Prolonged normoxic hypercapnia initially caused an increase in canine cerebral blood flow, as measured by the radioactive microsphere technique, accompanied by a decrease in cerebrovascular resistance. These effects persisted for 3 hours. An adaptive decrease in cerebral blood flow and increase in cerebrovascular resistance were seen when hypercapnia was maintained for an additional 3 hours. Regional variations occurred; those areas with the greatest initial hypercapnic blood flow (cortex, caudate nucleus) showed a greater rate of decay of flow over time. Cerebrospinal fluid pH, initially acidic during hypercapnia, increased over the subsequent 5 hours from 6.99 ± 0.02 to 7.13 ± 0.02. This was accompanied by an increase in the cerebrospinal fluid bicarbonate ion concentration from a normocapnic baseline of 19.6 ± 0.6 to 26.2 ± 4 mEq/l. Total and regional cerebral blood flow were linearly related to cerebrospinal fluid pH ($R^2 = 0.97$). Extrapolation of a full adaptive return of flow to baseline indicated a shift in the cerebrovascular sensitivity to extracellular hydrogen ion concentration during prolonged hypercapnia. (Stroke 1987;18:142-149)

**Materials and Methods**

Nineteen mongrel dogs (15-21 kg) were anesthetized with i.v. sodium pentobarbital (30 mg/kg), intubated, and mechanically ventilated with an inspired gas mixture of 50% O$_2$ and 50% N$_2$. Bilateral frontal and parietal scalp leads were placed to monitor the EEG, allowing anesthesia to be maintained with sodium pentobarbital infusion (approximately 4 mg/kg/hr) administered to avoid EEG burst suppression. Paco$_2$ was maintained at 38 ± 2 mm Hg during the 2-hour preparation period, and hypercapnia was later induced by the addition of inspired CO$_2$. End-tidal CO$_2$ was monitored continuously by capnometry (Hewlett Packard 4710A) and correlated with Paco$_2$. Pancuronium, 0.1 mg/kg i.v., was administered initially, and repeated as necessary (0.5-mg bolus) for immobility during hypercapnia. Pulmonary arterial blood temperature as measured by PA catheter thermistor was maintained near 38°C by surface heating or cooling.

Regional CBF was determined 6 times in each animal by a radioactive microsphere technique using carbonized 15 ± 5-μm spheres labelled with Ce$^{141}$, Gd$^{153}$, Sc$^{46}$, Sr$^{85}$, Nb$^{95}$, and Sn$^{113}$ (New England Nuclear) administered in random order. The microspheres, approximately 1.3 x 10$^6$ in number for each flow determination, were injected into the cardiac left ventricle via a catheter inserted retrogradely through the left femoral artery and positioned manometrically. Blood reference samples were obtained from catheters in the left femoral and brachial arteries at a withdrawal rate of 1.03 ml/min beginning 30 seconds prior to microsphere injection and continuing 3.0 minutes after injection. At the completion of each experiment, the brain was removed and divided into regions. The amount of isotope in the regions of the brain was determined by differential spectroscopy using a scintillation spectrometer (Packard Auto-Gamma Scintillation Spectrometer).
Systemic arterial pressure was monitored via the right brachial artery. Central venous (CVP), pulmonary arterial (PAP), and pulmonary artery wedge pressures (PAWP) were measured via a pulmonary artery catheter. Cardiac output (CO) was determined by thermodilution in triplicate. Sagittal sinus pressure (SSP) was measured from a catheter inserted through a burr hole into the midportion of the sagittal sinus and threaded 1 cm caudally. Systemic pressures were referenced from the level of the right atrium and SSP from the level of the external acoustic meatus.

Immediately preceding each blood flow measurement, arterial and sagittal sinus blood samples were analyzed for pH, Po2, Pco2, hematocrit, and hemoglobin. Cerebrovascular resistance (CVR) was calculated from the difference between mean arterial pressure (MAP) and SSP divided by total brain CBF. Systemic vascular resistance (SVR) was calculated by dividing the difference between MAP and CVP by the cardiac index (CI). CI was derived by dividing CO by the body weight in kilograms.

In 7 of the animals (Group 3), the occipital–Cl interspace was exposed via a dorsal midline occipitocervical incision. A 20-gauge teflon catheter was inserted approximately 1 cm into the cisterna magna and secured with cyanoacrylate for periodic aspiration of CSF. At sampling intervals corresponding to microsphere injections, the catheter was cleared and CSF was anaerobically aspirated into glass syringes for immediate analysis of Po2, Pco2, and pH (pH/Blood Gas Analyzer Model 713, Instrumentation Laboratory, Inc.).

Bicarbonate ion concentration was calculated from the Henderson-Hasselbalch equation. CO2 solubility and pK were temperature- and pH-corrected. On inspection of the arterial and sagittal sinus blood Pco2, measured Pco2 values for CSF were lower than expected and pH values higher. This was possibly due to residual (automated) rinse solution in the electrode chamber interacting with the poorly buffered CSF and to a memory effect at the CO2 electrode from calibration with gasses at a lower CO2 tension. Accordingly, a mock CSF (38°C) solution of NaHCO3 (20 mmol/l) and NaCl (140 mmol/l) was tonomtered with carbon dioxide and nitrogen for 30 minutes; egressed Pco2 was measured at 45 mm Hg and then at 85 mm Hg (Medical Gas Analyzer, Series 1100, Perkin-Elmer Corporation). Repeated samples at each CO2 tension were anaerobically withdrawn through a steel needle into a glass syringe and introduced into the blood gas analyzer. Mock CSF Pco2 values were consistently lower than expected (Appendix 1). Measured mock CSF Pco2 and pH values were then used to calculate bicarbonate ion concentrations, which were constant. Values seen were also similar to those bicarbonate concentrations calculated for the native CSF. Thereby, the error in the CSF pH and Pco2 values was assumed constant, allowing correction.

It has been shown that CSF Pco2, under conditions of both normocapnia and hypercapnia, can be accurately predicted from the equation:

\[
\text{CSF Pco}_2 = \left( \frac{\text{Paco}_2 + \text{Pvco}_2}{2} \right) + 1
\]

where PVco2 is that of the sagittal sinus blood. This value and the calculated bicarbonate ion concentration derived from CSF Pco2 and pH measurements were determined and then entered into the Henderson-Hasselbalch equation to derive a corrected CSF pH (Appendix 2). The values reported in this study are those calculated upon this basis.

Following the 2-hour normocapnic preparation period, baseline rCBF and physiologic measurements were made. Inspired CO2 was then added to raise Paco2 to the range of 80–85 mm Hg. We divided the animals into 3 groups varying the duration of hypercapnia at which CBF and physiologic variables were measured: Group 1 (n = 5): 20, 35, 65, 95, and 125 minutes; Group 2 (n = 5): 20, 50, 110, 230, and 350 minutes; Group 3 (n = 7): 60, 120, 180, 300, and 360 minutes.

An additional 2 animals (Group 4) underwent the identical surgical preparation as Group 3. These animals served as controls to assess for preparation deterioration. CBF and all other variables were measured during baseline normocapnia (Paco2 = 40 ± 1 mm Hg) and 30 minutes after onset of hypercapnia (Paco2...
Table 2. Cerebrovascular Variables for Groups 1 and 2 as a Function of Duration of Hypercapnia

<table>
<thead>
<tr>
<th>Mins</th>
<th>n</th>
<th>Total brain CBF (ml/100 g/min)</th>
<th>CVR (mm Hg/ml/100 g/min)</th>
<th>SSP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>48 ± 3</td>
<td>2.60 ± 0.23</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>248 ± 17</td>
<td>0.46 ± 0.03</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>35</td>
<td>5</td>
<td>240 ± 10</td>
<td>0.46 ± 0.04</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>298 ± 18</td>
<td>0.44 ± 0.04</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>65</td>
<td>5</td>
<td>225 ± 21</td>
<td>0.54 ± 0.05</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
<td>221 ± 23</td>
<td>0.58 ± 0.09</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>110</td>
<td>5</td>
<td>245 ± 32</td>
<td>0.58 ± 0.09</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>125</td>
<td>5</td>
<td>209 ± 17</td>
<td>0.58 ± 0.05</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>230</td>
<td>5</td>
<td>132 ± 28</td>
<td>1.26 ± 0.34</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>350</td>
<td>5</td>
<td>71 ± 8</td>
<td>1.82 ± 0.25</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

Normocapnic values were measured at 0 minutes; values are means ± SEM.

All values at 0, 230, and 350 minutes differ from values at 20, 35, and 50 minutes (p < 0.05).

All values at 20, 35, 50, 65, 95, 110, and 125 minutes differ from values at 0 minutes (p < 0.05).

= 85 ± 2 mm Hg). The Paco2 was then returned to normocapnic levels, and the animals remained anesthetized and immobilized for 5½ hours. Values were then taken a second time at normocapnia; hypercapnia was again reinstituted and all measurements repeated 30 minutes later.

All measurements other than CBF were made immediately prior to the injection of microspheres. Time intervals will henceforth be discussed as minutes after onset of hypercapnia. Other than measurement intervals, experimental conditions were identical for Groups 1 and 2 and, thus, the data from these 2 groups were combined for statistical analysis. Values are reported as mean ± SEM. Data were analyzed by regression analysis and by analysis of variance (Scheffe test) comparing the means of values over all time periods. Statistical significance was assumed when p < 0.05.

Results

Groups 1 and 2

Measurements were made in 10 animals while normocapnic (Paco2 = 39.3 ± 0.5 mm Hg) and at varying intervals subsequent to onset of hypercapnia (Paco2 = 82.6 ± 1.3 mm Hg). Values for physiologic variables are presented in Table 1. No significant variation was found over time with the exception of SVR, which showed a 57% increase over normocapnic baseline (0 minutes) after 230 minutes of hypercapnia and a 39% increase after 350 minutes.

Cerebrovascular values are presented in Table 2. Normocapnic CBF was 48 ± 3 ml/100 g/min. Twenty minutes after onset of hypercapnia, this was markedly increased (248 ± 17 ml/100 g/min) and remained relatively stable for the subsequent 2 hours. At 230 and 350 minutes, a significant reduction in flow was seen despite persistent hypercapnia. Hence, although total CBF had increased to 621% of baseline at 50 minutes, it was decreased to 148% of baseline flow 5 hours later. Flow was significantly decreased by 230 minutes in all regions except the brainstem, but reduction in that region reached significance at 360 minutes. Regional variations will be described more fully for Group 3.

CVR showed an inversely similar pattern, markedly decreasing 82% from baseline after 20 minutes of hypercapnia. Subsequently, a plateau was seen, and...
CVR later showed a significant increase after 230 and 350 minutes (Table 2). SSP also paralleled changes in flow, rising from an initial value of 3 ± 1 to 8 ± 1 mm Hg at maximum and returning to normal by 230 minutes.

Group 3

Despite steady-state hypercapnia, total CBF decreased from a peak flow of 313 ± 44 ml/100 g/min measured at 60 minutes to 88 ± 18 ml/100 g/min 5 hours later (Figure 1). Regional CBF measurements showed a trend similar to that in the first 10 dogs (Table 3). A regional variation was seen, although all regions showed a pattern of decreasing blood flow over time. Linear regression analysis was performed on rCBF (corpus callosum and cervical cord excluded) during hypercapnia as a function of time. This provided for each region the slope of the rCBF decrease, which was plotted against the respective 60-minute rCBF measurement, demonstrating the rate of decay of rCBF as a function of the initial hypercapnic flow value (R² = 0.89, p < 0.001). See Figure 2.

Acid–base values for arterial and sagittal sinus blood, and corrected values for CSF are given in Table 4. In one animal the CSF was bloody when aspirated at 300 minutes and was thus excluded from further analysis. CSF and arterial blood showed a decrease in pH of 0.27 and 0.30 units, respectively, when CO₂ was added as well as a trend for normalization of values over time. This was most evident in the CSF, which recov-

<table>
<thead>
<tr>
<th>Table 3. Regional CBF as a Function of Time During Steady-State Hypercapnia for Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total brain</td>
</tr>
<tr>
<td>Cerebral hemispheres</td>
</tr>
<tr>
<td>Total cerebral cortex</td>
</tr>
<tr>
<td>Left occipital</td>
</tr>
<tr>
<td>Left parietal</td>
</tr>
<tr>
<td>Left temporal</td>
</tr>
<tr>
<td>Left frontal</td>
</tr>
<tr>
<td>Right occipital</td>
</tr>
<tr>
<td>Right parietal</td>
</tr>
<tr>
<td>Right temporal</td>
</tr>
<tr>
<td>Right frontal</td>
</tr>
<tr>
<td>Caudate nucleus</td>
</tr>
<tr>
<td>Brainstem</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Corpus callosum</td>
</tr>
<tr>
<td>Cervical spinal cord</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, ml/100 g/min.
In all cases CBF after 60 and 120 minutes was significantly different (p < 0.05) from normocapnic values at 0 minutes.
In all cases except the caudate nucleus, CBF after 360 minutes was not different from normocapnic values.
*Different from normocapnic value (p < 0.05).
†Different from 60-minute value (p < 0.05).

<table>
<thead>
<tr>
<th>Table 4. Mean Temperature-Corrected Values for Acid–Base Chemistry in the Arterial and Sagittal Sinus Blood and CSF (Group 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mins</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>120</td>
</tr>
<tr>
<td>180</td>
</tr>
<tr>
<td>240</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>360</td>
</tr>
</tbody>
</table>

CSF values are corrected as explained in "Materials and Methods."
*Different from 60 minutes (p < 0.05).
†Different from 0 minutes (normocapnic values) (p < 0.05).
‡Different from 0, 60, and 120 minutes (p < 0.05).
Carbon dioxide, a potent cerebrovasodilator, initially caused a high CBF state accompanied by a similarly marked decrease in CVR in our experimental dogs. These parameters were maintained at a plateau for at least 125 minutes, but by 230 minutes a significant decrease in CBF associated with an increase in CVR occurred 0.14 units ($p < 0.05$), while arterial blood recovered 0.05 units (Figure 1). Bicarbonate ion concentration increased in the sagittal sinus blood and CSF but the increase reached significance in CSF only. Subsequent to the onset of hypercapnia, no significant variation in $Pco_2$ was observed in blood or CSF.

Figure 3 depicts the relation between mean CSF pH and mean total CBF for each measurement interval during hypercapnia. Linear regression analysis yielded an $R^2$ value of 0.97, $y = 11,769.38 - 1,639.52 \times$ CSF pH ($p < 0.01$). A similar comparison was made for each region of the brain (Table 3) with $R^2$ varying between 0.90 and 0.97, significant at $p < 0.01$ in all regions except the caudate nucleus and brainstem, where $p < 0.05$.

CBF as a function of Paco$_2$ for dogs in Group 4 is shown in Figure 4. Responsivity of the cerebral vasculature to hypercapnia appeared well-preserved despite prolonged immobilization, and thus no correction factor was applied to the time-dependent decay in CBF seen in Groups 1–3. CSF pH is reported for one dog in this group. Prior to the two normocapnic blood flows, CSF pH was 7.34 and 7.33, respectively. During the two hypercapnic intervals, CSF pH was 7.02 and 7.01, respectively.

**Discussion**

Carbon dioxide, a potent cerebrovasodilator, initially caused a high CBF state accompanied by a similarly marked decrease in CVR in our experimental dogs. These parameters were maintained at a plateau for at least 125 minutes, but by 230 minutes a significant decrease in CBF associated with an increase in CVR.

![Figure 2. Each point represents a region of the brain (see Table 3). The abscissa depicts the rCBF in ml/100 g/min measured after 60 minutes of hypercapnia. The ordinate represents the slope of the rCBF decrease as a function of time for the respective region. As can be seen, a higher 60-minute rCBF correlates with a greater rate of decrease in rCBF over time.](image1)

![Figure 3. Mean total CBF (Group 3) plotted as a function of CSF pH for each measurement interval during hypercapnia. * = normocapnic value. Linear regression equation: $y = 11,769.38 - 1,639.52$ (CSF pH); $R^2 = 0.97, p < 0.01$. Extrapolation of regression line to baseline CBF value (arrow) corresponds to a CSF pH of 7.14 (arrowhead), 0.12 units less than the initial normocapnic value of 7.26 (*).](image2)
was seen. This is consistent with the results of Agnoli et al., who found an adaptive decrement in flow 3 hours after onset of hypercapnia. Hence, measurements taken acutely during hypercapnia reflect only a short-lived steady-state in cerebrovascular hemodynamics.

Regional variation in the degree of adaptive response was seen. Differential reactivity of brain structures to \( \text{CO}_2 \) in regulation of blood flow has been previously shown. This may be associated with the degree of regional tissue vascularity or with regional variation in the sensitivity of resistance regulatory mechanisms. Either case is tenable to explain the finding that regions with higher initial flows show a larger proportionate adaptive decrease in flow over time.

Consistent with previous reports, the results of this experiment demonstrate a trend for normalization of CSF pH accompanied with an increase in bicarbonate ion concentration over a 6-hour period. The CSF (and the extracellular fluid within the brain) have almost negligible concentrations of protein or other non-bicarbonate buffers such as phosphates. Thus, changes in the bicarbonate concentration may be regarded as equal to the buffer base changes in the CSF.

CSF normally has a higher \( \text{CO}_2 \) tension and a lower \( \text{pH} \) than arterial plasma, accompanied with a CSF/plasma bicarbonate ratio of about 0.9. The results of this experiment during normocapnia are similar to this ratio (0.83). However, after 360 minutes of hypercapnia the bicarbonate ion ratio reached unity, indicating the presence of a regulatory mechanism other than passive flux of bicarbonate from the blood to CSF.

The details of this regulatory mechanism remain unclear but do not require the presence of an active ion pump. An electrical potential gradient has been demonstrated, being 4 mV positive for CSF in respect to the blood, which is sensitive to the plasma but not the CSF \( \text{pH} \). A shift in this potential difference, with an increase in the plasma bicarbonate concentration by renal mechanisms, may be adequate to explain regulation of the CSF \( \text{pH} \) in respiratory acidosis.

Fencl et al. showed an excellent correlation between the CSF hydrogen ion concentration and CBF. The results of our experiment support their finding for both total brain blood flow and all regions studied. However, if the regression line in Figure 3 is extrapolated to a normocapnic CBF value (48 ml/100 g/min), the corresponding CSF pH is 0.12 units less than that recorded prior to the inhalation of \( \text{CO}_2 \). This suggests that cerebrovascular sensitivity to extracellular \( \text{pH} \) may be altered as a component of the adaptive mechanism during prolonged hypercapnia.

The results of this experiment are inconsistent with the argument that time-dependent decay of CBF is due to physiological deterioration of the preparation. Group 4 demonstrated an unaltered cerebrovascular reactivity to severe hypercapnia after a 5½-hour period of immobilization (Figure 4). In addition, although brain water content was not measured, the significant decrease in sagittal sinus pressure from 8 ± 1 to 3 ± 1 mm Hg seen in Groups 1 and 2 does not support the presence of brain edema, which could impede flow via increased intracranial pressure. Finally, under conditions of maximal or near-maximal cerebral vasodilation, CBF becomes pressure-dependent. Mean arterial pressure remained unchanged in these animals over time (Table 1) and thus could not explain decreased flow secondary to hemodynamic instability.

In conclusion: Normoxic hypercapnia in dogs initially causes a dramatic increase in CBF accompanied by a decrease in CVR. This persists for 3 hours, followed by an adaptive decrease in CBF and increase in CVR. Regional variation occurs; areas with the greatest initial hypercapnic blood flow show a greater rate of decay in flow over time. CSF \( \text{pH} \), initially more acid under hypercapnia, increases over time accompanied by an increase in CSF bicarbonate, probably due to an adaptive regulatory mechanism within the brain. The
time-dependent changes seen did not appear to be a function of preparation deterioration. Total and re-
time-dependent changes seen did not appear to be a

148 Stroke

that a shift in the cerebrovascular sensitivity to extra-
cellular hydrogen ion concentration occurs during pro-

Acknowledgments

The authors are deeply indebted to Dr. Bo K. Siesjö
for reviewing the manuscript during its preparation
and to LaVerle Vust for providing skillful technical as-
sistance in the laboratory.

References

1. Kety SS, Schmidt CF: The effects of altered arterial tensions of
carbon dioxide and oxygen on cerebral blood flow and cerebral
oxygen consumption of normal young men. J Clin Invest
1948;27:484-491

2. Reivich M: Arterial PCO2 and cerebral hemodynamics. Am J
Physiol 1964;206:25-35

3. Wilson DA, Traystman RJ, Rapela CE: Transient analysis of
the canine cerebrovascular response to carbon dioxide. Circ
Res 1985;56:596-605

4. Agnoli A, Boffo L, Nardini M, Battistini N, Feischi C: Ac-
climatizzazione del flusso sanguigno cerebrale nella acidosis re-
spiratoria norosica cronica. Boll Soc Ital Biol Sper
1968;44:709-712

5. Skinhoj E: Regulation of cerebral blood flow as a single func-
tion of the interstitial pH in the brain. Acta Neurol Scand
1966;42:604-607

6. Betz E, Heuser D: Cerebral cortical blood flow during changes
23:726-734

7. Severinghaus JW, Lassen N: Step hypocapnia to separate arte-
rial from tissue PCO2 in the regulation of cerebral blood flow.
Circ Res 1967;20:272-278

of local pH, PCO2, and bicarbonate on pial vessels. Stroke
1977;8:358-360

9. Nilsson B, Siesjö BK: Evidence against H+ as a regulator of
cerebral blood flow, in Owman C, Edvinsson L (eds):
Neurogenic Control of the Brain Circulation. Elmsford, NY,
Per-

ganmon Press, 1977, pp 295-300

10. Merwarth CR, Sicker HO: Acid–base changes in blood and
cerebrospinal fluid during altered ventilation. J Appl Physiol
1961;16:1016-1018

11. Bleich HL, Berkman PM, Schwartz WB: The response of
cerebrospinal fluid composition to sustained hypercapnia. J
Clin Invest 1964;43:11-16


**Key Words**: • brain–blood flow • cerebrospinal fluid pH • hypercapnia
Time-dependent effects of prolonged hypercapnia on cerebrovascular parameters in dogs: acid-base chemistry.
D S Warner, D M Turner and N F Kassell

Stroke. 1987;18:142-149
doi: 10.1161/01.STR.18.1.142

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/18/1/142

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

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

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