Effects of Glucose and PaO₂ Modulation on Cortical Intracellular Acidosis, NADH Redox State, and Infarction in the Ischemic Penumbra

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Background and Purpose—During focal cerebral ischemia, the ischemic penumbra or border-zone regions of moderate cortical blood flow reductions have a heterogeneous development of intracellular cortical acidosis. This experiment tested the hypotheses that (1) this acidosis is secondary to glucose utilization and (2) this intracellular acidosis leads to recruitment of potentially salvageable tissue into infarction.

Methods—Brain pHᵢ, regional cortical blood flow, and NADH redox state were measured by in vivo fluorescent imaging, and infarct volume was assessed by triphenyltetrazolium chloride histology. Thirty fasted rabbits divided into 6 groups of 5 each were subjected to 4 hours of permanent focal ischemia in the presence of hypoglycemia (≤2.8 mmol/L), moderate hyperglycemia (≈11 mmol/L), and severe hyperglycemia (>28 mmol/L) under either normoxia or moderate hypoxia (PaO₂ ≈50 mm Hg).

Results—Preischemic hyperglycemia led to a more pronounced intracellular acidosis and retardation of NADH regeneration than in the hypoglycemia groups under both normoxia and moderate hypoxia in the ischemic penumbra. For example, 4 hours after ischemia, brain pHᵢ in the severe hyperglycemia/normoxia group measured 6.46, compared with 6.84 in the hypoglycemia/normoxia group (P<0.01), and NADH fluorescence measured 173% compared with 114%. Infarct volume in the severe hyperglycemia/normoxia group measured 35.1±6.9% of total hemispheric volume, compared with 13.5±4.2% in the hypoglycemia/normoxia group (P<0.01).

Conclusions—Hyperglycemia significantly worsened both cortical intracellular brain acidosis and mitochondrial function in the ischemic penumbra. This supports the hypothesis that the evolution of acidosis in the ischemic penumbra is related to glucose utilization. Furthermore, the observation that hypoglycemia significantly decreased infarct size supports the postulate that cortical acidosis leads to recruitment of ischemic penumbra into infarction.

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Key Words: acidosis ■ redox, NADH ■ cerebral infarction ■ glucose ■ cerebral ischemia, focal ■ rabbits

When the cortical surface is imaged during focal cerebral ischemia, there is variation in the development of intracellular acidosis.1–3 In regions of severe cerebral blood flow reductions or evolving infarction, the decline in intracellular pH (pHi) is relatively uniform and profound. In the border-zone region of moderate ischemia surrounding the evolving infarction, however, there is a heterogeneous distribution of intracellular acidosis. The significance of this intracellular acidosis in the ischemic penumbra is unclear.

The mechanisms by which acidosis contributes to neuronal injury may include facilitating free radical formation, activating pH-dependent endonucleases with DNA fragmentation, or altering intracellular Ca²⁺ regulation.4–10 Despite the probable deleterious effects of acidosis, the effects of hyperglycemia in focal cerebral ischemia remain controversial.11–14 For example, it has been published that hyperglycemia reduces, does not alter, or increases damage after transient focal cerebral ischemia.11–14 It has also been published that hypoglycemia decreases the degree of pan-necrosis.13 It is possible that varying effects of hyperglycemia may be due to differences in collateral blood flow and therefore the degree of lactic acid production.13 The effects of hyperglycemia may also be dependent on reperfusion.16 Adding to the controversy are in vitro observations that acidosis may ameliorate neuronal injury caused by glutamate and anoxia.17,18

This experiment tested the hypotheses that (1) the development of cortical intracellular acidosis in the ischemic penumbra is a result of glucose utilization and (2) this acidosis leads to recruitment of potentially salvageable tissue into infarction. To test this hypothesis, in vivo fluorescence imaging was used to measure brain pHᵢ, regional cortical blood flow (rCBF), and the NADH redox state in the New

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Zealand White rabbit. Histological assessment of infarction was performed in the acute setting with tetrazolium staining.

Materials and Methods

Animal Preparation

After review and approval by the Institutional Animal Care and Use Committee, 30 New Zealand White rabbits weighing between 3.5 and 4.5 kg that had been fasted overnight were induced with sodium pentothal 40 mg/kg, operated on under 2.5% halothane anesthesia, and studied under 1.5% halothane anesthesia, respectively. A tracheotomy was performed, and the animals were placed on a Harvard respirator (Harvard Apparatus). The animals were given 0.15 mg/kg pancuronium bromide (Pavulon, Pharmacia & Upjohn Inc) to prevent surface oxygenation and to keep the brain moist. Blood loss for the surgical preparation did not exceed 5 mL.

After the surgical preparation, the animal was moved from the operating table and placed on an intravital-type microscope stand. One of the major areas of interest was focused on an area centered around the suprathyman gyrus, with 1.5 cm² of cortex imaged for brain pH, rCBF, and NADH fluorescence measurements. Arterial blood pressure was measured by a Statham strain gauge attached to the femoral artery catheter and recorded on a Grass model 78 polygraph. The animals were kept normothermic (38.8±0.5°C) by use of a heating blanket (K-Pad, Gorman-Rupp), and core body temperature was monitored with a rectal digital thermometer. PaO₂, PaCO₂, and pH (arterial) measurements were performed on a London Radiometer blood gas analyzer (PHM-73). Serum glucose and lactate were measured on a YSI 2300 Stat glucose/lactate analyzer. Brain temperature was not measured in this study; however, it was maintained at core body temperature with an air heater (Air-Therm, WPI). This device blows temperature-regulated air at a low constant velocity toward the surface of the brain. The temperature at the surface of the brain is regulated to within 0.2°C of core body temperature.

Severe focal cerebral ischemia of the parietal cortex was induced by cautery of the right middle cerebral artery (MCA). To reduce the potential for collateral blood flow, the right vertebral artery, and the anterior segment of the MCA were cauterized before the surgical procedure and through the experimental procedure with supplemental CO₂ and O₂. A craniectomy was performed with a high-speed air drill (Hull Surgical, Division of Zimmer) with the aid of an Olympus operating microscope. 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 Saran Wrap to prevent surface oxygenation and to keep the brain moist. Blood loss for the surgical preparation did not exceed 5 mL.

Histological Analysis

At the end of each experiment, the brain was sliced into 4-mm coronal sections, yielding slices (Figure 1) that represent 2 anterior locations, 2 central locations, and 2 posterior

In Vivo Video Fluorescent Instrumentation

Instrumentation was designed to perform serial panoramic video imaging of cortical brain pH, rCBF, and umbelliferone fluorescence. The optical characteristics were such that the majority or a portion of the exposed hemisphere, pH, and rCBF could be studied simultaneously through a large craniectomy by varying the degree of magnification. The use of umbelliferone as a noninvasive in vivo technique for measuring brain pH, and CBF has been described previously. Umbelliferone is nontoxic, fat soluble, and freely diffusible across the blood-brain barrier, and it rapidly equilibrates across cell membranes and is distributed through the cytoplasm as an uncharged molecule. Umbelliferone was prepared for injection by dissolving 0.2 g of indicator in 200 mL of 5% glucose-saline solution at 90°C for 30 minutes. The solution was then filtered through a 0.22-mm filter before injection. For each measurement, 1.0 mL of umbelliferone was injected retrogradally through a catheter placed in the right lingual artery. The measurements were separated by 30-minute intervals to allow for sufficient clearance of the indicator out of the brain tissue. The pH-sensitive indicator umbelliferone has 2 fluorophores, anionic and isobestic. The anionic and isobestic 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, whereas the fluorescence of the isobestic form varies directly only with the indicator concentration. Therefore, it is possible to create a nomogram from the ratio of 340- to 370-nm excitation to determine brain pH. NADH fluorescent images were acquired before umbelliferone was injected into the lingual artery for correction of background fluorescence. Intrinsc NADH fluorescence images excited at 370 nm were stored for later analysis of mitochondrial function. The scale factor for the percent change in NADH fluorescence from baseline is set so that at 100%, the level represents the level of NADH fluorescence in normal brain, whereas an increase to 300% represents brain death. The scale factor is confirmed by random measurement of NADH fluorescence levels at death and comparing it with baseline nonischemic values in the same animal. It is important to note that a primary source of artifact in the measurement of NADH fluorescence is hemoglobin interference. It has shown previously that use of bright-field illumination, as used in this optical system to reduce scatter, minimizes the effect of this type of interference.

Sundt et al, in a monkey model, correlated biopsy analysis of NADH and NADPH with that of NADH fluorescence measurements in normal brain and brain at death. Use of both techniques showed that there was a close relationship in the degree of change between normal brain and brain at death. The images from the 340-nm excitation were processed to compute rCBF from the 1-minute initial slope index with a partition coefficient of unity for umbelliferone. The rCBF image was then displayed and stored on tape for final analysis. Fast Fourier transformation of paired images (Figure 1) that represented 2 anterior locations, 2 central locations, and 2 posterior
locations. The sections were then immersed in a 37°C solution of 2% 2,3,5-triphenyltetrazolium chloride (TTC) in saline. To enhance TTC penetration, the sections were suspended within the TTC solution for 30 minutes in a shaker bath maintained at 37°C. Sections were removed from the TTC solution, placed flat on one another in separate fixation cassettes, and stored in 10% buffered formalin. Photographic slides of each section were taken 1 week later. For assessment of the amount of tissue damage, each section was photographed, and the area of infarction was identified, traced, and digitized. The total area of the hemisphere was also determined.

**Definition of Ischemic Penumbra**

The purpose of this experiment was to determine the effects of glucose and oxygen manipulations on brain pH_i and NADH redox state in the ischemic penumbra. Determination of the location of the ischemic penumbra was made as follows. Thirty minutes after the onset of ischemia, in the immediate proximal distribution of the MCA along the sylvian fissure, there is a relatively small region of cortex in which rCBF declines to \(<12 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}\), or an \(\approx 80\%\) reduction in rCBF values compared with preischemic measurements. Under normoglycemic conditions, this cortex has pH reductions to \(\approx 6.60\). This region has previously been demonstrated to be an evolving infarction with necrosis under light microscopic examination. Distal to this zone of severe rCBF reductions is parietal cortex exposed by the craniectomy that has initial rCBF reductions of \(\approx 20 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}\). This cortex was defined as the ischemic penumbra in this experiment, and the tissue was analyzed by fluorescence imaging and histology. For example, Figures 2 and 3 are composite images of this cortex, which is distal to the smaller zone of early evolving infarction. Within this ischemic penumbra, there is a heterogeneous development of acidosis, which is the issue of interest for this experiment.

**Statistical Analysis**

Separate analyses were carried out for each of the 4 variables under study: pH_i, rCBF, NADH fluorescence, and infarct volume. ANOVA was used to test the statistical significance of differences between groups. Results were considered statistically significant at a value of \(P<0.05\). Data are presented as mean ± SEM. All analysis was conducted with CSS (Statsoft) statistical software.

**Results**

**Systemic Parameters, Nonischemic Control Group, and Video Acquisition**

There were no significant differences over time between animals studied in each of the 6 groups in the measurements of \(\text{Paco}_2\), \(\text{Pao}_2\), \(\text{pH}_i\), mean arterial blood pressure, body temperature, glucose, and hematocrit (Table). The control sham-operated nonischemic animals had stable \(\text{pH}_i\), rCBF, and NADH fluorescence measurements throughout the experiment, thus confirming the stability of the preparation. The blood serum levels of \(\text{Pao}_2\), \%Sco_2, and lactate in the moderate hypoxia groups were significantly different from those of the normoxia groups \((P<0.01)\). Figures 2 and 3 show composite video pictures of 2
typical experiments: a hypoglycemic/moderately hypoxic animal and a hyperglycemic/normoxic animal, respectively.

**Experimental Groups (Figure 4)**

**Normoxic Groups**

Brain pH. Baseline preischemic brain pH was uniform over the exposed cortex in all groups, measuring 6.96±0.03. Within the ischemic penumbra, there was a heterogeneous development of acidosis, as illustrated in an example experiment, Figure 3. After 2 hours of ischemia, overall brain pH, in the ischemic penumbra declined to 6.65±0.04 and 6.56±0.10 in the moderate and severe hyperglycemia groups, respectively (P<0.01 compared with preischemic values). In the hypoglycemia group, brain pH declined to 6.88±0.03, which was not significantly different from preischemic values. After 4 hours of ischemia, brain pH declined further, to 6.47±0.06 in both the moderate and severe hyperglycemia groups (P<0.01). In the hypoglycemia group, brain pH was 6.84±0.09, not significantly different from preischemic values. Figure 5 depicts a series of brain pH histograms of a hyperglycemic/normoxic animal (Figure 3), demonstrating that before occlusion, brain pH was relatively homogeneous and then became markedly heterogeneous after the onset of focal ischemia.

Regional Cortical Blood Flow. rCBF in all groups was 51.5±3.3 mL · 100 g⁻¹ · min⁻¹ before ischemia. After 2 hours of ischemia, rCBF in the ischemic penumbra fell significantly (P<0.01) in all groups studied, to ≈15 mL · 100 g⁻¹ · min⁻¹. After 4 hours of ischemia, rCBF declined further to ≈10 mL · 100 g⁻¹ · min⁻¹ in all groups (P<0.01). Figure 5 depicts a series of rCBF histograms of a hyperglycemic/normoxic animal (Figure 3) demonstrating that before occlusion, rCBF was relatively heterogeneous and then became more heterogeneous after MCA occlusion.

**NADH Fluorescence.** NADH fluorescence was uniform over the exposed cortex in all groups, measuring 105.3±2.9% before ischemia. After 2 hours of ischemia, NADH fluorescence levels in the ischemic penumbra increased to ≈152% in both the moderate and severe hyperglycemia groups (P<0.01). In the hypoglycemia group, NADH fluorescence increased to 141±12.8% (P<0.01). After 4 hours of ischemia, NADH fluorescence increased further, to 148±7.1% and 173±16.7% in the moderate and severe hyperglycemia groups, respectively (P<0.01 compared with preischemic values). However, in the hypoglycemia group, NADH redox state improved, measuring 114±7.5%, which was not significantly different from preischemic values. Figure 5 depicts a series of NADH redox state histograms of a hyperglycemic/normoxic animal (Figure 3) demonstrating increased heterogeneity during the period of occlusion.

**Areas of Infarction as Measured With TTC.** Infarct volume in the moderate and severe hyperglycemia groups was 30.1±1.9% and 35.1±3.1% of total hemisphere volume. The hypoglycemia group showed a significantly (P<0.01) smaller infarct volume (13.5±1.9%) than the other 2 groups. Analysis showed that
hemispheric volumes in all normoxic study groups were not significantly different, indicating that there was no significant early edema formation, which might alter infarct volumes.

**Moderately Hypoxic Groups**

**Brain pH**. Brain pH was uniform over the entire exposed cortex in all groups, measuring 7.00±0.03 before ischemia. After 2 hours, brain pH in the ischemic penumbra fell significantly in the moderate and severe hyperglycemia groups, to 6.50 (P<0.01 compared with preischemic values). In the hypoglycemia group, brain pH declined to 6.87±0.09, which was not statistically different from preischemic values. After 4 hours of ischemia, brain pH declined further, to 6.43±0.03 and 6.19±0.13 in the moderate and severe hyperglycemia groups, respectively (P<0.01 compared with preischemic values). In the hypoglycemia group, brain pH measured 6.91±0.06, which was not statistically different from preischemic values. Figure 6 depicts a series of histograms of a hypoglycemic/moderately hypoxic animal (Figure 2) demonstrating homogeneity of pH before occlusion and throughout the ischemic period.

**Regional Cortical Blood Flow**. rCBF in all groups was 48.3±3.3 mL · 100 g⁻¹ · min⁻¹ before ischemia. After 2 hours of ischemia, rCBF fell significantly in the ischemic penumbra (P<0.01) in all groups studied, to 16 mL · 100 g⁻¹ · min⁻¹. After 4 hours of ischemia, rCBF further declined to 11 mL · 100 g⁻¹ · min⁻¹ in all groups (P<0.01). Figure 6 depicts a series of rCBF histograms of a hypoglycemic/moderately hypoxic animal (Figure 2) demonstrating that before occlusion, rCBF was relatively heterogeneous and then became more heterogeneous with reductions in rCBF after MCA occlusion.

**NADH Fluorescence**. NADH fluorescence was uniform over the exposed cortex in all groups, measuring 101.9±2.8% before ischemia. After 2 hours of ischemia, NADH fluorescence levels in the ischemic penumbra increased to 173% in both the moderate and severe hyperglycemia groups (P<0.01 compared with preischemic values). In the hypoglycemia group, NADH fluorescence increased to 111±9.4%. After 4 hours of ischemia, NADH fluorescence increased further, to 167±10.9% and 197±22.1% in the moderate and severe hyperglycemia groups, respectively (P<0.01 compared with preischemic values). In the hypoglycemia group, NADH redox state increased slightly, to an overall increase of 130±13.4%, which was not statistically different from preischemic values. Figure 6 depicts a series of NADH redox state histograms of a hypoglycemic/moderately hypoxic animal (Figure 2) demonstrating very little change in the histograms during ischemia.

**Areas of Infarction as Measured With TTC**. Infarct volume in the moderate and severe hyperglycemia groups measured 30.4±1.2% and 35.0±1.2% of total hemisphere volume. The hypoglycemia group showed a significantly smaller infarct volume (21.4±3.6%) compared with the other 2 groups.
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A number of investigators have studied the effects of hyperglycemia in models of focal cerebral ischemia. These studies have shown conflicting results, either worsening of ischemic damage\(^{11,15,28–32}\) or amelioration of ischemic damage.\(^{13,33–37}\) The discrepancy in these studies could be explained in part by the degree of collateral blood supply. In models of focal “incomplete” cerebral ischemia, in which there was some collateral flow (“trickle flow” phenomenon), hyperglycemia appeared to exacerbate ischemic damage.\(^{15,31,38}\) However, in other studies of focal cerebral ischemia, in which there was a greater amount of collateral blood flow, the effects of hyperglycemia on ischemic damage were less.\(^{13,34,35}\) In our study of the ischemic penumbra, increasing the serum glucose levels before the onset of focal ischemia resulted in greater brain intracellular acidosis, increased the heterogeneity of pHi, adversely effected NADH redox state, and significantly increased infarct volume compared with the hypoglycemia group. The increased heterogeneity of pHi with increasing plasma glucose concentration observed in this study is in agreement with that of LaManna et al.\(^2\) In a model of cardiac arrest and using neutral red histophotometry, they showed that when the plasma glucose concentration was increased, there was greater acidosis and increased heterogeneity because of greater accumulation of tissue lactate. They also demonstrated that during ischemia, pHi and tissue lactate accumulation are linearly correlated. Griffith et al\(^1\) also showed increased heterogeneity during ischemia in a model of global ischemia.

**Hypoglycemia**

Preischemic moderate hypoglycemia in models of focal cerebral ischemia has not been studied as extensively as in models of global and forebrain ischemia. It is known that hypoglycemic coma without occlusion of the MCA results in neuronal damage in selective areas of the brain.\(^{39–41}\) Kristián et al\(^{41}\) showed that during hypoglycemic coma, brain pH\(_i\), did not become acidic when the animals were normocapnic, although there were selective areas of neuronal damage. When the animals were made hypercapnic, however, brain pH\(_i\) became more acidic, with greater regions of neuronal damage and pan-necrosis. In a model of forebrain ischemia, Smith et al\(^2\) showed that during ischemia, brain pH\(_i\) was less acidic in animals with hypoglycemia than in animals with normoglycemia, 6.37\(\pm\)0.04 versus 6.15\(\pm\)0.06. In this study, the plasma glucose level during hypoglycemia was 4.6\(\pm\)0.1 mmol/L. Conversely, in a model of global ischemia using 4-vessel occlusion, Nagai et al\(^{42}\) noted that pH\(_i\), which became acidic during ischemia, was not different in either the normoglycemic or hypoglycemic setting. The plasma
Glucose level was not specified in that study. There have been several reports in which insulin reduced ischemic damage.43–45 Hamilton et al 45 showed that by reducing the blood glucose to minimum values but within the physiological range (moderate hypoglycemia), infarct volume could be significantly reduced. In this study, untreated animals had a cortical infarct volume of 39.9 ± 7.3 mm³, whereas the insulin-treated animals had reductions in infarct volume to 22.5 ± 3.1 mm³ (43.5%). This is in close agreement with our study, in which we showed a reduction in infarct volume by 47.7%. Furthermore, this experiment demonstrates that hypoglycemia decreases pH_i heterogeneity changes during ischemia. This supports the suggestion that moderate hypoglycemia or the avoidance of hyperglycemia may be beneficial for neurosurgical procedures in preventing ischemic damage as a result of temporary arterial occlusion.46

Moderate Hypoxia/Ischemia

Many studies have investigated the effects of hypoxia on the brain.46–53 Some of these studies were done in animals made hypoglycemic or hyperglycemic.50,51 Other studies used the Levine rat preparation, which is a model of global ischemia with hypoxia as a method to further exacerbate tissue damage.54 To the best of our knowledge, no studies of moderate hypoxia in focal cerebral ischemia have been performed. In our study, we noted 3 distinct findings in animals studied with moderate hypoxia: (1) there was no difference in infarct volume, pH_i, or NADH redox state between the normoxic and moderately hypoxic animals during moderate hyperglycemia; (2) moderate hypoxia exacerbated brain intracellular acidosis compared with the normoxic animals in the severe hyperglycemia group; and (3) in hypoglycemia groups, although brain pH_i was slightly but not significantly alkalotic in the moderate hypoxia group, infarct volume was 180% greater than in the normoxia group. It has been documented that during moderate hypoxia (Pao₂ =45 to 55 mm Hg), there are biochemical alterations in brain tissue levels of energy metabolites, NADH redox state, and pH_i, ATP, ADP, and AMP do not change unless the Pao₂ is <25 mm Hg, whereas phosphocreatine, NADH redox state, and pH change at <35 mm Hg.52,55 However, the lactate and pyruvate levels begin
to increase when the Pao2 is <50 mm Hg. This would explain in part why the severely hyperglycemic/moderately hypoxic animals were markedly more acidic than the severely hyperglycemic/normoxic animals. It is interesting to note that the increase in NADH fluorescence between the hypoglycemia and hyperglycemia groups was not enhanced with the addition of moderate hypoxia. The results of this study suggest that one adverse effect of acidosis is increased damage to mitochondria. It has been shown previously that during complete and incomplete ischemia, lactic acidosis prevents normalization of mitochondrial respiration.56,57 Wagner et al58 demonstrated that a combination of transient anoxia in hyperglycemic cats caused altered mitochondrial respiration. Our study would support the above findings and conclusion that acidosis does adversely alter mitochondrial function during the acute ischemic insult. In fact, the observation that changes in systemic O2 did not significantly alter NADH fluorescence supports the contention that the observed effects were not due to a failure of substrate delivery for aerobic metabolism but rather a direct effect of hyperglycemia on the mitochondria. The mechanisms by which acidosis might adversely affect mitochondria have been expertly discussed elsewhere.9 The observation that the apparent adverse

Figure 5. Composite histogram of the experiment in Figure 3. Before ischemia, the distribution of rCBF is quite heterogeneous compared with brain pH or NADH redox state. Over the next 2 hours after MCA occlusion, rCBF becomes less heterogeneous, then at hours 3 and 4 becomes more heterogeneous, but with rCBF values significantly less than preischemic values. After occlusion of the MCA, brain pH becomes very acidotic and heterogeneous over the 4-hour period. The distribution of NADH redox state becomes more heterogeneous over this same time frame.
effects of hyperglycemia on NADH redox state occurred acutely in our experiment would suggest that free radical formation was not the cause but rather that other mechanisms, such as mitochondrial calcium overload, were at play.

TTC Staining Technique
Tetrazolium salts have been used to determine the area and degree of infarction in myocardial tissue obtained from patients. This technique has been extended for use in experimental animals in the study of cerebral injury as a result of unilateral temporary or permanent MCA occlusion. TTC is a water-soluble salt that is reduced to formazan by the enzyme succinate dehydrogenase in mitochondrial tissue. This in turn stains a deep red color in normal tissue. In ischemic tissue in which mitochondria have been damaged, however, there would be a lack of staining, i.e., tissue will be a white or pale color.

The reliability of TTC staining as an early marker (<24 hours after MCA occlusion) of ischemic damage has been controversial.

**Figure 6.** Composite histogram of experiment in Figure 2. Before ischemia, the distribution of rCBF is quite heterogeneous compared with brain pH, or NADH redox state. Over the next 2 hours after MCA occlusion, rCBF becomes less heterogeneous, then at hours 3 and 4 becomes more heterogeneous, but with rCBF values significantly less than preischemic values. The distribution of pH is unchanged during the 4-hour period of occlusion. The distribution of NADH redox state became less homogeneous over the 4-hour period of occlusion.
Infarction can be detected as early as within 1 to 2 hours; however, the color differences between red and white are subtle, making it difficult to delineate the extent of infarction. At infarct times of ≥3 hours, the infarcted tissue becomes distinctly delineated even before development of histological evidence of infarction. Hatfield et al. showed that 5 to 20 minutes after MCA occlusion, the area of damage as assessed by hematoxylin-eosin staining was significantly smaller than that assessed by TTC. At 24 hours, infarct size was not significantly different from the 3 to 4 hours post–MCA occlusion group. It can be postulated that when the animals are killed 5 to 20 minutes after MCA occlusion, the cerebral metabolic rate of oxygen is reduced immediately after occlusion. TTC can overestimate infarct size because, although mitochondrial function is compromised, it may potentially recover. Peri-infarct edema can also affect assessment of infarct volume. Other studies have also shown similar results.

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pathol. 1987;73:131–137.
Intraischemic hyperglycemia has been widely demonstrated to exacerbate brain injury in a variety of animal models by enhancement of intracellular acidosis, consequent loss of ion homeostasis, and through bioenergetic failure. A host of potential cellular mechanisms have been identified, with particularly good evidence for the involvement of iron-catalyzed production of reactive oxygen species. It is increasingly clear that lactic acidosis amplifies multiple subcellular, and potentially mitochondrial, injury cascades during cerebral ischemia. However, results in focal cerebral ischemia have not completely agreed on the deleterious effect of high glucose, in part because glycemic state may alter collateral blood flow and may therefore influence the fate of penumbral tissues. The preceding article uses sophisticated, panoramic video imaging to evaluate ischemic homeostasis, and tissue infarction may or may not be causal. Further, because the animal model is quite severe and not reversible, the investigators are limited to study of acute pathophysiology without reperfusion. This is important, because hyperglycemic ischemia has delayed effects that are of clinical interest, such as exaggerated cerebral edema, seizure development, and transformation of selective neuronal injury to pan necrosis. Nevertheless, the temporal and spatial measurement resolution of the experimental approach allows for excellent description and correlation of important, physiological variables during vascular occlusion. The data provide remarkable spatial detail and fresh quantification of tissue pH/perfusion heterogeneity that has previously been speculated to develop during hyperglycemic stroke. Although no specific cellular mechanism is elucidated, the study strongly supports the prevailing concept that acidosis recruits potentially salvageable brain into a state of no return.

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