fluorescence increased steadily to 208±27% (p<0.05), coinciding with the postischemic hyperperfusion. After this period, NADH fluorescence declined for the remainder of the experiment but remained significantly greater than the preischemic control values (p<0.05, Figure 5).

Discussion

Due to the vascular anatomy, with a patent circle of Willis and a well-defined anastomosis between the vertebral and anterior spinal arteries, the New Zealand White rabbit is better suited to be a model of incomplete than complete global ischemia. In addition to sectioning of the basilar artery and clamping of the common carotid arteries, the rabbit model for global ischemia described by Ames et al requires constriction of the neck with a pneumatic cuff inflated to 350 mm Hg during the ischemic period. This arrangement is not compatible with the use of a lingual artery catheter for the intracarotid injection of umbelliferone and xenon-133 used in this experiment. Some investigators have used a technique of graded carotid occlusion to produce incomplete global ischemia. This model, which in-
volves exposure of the vertebral arteries at C-1 and coagulation with electrocautery long enough to extend to the junction with the anterior spinal artery, proved very successful for the study of 40 minutes of reperfusion. In our model, the vertebral artery circulation was interrupted by clipping of the innominate and left subclavian arteries. This method, rather than a more distal occlusion using electrocautery, was performed to avoid the possibility of distal propagation of thrombus into the vertebral artery, which might result in brain stem ischemia and associated hemodynamic instability. A patent anastomosis between the anterior spinal and vertebral arteries provides a route for cerebral perfusion during the period of cerebral ischemia, which results in a model of incomplete ischemia.

The method of blood flow measurement differs from that in some other models of incomplete and complete global ischemia in that CBF was measured in that ischemic period. Other studies have injected a diffusible indicator such as xenon-133 into the cerebral circulation immediately before vessel occlusion and used this as a measure of CBF during ischemia. This technique assumes that following occlusion a collateral circulation does not develop.

Cerebral Blood Flow

During vessel occlusion CBF flow fell to 12% of the preischemic control value, most likely representing blood flow from the collateral circulation through the anterior spinal arteries. After blood flow restoration at 15 minutes, rCBF-umbellifere and 133Xe-rCBF increased to control levels and then declined, with a reduction of approximately 50% by 30–45 minutes. The initial hyperemia described by other authors occurring soon after reflow following incomplete and complete global ischemia was not detected in our study.10,11,30 This may be due to the fact that in the experimental paradigm blood pressure was controlled by a monitored reinfusion of blood, which prevented postsischemic hypertension. It is also possible that this may have been related to the time of the first measurement of rCBF-umbellifere and 133Xe-rCBF, which was performed 15 minutes after reflow was established and might have missed an early postsischemic hyperperfusion. Kågström et al11 showed increases of >350% in CBF measured 5
minutes after reperfusion in fasted and fed rats. In that study, the authors also showed that the decline in CBF was greater in the fed than in the fasted group during hypoperfusion. Ginsberg et al. showed that the incidence of hyperemia is greater with 30 minutes than with 15 minutes of ischemia in cats. Allen et al. observed 15.5% and 6.5% increases in CBF after 5 minutes of reperfusion as a result of 15 and 30 minutes of complete ischemia, respectively, in gerbils. The delayed hypoperfusion observed in this experiment was similar to that reported by other investigators after incomplete and complete global ischemia. A novel finding in this experiment is that the hypoperfusion is an ongoing uniform reduction in CBF across the cortical surface that is unrelated to surface vasculature.

**Brain pHi Measurements**

Incomplete global ischemia is considered to be more deleterious than complete global ischemia due to the delivery of glucose under anaerobic conditions, which results in excess lactic acid production. Standard practice with techniques including [14C]5,5-dimethyl-2,4-pyrrolidone (DMO), phosphorus-31 nuclear magnetic resonance (31P-NMR), neutral red, umbelliferone, and CO₂ considers measurements of pHi to be within the cytoplasm as a heterogeneous summation of all pHi pools. Following 15 minutes of incomplete global ischemia, pHi fell to 6.61 ± 0.02. This average pHi approximates that reported after similar periods of complete global ischemia. However, although the overall pattern of acidosis was homogeneous, there were acidic foci...
with pH, measuring 6.40±0.10. These small acidotic foci may represent the predicted effects of glucose delivery during anaerobic metabolism.45,7 This heterogeneity of the brain pH, response is similar to that reported by Griffith et al17 for global ischemia. They used neutral red and 31P-NMR to detect areas of acidosis within a pH, of 6.0–7.2 (mean, 6.7). Using 31P-NMR in normoglycemic cats, Chopp et al17 found the mean pH, to be 6.1. However, this value was representative of a larger volume of tissue than in this current experiment.

Although the overall brain pH, was not significantly different from the control value after 30 minutes of reperfusion, pH, at 3 hours declined significantly to 6.88±0.06 (p<0.05). Using bilateral occlusion in gerbils, Allen et al13 found that during ischemia pH, decreased to 6.56 after 15 minutes and had fully recovered by 20 minutes after reperfusion. While the initial rise in pH, following incomplete global ischemia indicates that mechanisms for acid–base homeostasis had been initiated, the failure to detect an alkaline shift to above baseline control values and the trend toward recurrent intracellular acidosis suggest that during reperfusion mechanisms of pH regulation were operating below the level following global ischemia.36 These differences in pH, regulation suggest that a greater degree of cell injury occurred following incomplete than complete global ischemia.

**Measurement of NADH**

The measurement of in vivo NADH fluorescence was used as an indicator of cellular metabolism in this study. The NADH fraction measured was intramitochondrial, with very little or none originating from the cytoplasm. It is of importance that the primary source of artifact in

![Figure 4](image-url)  
**FIGURE 4.** Graph of mean±SEM regional cerebral blood flow (rCBF) as measured by xenon-133 before, during, and after severe incomplete global ischemia in rabbits. ***p<0.005 different from control.

![Figure 5](image-url)  
**FIGURE 5.** Graph of mean±SEM alterations in reduced nicotinamide adenine dinucleotide (NADH) fluorescence expressed as percentage of control before, during, and after severe incomplete global ischemia in rabbits. **p<0.01, *p<0.05 different from 100%.
these measurements is hemoglobin interference. Hemoglobin is a strong absorber of the exciting light, thereby reducing the emitted fluorescence. If there is a large amount of blood in the field of view, then NADH fluorescence as a measure of metabolism will be erroneous. The imaging system allows us to analyze only those areas that are devoid of the larger surface conducting vessels, thereby reducing the amount of hemoglobin error.16

The level of NADH fluorescence seen during occlusion represented limited oxygen availability to tissue mitochondria. Upon blood flow restoration, there was a very fast oxidation of NADH to a point of undershoot from the original baseline value. This is a possible result of hyperoxygenation during the initial hyperemia, although CBF was not markedly elevated over the baseline value. There was a delayed but greater rise in NADH fluorescence 60 minutes after blood flow restoration than during ischemia, with eventual normalization to baseline levels 45 minutes later. This might have been due to the hyperoxygenation of venous blood37 with limited oxygen utilization due to increased anaerobic metabolism. Urbanics et al38 found that in middle cerebral artery occlusion in cats, regions of severe ischemia showed increased levels of NADH fluorescence whereas areas of mild ischemia showed reduced levels of NADH fluorescence.

Although this study showed significant heterogeneity in NADH fluorescence across the cerebral cortex, we did not visualize a pattern as described by other investigators.39 There was no clear correlation between the heterogeneous pattern of brain pH1 changes and that of NADH fluorescence. Furthermore, there was no evidence that these heterogeneous changes were due to a vascular phenomenon since the changes in cortical blood flow were relatively uniform across the brain’s surface. Finally, the dissimilarity between brain pH1 and NADH fluorescence changes suggest that there is a differential effect between cytoplasmic and mitochondrial metabolism during and after incomplete global ischemia.

Mechanisms of Cell Injury

A possible explanation why incomplete global ischemia is more deleterious than complete global ischemia may be related to the severity of brain acidosis and the rate of development of postischemic hyperperfusion. In the former we observed hyperperfusion to be established at approximately 30 minutes, whereas in global ischemia hyperperfusion, beginning 15–30 minutes after ischemia, becomes fully established 45–60 minutes after reflow.32–34 This may be due to a more severe brain acidosis during incomplete global ischemia from continued glucose delivery under anaerobic conditions, resulting in glial edema and vascular capillary bed compression.40–43 The faster the development of hyperperfusion with concomitant reduction in glucose and oxygen delivery, the greater the ischemic stress placed on the recovering brain. This postulate is supported by the increase in NADH fluorescence following the development of hyperperfusion that presumably was due to a decrease in tissue O2 availability. The maximum level of NADH fluorescence measured during reperfusion, which was greater than that seen after 15 minutes of occlusion, may reflect the degree of stress imposed on the cell by the early development of hyperperfusion.5 Based on the reduction of the NAD/NADH couple, the cortical energy state may be most deficient 60–90 minutes following reperfusion and possibly more susceptible to metabolic stress.

The apparent heterogeneity of brain pH1 and NADH responses during and after the ischemic injury merits reemphasis. It has been previously suggested that there is compartmentalization of brain pH during an ischemic injury.37 The spatial resolution of this umbiliferalere instrumentation demonstrated that this differential response of brain pH1 was not due to local changes in cortical blood flow. Although it has been demonstrated that astrocytes can develop extreme acidosis during brain injury, it is unlikely that this is the explanation of the acidic foci observed in this current experiment.44 The heterogeneous pattern of brain pH1 and NADH fluorescence suggests that there may exist a selective vulnerability of cortical tissue to an ischemic insult.

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References

Application of modern methodology has begun to change our understanding of the role of intracellular pH (pHi) in physiology and pathology in the central nervous system. In the past, it was simplistically thought that under normal physiological conditions, pHi was constant and homogeneous and that insults such as ischemia resulted in acidification to the detriment of the tissue. Today, the major quantitative techniques used for determining pHi are $^{31}P$-labeled nuclear magnetic resonance spectroscopy and ion-selective-H+ microelectrodes. Observations made with these techniques have suggested that there is significant temporal and spatial heterogeneity of pHi, even for normal function, but these methods, in isolation, are inadequate for the purpose of addressing the fundamental issues of brain tissue acid-base balance. It is becoming clear that to understand the mechanisms underlying any proposed expanded role for protons in the brain it will be necessary to analyze pHi under physiological and pathological situations using multiple, complementary methods, especially those that preserve spatial and temporal distinctions.

The article by Tomlinson et al reports the application of an optical imaging method that permits quantitative determination of pHi in situ in rabbit brain under experimental conditions that allow the simultaneous measurement of other cerebral metabolic and vascular variables. Their data lead to the speculation that heterogeneous cerebral acidosis within the first critical hour of incomplete global ischemia is correlated with selective neuronal vulnerability. Studies such as these could help to provide definitive evidence regarding the important yet unresolved issue of whether and how intracellular acidosis affects survival of neurons after cerebral ischemia.

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Brain pHi, cerebral blood flow, and NADH fluorescence during severe incomplete global ischemia in rabbits.
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