Experimental Model for Systematic Study of Impaired Microvascular Reperfusion

JOHN M. HALLENBECK, M.D., AND MARK E. BRADLEY, M.D.

SUMMARY A technique is described for reliably producing quantifiable impairment of microvascular reperfusion of the brain after ischemia in dogs. The technique is derived from an analysis of the cerebrospinal fluid compression ischemia model as a Starling resistor. It is proposed that this model would be useful in a systematic study of post-ischemic impairment of reperfusion.

IN DEATHS due to dysbaric cerebral air embolism observed by the authors, a course suggesting progressive impairment of microcirculatory reperfusion has repeatedly occurred. Recompression to a simulated 165 feet in a chamber has usually been delayed for at least 5 minutes in these cases but, when instituted, should have eliminated the mechanical obstruction of cerebral vessels by bubbles. During the 15 to 30 minute interval at 165 feet called for by standard Navy treatment tables, these patients characteristically regained consciousness and demonstrated varying degrees of recovery. However, beginning about 30 minutes to 2 hours after the initial ictus, the clinical state progressively deteriorated, often with the patient still under pressure, but, when instituted, should have eliminated the mechanical obstruction of cerebral vessels by bubbles.

It follows that an experimental model permitting study of the factors that influence the adequacy of post-ischemic microcirculatory reperfusion would have potential therapeutic relevance. Models of cerebral ischemia in animals abound. Most of these models involve preliminary surgery which ranges from major to mutilating. The consequence tissue damage introduces the probability of alteration: in vivo intracisternal production of spasm by serotonin and blood and its experimental outcome, a circumstance for which the term "physiologic reactance" has been used. Indeed, Bergstrom has demonstrated circulating platelet aggregates in the hamster cheek-pouch minutes after crush injury to the thigh and has classified these models into 4 phases the serial response of blood tissue injury. Borgeest has noted intravascular aggregation of red cells 1-3 hours after blunt leg trauma in rabbits. The latency for all of these changes was much shorter in the vicinity of the tissue damage. Most of the surgical trauma can be avoided i cerebrospinal fluid compression (CSP) stimulation is used to induce ischemia, but when the brain is rendered bloodless by raising cerebrospinal fluid pressure (CSFP) above systolic arterial pressure, the subsequent reperfusion seems uniform without evidence of "no-reflow." Several studies have im

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The experiments reported were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals." Institute of Laboratory Animal Resources, National Research Council, DHEW Pub. No. (NIH) 74-23.
plicated intravascular blood as a factor that somehow adversely influences post-ischemic reperfusion. By subjecting the CSF compression model of ischemia to a Starling-resistor analysis, it was possible to find a relationship between systemic arterial pressure (SAP) and CSFP that would cause global neuraxis ischemia with a cyclic ebb and flow of blood into and out of the cerebral vessels coincident with fluctuations of SAP.

Methods

Five splenectomized, conditioned male mongrel dogs weighing 7–12 kg were anesthetized with intravenous pentobarbital sodium 25–30 mg/kg. A schematic diagram of the preparation is shown in figure 1. The animals were intubated and connected to a Bird respirator. End-tidal CO₂ concentration was continuously monitored by a Beckman LB-2 CO₂ analyzer to assist maintenance of the Pco₂ in the normal range. Core temperature was maintained by means of a tympanic membrane thermistor connected to a YSI telethermometer (Yellow Springs Instrument Company) and the temperature was maintained at 38°C with an infrared light and heating pads. Two catheters were placed in the right femoral artery. One was directed proximally into the aorta and the other was directed into the distal femoral artery. When these two catheters were later joined through a Y-connector, the arterial flow to the right hindleg was externalized permitting rapid sampling of arterial blood, a required operation in the ¹⁴C-antipyrine autoradiographic blood flow assay. A right ventricular catheter was placed via the right femoral vein. A 1000 ml bottle of Elliott's solution B, a mock cerebrospinal fluid, was attached to a line that went through a pulley on the ceiling so that the bottle could be raised or lowered at will. Tubing from this bottle passed to a Y-connector from which one arm went to a strain gauge. Bottle pressure was thereby continuously recorded on a Gilson polygraph. The other arm of the Y-connector was attached to tubing which led through a copper coil placed in a 38°C water-bath. From the copper coil, a short piece of plastic tubing led to a cisternal needle. The animals were anesthetized with intravenous pentobarbital sodium 25–30 mg/kg. A schematic diagram of the experimental arrangement is shown in figure 1.

![Diagram](attachment:diagram.png)

**Figure 1.** A schematic diagram of the experimental arrangement.

The two animals studied during CSF compression ischemia to determine the extent of blood flow interruption

Statham strain gauge connected to the proximal femoral catheter. An ECG lead was continuously monitored for cardiac rate and rhythm.

The cisternal needle was carefully placed by percutaneous puncture and phenolamine 10 mg IV was administered to combat the sympathetic nervous system mediated rise in SAP that accompanies intracranial hypertension (Cushing response). The aortic pressure recording on the polygraph was switched to electronic mean. The bottle containing the Elliott's solution B was then raised until its line pressure equalled the mean systemic arterial pressure (MSAP). If reactive systemic arterial hypertension supervened, blood was withdrawn into heparinized 50 ml syringes until the MSAP was in the 120 mm Hg to 140 mm Hg range. As CSF compression ischemia was continued, the mean arterial pressure characteristically fell after 4 to 8 minutes into the 70–90 mm Hg range. Subsequently, the mean arterial pressure was maintained between 70 and 90 mm Hg by reinfusing the withdrawn blood and infusing saline or levaterenol bitartrate (Levophed, Winthrop) 1 mg/250 cc of 5% dextrose in water as necessary. After 35 minutes of CSF compression ischemia the bottle of Elliott's solution B was lowered so that its line pressure equaled 10 mm Hg and the systolic SAP was kept between 110 and 120 mm Hg for 30 minutes. This represented the post-ischemic recirculation period. At the conclusion of this recirculation period, a ¹⁴C-antipyrine autoradiographic blood flow measurement was performed. Essentially, this required intravenous infusion of 100 μg/kg of ¹⁴C-antipyrine for one minute with serial sampling of arterial blood repeated every 4 to 6 seconds. The cardiac arrest which terminated this procedure was produced by injecting a bolus of 20 cc of saturated potassium chloride through the right femoral vein catheter into the right ventricle. The brain, spinal cord, and heart were then removed, frozen in liquid freon suspended over liquid nitrogen and cut into 20 micron sections. The tissue concentration of the isotope was determined autoradiographically. Local blood flow was calculated from the following formula:

\[
C(T) = \lambda k_v \left( \frac{C_s}{C_v} \right) e^{-k_v T + \lambda T}
\]

where \(C(T)\) = the concentration of tracer substance in the tissue at time \(T\); \(\lambda\) = the tissue-blood partition coefficient for the tracer material; \(k_v\) = the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that tissue; and \(C_s\) = the concentration of tracer substance in the arterial blood.

Blood gases and blood pH were frequently monitored and blood was drawn for hematocrit and platelets initially as a baseline control (C), prior to ischemia (PI), prior to the post-ischemic recirculation period (PR), and just prior to the blood flow study (PF).

Two splenectomized male mongrel dogs were subjected to the same procedure except that after 12 and 15 minutes of CSF compression ischemia respectively, autoradiographic blood flow procedures were performed to measure the extent of blood flow interruption with the CSFFP maintained equal to MSAP during the flow study.

**Results**

The two animals studied during CSF compression ischemia to determine the extent of blood flow interruption
both had absent flow in the parenchyma of the neuraxis. Therefore, setting CSFP equal to MSAP and, by definition, reducing the cerebral perfusion pressure (CPP) to zero produced a state of global central nervous system (CNS) ischemia rather than oligemia in these animals.

Such variables as hematocrit, platelets, PO$_2$, PCO$_2$, pH, systolic blood pressure, diastolic blood pressure, and heart rate for the 5 animals exposed to 35 minutes of ischemia and 30 minutes of recirculation are shown in table 2. The average blood flow for each brain area and heart in ml/100gm/min from the group of 5 animals is shown in table 3. Foci of extremely low flow, which are felt to correspond to areas of impaired perfusion, are displayed in fig. 2. The focal variations are displayed in table 3. Foci of extremely low flow, which are felt to correspond to zones of impaired microvascular perfusion noted in other models,

<table>
<thead>
<tr>
<th>Areas and Heart in ml/100gm/min From the Group of 5 Animals</th>
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<tbody>
<tr>
<td>Anterior Centrum Ovale</td>
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<tr>
<td>Auditory Cortex</td>
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<tr>
<td>Caudate Nucleus</td>
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<tr>
<td>Corpus Callosum</td>
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<td>Hippocampus</td>
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<td>Internal Capsule</td>
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<td>Middle Centrum Ovale</td>
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<td>Sensorimotor Cortex</td>
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<td>Thalamus</td>
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<td>Visual Cortex</td>
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<td>Posterior - Middle Cerebral Watershed Cortex</td>
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<tr>
<td>Anterior Association Cortex</td>
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<td>*Mean ± standard error of mean.</td>
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The average local blood flows for various neuroanatomic areas and the free wall of the left ventricular myocardium are shown in table 2. The average blood flow for each brain structure was somewhat low relative to flows measured in barbiturate anesthetized animals and no tendency toward reactive hyperemia was induced by the state of temporary ischemia. The major contrast with the uniform depression of blood flow caused by anesthesia alone was the extreme heterogeneity of blood flows in post-ischemic animals with large focal variations often encountered within single neuroanatomic structures. The focal variations are displayed in table 3. Foci of extremely low flow, which are felt to correspond to zones of impaired microvascular perfusion noted in other models, ranged in incidence from infrequent and confined to several structures (figs. 2-4) to ubiquitous (fig. 5). No focal areas of flow heterogeneity were noted in spinal cord gray matter, spinal cord white matter or free wall of the left ventricular myocardium.

![Figure 2. An autoradiogram showing low blood flow in the dorsolateral cortex and a region of impaired perfusion in the caudate nucleus. The animal received 35 minutes of CNS ischemia followed by 30 minutes of recirculation.](http://stroke.ahajournals.org/)
FIGURE 3. An autoradiogram showing impaired microvascular perfusion in the posterior cerebral — middle cerebral watershed cortex. The animal received 35 minutes of CNS ischemia followed by 30 minutes of recirculation.

Discussion

The results indicate that this technique for producing global CNS ischemia does produce focal areas of extremely low blood flow after an ischemic interval of 35 minutes followed by 30 minutes of general recirculation. We have noted that dogs anesthetized with pentobarbital sodium without exposure to transient CNS ischemia have uniformly reduced brain blood flows relative to awake, unrestrained animals\textsuperscript{11} that contrast with the present series by the absence of focal areas of extremely low flow. However, such dogs constitute an inappropriate comparison group for the present series since their brain blood flows do not reflect the potentially large influence of prior ischemia. A more appropriate comparison would be made between two groups exposed to CSF compression ischemia as outlined but differing with regard to some operation designed to prevent impaired microvascular reperfusion. One such comparison, in which one group was subjected to glass-wool filtration of each animal's blood and the other group was handled in an otherwise identical manner, revealed greatly increased post-ischemic brain flows in the filtered group.\textsuperscript{18}

FIGURE 5. An autoradiogram showing virtual absence of reflow in a section of brain. The animal received 35 minutes of CNS ischemia followed by 30 minutes recirculation.

Early studies with several dogs subjected to CNS ischemia with the CSFP 20 mm Hg higher than systolic SAP for 35 minutes or longer had relatively uniform reperfusion suggesting that suprasystolic CSF compression ischemia had limited usefulness for the study of impaired microvascular reperfusion. The general CSF compression ischemia model was then analyzed as being similar to a Starling resistor, i.e., being composed essentially of collapsible vessels contained in a rigid skull and surrounded by a total tissue pressure that is closely approximated by CSFP. In its classical sense, a Starling resistor consists of a collapsible tube in a pressure chamber.\textsuperscript{14} Figure 6 applies the Starling resistor archetype to the CSF compression ischemia model. When SAP (P\textsubscript{1}) exceeds CSFP (P\textsubscript{3}) which exceeds CNS venous pressure (P\textsubscript{2}), flow is proportional to the difference between P\textsubscript{1} and P\textsubscript{3} rather than the difference between P\textsubscript{1} and P\textsubscript{2}.\textsuperscript{15} When P\textsubscript{2} exceeds both P\textsubscript{1} and P\textsubscript{3}, there is no flow. It seemed theoretically possible that if the CSFP was set at some intermediate point between the maxima and minima of a fluctuating vessel inlet pressure, neuraxis circulation could conceivably be interrupted while preserving a cyclic ebb and flow of blood into and out of inlet vessels coincident with fluctuations of SAP. A necessary condition for such an occurrence would be that the time interval spent above CSFP by the fluctuating SAP during each cardiac pulse should be considerably shorter.
that antipyrine is not an ideal tracer for blood flow measure-
ment because its uptake in brain is diffusion limited par-
ticularly at high flow rates, leading to systematic underesti-
mates of local CBF.14 However, the method is more accurate at low flows such as those in question28 and does permit assess-
ment of patterns of heterogeneous flow change.27 Until a bet-
ter tracer is developed, the ability of the 4C-antipyrine auto-
radiographic technique to detect a pattern of flow change char-
acterized by very low flows interspersed with normal or high flows warrants its use in this type of study.

There is good evidence that in global ischemia, definite
neuronal damage occurs after 5–15 minutes in the absence of
impaired microvascular reperfusion.25-28 This renders un-
likely the suggestion that the first irreversible change during
global cerebral ischemia occurs in the vessels with irrevers-
able neuronal damage following secondarily.18 However, the
possibility remains that a common mechanism is shared by
impaired microvascular reperfusion occurring after
relatively long periods of ischemia and progressive deterio-
ration of microcirculatory flow in regions of acute tissue
damage.21-24 The fundamental fact may be that a blood flow
of less than some critical value in a zone of acute tissue
damage tends to shut itself off by some mechanism other
than clot formation. Such a mechanism could explain the
observed failure over time of collateral circulation in focal
cerebral ischemia.29

The mechanism underlying the phenomenon of impaired
microvascular reperfusion should be worked out whether or
not one believes it has an important role in the process of
ischemic tissue damage. The ultimate demonstration that
impaired microvascular perfusion is irrelevant to the genesis
of neuronal infarction in cerebral ischemia would be to pre-
vent impaired perfusion and document that it fails to in-
fluence the course and outcome of graded ischemic insults.
If, however, prevention of impaired perfusion should prove
beneficial, a new approach to therapy could emerge.

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FIGURE 6. A schematization of the Starling resistor concept as
applied to the CSF compression ischemia model. With the pressure
relationships shown, flow would be proportional to the $P_1 - P_3$
gradient (transmural pressure) rather than the $P_1 - P_2$ gradient.

Variable CSFP
(P3)

SAP (P1)

CNS Venous
Pressure (P2)

$P_1 > P_3 > P_2$
Respiratory Influence on the Total and Regional Cerebral Blood Flow Responses to Intracranial Hypertension

Asrar B. Malik, Ph.D., John A. Krasney, Ph.D., and George J. Royce, Ph.D.

SUMMARY The effect of respiration on the cerebrovascular response to elevated intracranial pressure (ICP) was studied in anesthetized dogs. Total and regional cerebral blood flows were measured using labeled microspheres. In spontaneously breathing dogs total and regional cerebral blood flows increased when cerebral perfusion pressure was reduced to 20 mm Hg. The increase in regional flows was greater in the supratentorial areas than in the infratentorial areas. The increase in cerebral flow in spontaneously breathing dogs was associated with the development of hypoxemia and respiratory acidosis secondary to depression of ventilation. Elevation in ICP while regulating PO2, PCO2, and pH by controlled ventilation resulted in decrease in total and regional cerebral blood flows. The decrease in regional flows was greater in the supratentorial areas. Induction of respiratory acidosis during elevated ICP in the controlled ventilated dogs with 5% CO2 in air gas mixture, reversed the decrease in cerebral flows. The results suggest that the increase in cerebral blood flow during elevated ICP in spontaneously breathing dogs is secondary to the development of hypoxemia and respiratory acidosis since cerebral vessels retain responsiveness to increased Paco2 when the vessels are dilated due to elevated ICP. The results also indicate that the regional cerebrovascular response to elevated ICP is non-uniform.

ELEVATION IN THE intracranial pressure (ICP) in spontaneously breathing animals results in hypoventilation which is induced by depression of the medullary respiratory centers.1,2 The decrease in ventilation is associated with decreases in arterial PO2 and pH, and increase in arterial CO2.3,4 The blood gas and pH alterations may affect the cerebrovascular response to intracranial hypertension.5 Data from patient and animal studies suggest that cerebral vessels retain their reactivity to carbon dioxide during cerebral vasodilation and reduced blood flow.6,7 Therefore, the potential exists for the alteration in the cerebrovascular response to elevated ICP due to the development of hypoxemia and respiratory acidosis. The purpose of the study was to determine the role of blood gas and pH alterations occurring during elevated ICP in spontaneously breathing dogs on the total and regional cerebrovascular response to elevated ICP.

Methods
Fifteen mongrel dogs (mean weight 11.2 ± 1.5 kg) were anesthetized with 30 mg/kg sodium pentobarbital and intubated. Teflon catheters were positioned in a femoral artery
Experimental model for systematic study of impaired microvascular reperfusion.
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