Regional Brain Blood Flow in the Conscious Gerbil

ROBERT L. VAN UITERT, M.D. AND DAVID E. LEVY, M.D.

SUMMARY Regional brain blood flow was determined in 23 awake, unparalyzed gerbils with a simplified indicator-fractionation technique. The use of intravenous [14C]-butanol, an indicator that is freely diffusible into the brain, eliminated the need for repetitive sampling of arterial and cerebral venous blood and reduced the period of indicator circulation to 10 seconds. Gerbils spontaneously breathing room air (Paco2 = 32 ± 1 (SE) mm Hg) had blood flows in whole cerebrum, cerebellum, and brainstem of 102 ± 4, 93 ± 5, and 114 ± 6 ml/100gm/min respectively. Cerebral blood flow increased linearly with elevations in Paco2 (r = 0.969) and averaged 3.14 ± 0.17 ml/100gm/min per mm Hg increase in Paco2. Interpolated cerebral blood flow at a Paco2 of 40 mm Hg was 127 ± 2 ml/100gm/min. This technique is easy and convenient to use, involves no intracranial surgery, requires steady state conditions for only 10 seconds, and minimizes blood loss in small animals. In more discrete brain regions a less volatile indicator is needed.

QUANTITATIVE INFORMATION about regional brain blood flow is needed to interpret studies of regional metabolism in experimental stroke. Most blood flow methods that do not involve invasive intracranial procedures are difficult to use in the conscious, unparalyzed, small laboratory animal because they require either prolonged periods for equilibration of indicators or the withdrawal of relatively large amounts of blood (e.g., 133Xenon washout). Refinements of regional brain blood flow techniques have reduced the necessary steady state equilibration period to one minute or less but still have required both the collection of multiple blood samples and either a constant intravenous infusion of an indicator or the injection of an exact amount of indicator coupled with a calculation of cardiac output. Furthermore, antipyrine, the indicator most often used in the more recent techniques, is not ideal because its passage from blood into brain tissue is limited by slow diffusion through the blood-brain barrier as well as by brain blood flow. Use of this indicator thus results in an underestimation of blood flow at rates greater than 95 ml/100gm/min; this includes the normal range of cerebral blood flow found in small rodents.

Many of the practical difficulties of these rapid blood flow techniques could be overcome if a more freely diffusible indicator were employed. Completion of the flow determination before indicator appeared in brain venous blood would eliminate the usual requirement that frequent venous indicator contents either be directly measured or mathematically derived from arterial contents throughout each blood flow determination. Arterial indicator content could then be integrated mechanically by a method described by Scheinberg and Stead. These modifications would not only avoid the inconvenience and possible hemodynamic consequences of repetitive blood sampling but would also greatly simplify the calculation of brain blood flow.

Raichle et al. have shown that even at high flow rates, alcohols with four or more carbons are virtually 100% extracted from the blood by the brain, and they thus recommended butanol as a suitable indicator for measuring regional brain blood flow. Schaefer et al. reported in abstract that [14C]-butanol could be used in a modification of Goldman and Sapirstein's indicator-fractionation technique to measure blood flow in the rat. The present paper provides details of a similarly modified indicator-fractionation method as applied to the measurement of regional brain blood flow in the conscious, spontaneously breathing gerbil. This is an animal widely used in stroke research but so small that measurements of blood flow previously have not been possible without intracranial surgery.

Methods

Theory

After injection of an indicator that is freely diffusible and reaches instantaneous equilibrium between blood and tissue, the blood flow to any tissue can be calculated from the Fick equation as follows:

$$F = \frac{Q(T)}{\int_0^T (Ca-Cv)dt}$$

where:

- $F$ = blood flow to any tissue;
- $Q(T)$ = quantity of indicator present in the tissue at time $T$;
- $Ca$ = concentration of indicator in arterial blood entering the tissue; and
- $Cv$ = concentration of indicator in venous blood leaving the tissue.

For any tissue with a negligible venous outflow of indicator between the time of indicator injection ($t = 0$) and sampling ($t = T$),

$$\int_0^T (Ca-Cv)dt \approx \int_0^T Cadt$$

$Ca$ will be the same for all organs at any given time if blood leaving the left ventricle of the heart contains a homogeneous mixture of the indicator and arrives simultaneously at all organs. The relationship of the indicator accumulated by different organs (i and ii) to the blood flow in these organs can then be derived from equation (1) as follows:

$$\int_0^T Cadt = \frac{Q_i(T)}{F_i} = \frac{Q_{ii}(T)}{F_{ii}}$$

An "artificial organ" with known blood flow can be created by withdrawing arterial blood into a syringe at a known, constant rate, such an organ will have no venous outflow of indicator. If the indicator extraction by the brain is complete, the outflow of indicator from the brain also will be negligible during a suitably chosen brief period of time from...
by guest on October 15, 2017 http://stroke.ahajournals.org/ Downloaded from

Brain blood flow can thus be calculated by determining the indicator content of the measured mass of brain tissue at time T and of the blood withdrawn into the syringe at a constant, known rate \( F_s \) from \( t = 0 \) to \( t = T \).

**Operative Procedures**

Adult male Mongolian gerbils (Meriones unguiculatus) weighing 50–70 gm were anesthetized with halothane (1.5–2.0%), and PE-10 polyethylene catheters were inserted into the tail artery and through the right external jugular vein into the superior vena cava. The catheters were filled with dilute heparinized saline (1000 IU/ml) prior to sealing the catheter tips with melted polyethylene. The arterial cannula was sutured to the ventral tail tendons prior to skin closure; the venous catheter was passed through a puncture wound in the skin and secured to the skin of the nape. Both catheters remained patent for at least 48 hours in over 75% of the animals. During the 50–90 minute surgical procedure, the animals breathed spontaneously and rectal temperature was maintained at 37°C with a thermistor-controlled heat lamp. Gerbils recovered from anesthesia within 5 minutes, and each animal within 1 minute of blood flow determination.

The brain was rapidly removed from the skull, and four brain regions (right and left cerebral hemispheres, cerebellum and brainstem) were separated within 50 seconds of the decapitation. Each brain region was homogenized by forcing it through a 26-gauge needle into a pre-weighed scintillation vial containing 1 ml of a 1:2 mixture of Protosol (New England Nuclear) and ethanol. After the vials were weighed, 10 ml of a solution of Omnifluor in toluene (New England Nuclear; 4 g/liter) were added, and the capped vials were heated at 50°C for at least 12 hours to solubilize the tissue. The arterial blood in the PE-50 catheter was expelled into a scintillation vial containing 0.5 ml of Protosol-ethanol, and 10 ml of Omnifluor in toluene were added. Radioactivity of all samples was assayed in a Searle Mark III liquid scintillation counter, and blood flow in ml/100gm/min was calculated according to equation (4).

Cerebral Blood Flow

Forty-eight hours after catheter placement, the conscious, behaviorally normal gerbils were loosely restrained by passing a 3/4-inch segment of Tygon tubing around the abdomen and taping this "girdle" to an animal board. The head was restrained by hooking the maxillary incisors with a clip attached to the animal board. The arterial cannula was opened, flushed with dilute heparinized saline, and then used to record blood pressure (Statham P23Db transducer) and to sample arterial blood anaerobically for determination of pH (Radiometer), Paco2, and Pao2 (L. Eschweiler and Co.). All 23 animals used in the study met the following criteria while spontaneously breathing room air: mean blood pressure > 80 mm Hg, rectal temperature between 36.5° and 38°C, Pao2 > 70 mm Hg, pH > 7.37, and calculated base excess < 3 meq/l. During the 50-90 minute surgical procedure, the animals breathed spontaneously and rectal temperature was maintained at 37°C with a thermistor-controlled heat lamp. Gerbils recovered from anesthesia within 5 minutes, and each animal within 1 minute of blood flow determination.

The brain was rapidly removed from the skull, and four brain regions (right and left cerebral hemispheres, cerebellum and brainstem) were separated within 50 seconds of the decapitation. Each brain region was homogenized by forcing it through a 26-gauge needle into a pre-weighed scintillation vial containing 1 ml of a 1:2 mixture of Protosol (New England Nuclear) and ethanol. After the vials were weighed, 10 ml of a solution of Omnifluor in toluene (New England Nuclear; 4 g/liter) were added, and the capped vials were heated at 50°C for at least 12 hours to solubilize the tissue. The arterial blood in the PE-50 catheter was expelled into a scintillation vial containing 0.5 ml of Protosol-ethanol, and 10 ml of Omnifluor in toluene were added. Radioactivity of all samples was assayed in a Searle Mark III liquid scintillation counter, and blood flow in ml/100gm/min was calculated according to equation (4).

Cerebral Blood Flow was determined in three groups of gerbils with different arterial CO2 tensions. The first group breathed room air throughout the experiment. The other two groups first breathed room air while arterial blood pressure and blood gases were measured and then were switched to mixtures of oxygen-nitrogen-carbon dioxide designed to produce either moderate hypercapnia (Paco2 = 45 mm Hg) or marked hypercapnia (Paco2 = 60 mm Hg). The gas mixture was delivered to the animals through a loosely fitting plastic nose cone that permitted easy venting of the expired gas. Animals in the two hypercapnic groups met the established criteria for study prior to exposure to CO2 gas and then breathed the CO2 gas mixture for 5 minutes before the blood flow determination. A final blood sample for blood gas measurement was drawn from each animal within 1 minute of blood flow determination. Blood samples collected from each animal totaled less than 10% of the gerbil's estimated blood volume of 4.5 ml.

**Results**

All 23 of the previously cannulated gerbils used for blood flow measurements met the stated physiological criteria at the time of the experiment. Table 1 shows the mean arterial blood gases, blood pressure, and rectal temperature of these

---

*The diffusion of butanol within brain was shown to be minimal by directly injecting a small volume of C-butanol (containing approximately 20,000 dpm) into the right frontal cerebral cortex of gerbil brains 1 mm from the midline. Thirty or sixty seconds after injection, the cerebral hemispheres were separated, and the right cerebral hemisphere was sectioned along a coronal plane 2 mm posterior to the injection site. After 30 seconds, 93.5 ± 1.7 (SE)% of the indicator remained in the right frontal portion of brain, and only 3.8 ± 1.4% had crossed to the left cerebral hemisphere (N = 3). After 60 seconds, 90.5 ± 1.7% of the indicator was present in the right frontal region, and again, 3.8 ± 1.4% had crossed the midline (N = 3).
gerbils and also includes similar data from 5 normal, unoperated gerbils subjected to direct, percutaneous left ventricular cardiac puncture. All animals were unanesthetized and spontaneously breathed room air, but probably because they were restrained, both unoperated and operated gerbils hyperventilated slightly, resulting in a mean $\text{Paco}_2$ of $33 \pm 2$ and $32 \pm 1$ mm Hg, respectively. Physiological data for the 11 gerbils subsequently made hypercapnic are also shown in table 1; the higher $\text{Paco}_2$ in these animals resulted from breathing a gas mixture containing 30% oxygen in addition to the carbon dioxide.

The mean cerebral hemispheric blood flow (CBF) of experimental gerbils breathing room air was $102 \pm 4$ ml/100gm/min (table 2). The blood flow in the left and right cerebral hemispheres of the animals never differed by more than $6 \pm 1$ ml/min. Blood flow to the cerebellum averaged 8% below and to the brainstem 9% above the CBF.

Blood flow in each of the four brain regions increased in response to graded hypercapnia (table 2). Mean cerebral hemispheric flow was linearly related to $\text{Paco}_2$ (fig. 2). The response averaging $3.14 \pm 0.17$ ml/100gm/min for each mm Hg increase in $\text{Paco}_2$ over the range of 28 to 72 mm Hg. Calculated CBF at the "normal" $\text{Paco}_2$ of 40 mm Hg was $127 \pm 2$ ml/100gm/min; hence the CBF response was 2.4% of the normocapnic flow per mm Hg change in $\text{Paco}_2$.

The relationship of mean cerebral blood flow to blood pressure was determined in 10 criteria gerbils and 24 non-criteria gerbils (fig. 3). Non-criteria animals were either mildly hypotensive ($\text{BP} = 70-80$) or acidotic ($\text{pH} = 7.28-7.37$), but the $\text{Paco}_2$ and regional brain blood flow were the same as in the criteria gerbils (table 2). Blood flow could not be determined in all animals with an arterial pressure below 70 mm Hg because the low pressure precluded withdrawal of arterial blood swiftly enough to permit blood flow measurement. No change in CBF was found with changes in blood pressure between 70 and 110 mm Hg.

**Discussion**

The gerbil brain blood flow values obtained by the technique described in this paper compare favorably with values found in other rodents under similar physiological conditions. For gerbils with $\text{Paco}_2 = 40$ mm Hg, CBF is $127 \pm 2$ ml/100gm/min; studies on the normocapnic rat have yielded CBF values ranging from 100 to 130 ml/100gm/min when $^{133}$xenon,$^{1}$ $^{15}$C-antipyrine$^{13}$ or

**Table 1. Physiological Data**

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>$\text{Paco}_2$ (mm Hg)</th>
<th>pH</th>
<th>$[\text{HCO}_3]^{#}$ (meq/l)</th>
<th>$\text{Paco}_2$ (mm Hg)</th>
<th>Mean BP (mm Hg)</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoperated</td>
<td>5</td>
<td>$33 \pm 2$</td>
<td>7.41</td>
<td>$0.02$</td>
<td>$22 \pm 1$</td>
<td>85 $\pm 4$</td>
<td>37.2 $\pm 0.1$</td>
</tr>
<tr>
<td>Experimental (Room air)</td>
<td>23</td>
<td>$32 \pm 1$</td>
<td>7.41</td>
<td>$0.02$</td>
<td>$21 \pm 1$</td>
<td>81 $\pm 2$</td>
<td>90 $\pm 2$</td>
</tr>
<tr>
<td>Moderate hypercapnia</td>
<td>5</td>
<td>$46 \pm 1$</td>
<td>7.30</td>
<td>$0.03$</td>
<td>$23 \pm 1$</td>
<td>175 $\pm 6$</td>
<td>92 $\pm 7$</td>
</tr>
<tr>
<td>Marked hypercapnia</td>
<td>6</td>
<td>$60 \pm 1$</td>
<td>7.13</td>
<td>$0.03$</td>
<td>$20 \pm 1$</td>
<td>148 $\pm 4$</td>
<td>85 $\pm 1$</td>
</tr>
</tbody>
</table>

All values are mean $\pm$ SE.

$^{\#}[\text{HCO}_3]$ was determined from the nomogram for acid-base balance of human blood$^{14}$.
**TABLE 2 Regional Brain Blood Flow**

<table>
<thead>
<tr>
<th>Condition</th>
<th>N*</th>
<th>Paco2 (mm Hg)</th>
<th>Blood flow (ml/100gm/min)</th>
<th>Left</th>
<th>Right</th>
<th>Cerebellum</th>
<th>Brainstem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental (Room air)</td>
<td>10</td>
<td>32 ± 1***</td>
<td>102 ± 4*** 102 ± 3***</td>
<td>93 ± 5***</td>
<td>114 ± 6**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate hypercapnia (Fico2 = 26 ± 1)</td>
<td>5</td>
<td>46 ± 1</td>
<td>142 ± 4      142 ± 6</td>
<td>126 ± 6  150 ± 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marked hypercapnia (Fico2 = 50 ± 1)</td>
<td>6</td>
<td>60 ± 1***</td>
<td>191 ± 7*** 190 ± 6***</td>
<td>184 ± 7*** 206 ± 8**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-criteria animals</td>
<td>24</td>
<td>51 ± 1</td>
<td>97 ± 2       99 ± 2</td>
<td>90 ± 3    102 ± 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SE

*Table does not include two gerbils with Paco2 of 39 and 72 as they did not conveniently fit into any of the stated conditions. These animals are included in fig. 2.

***p < 0.001 compared to the moderate hypercapnic group.

All reported studies have used the invasive hydrogen clearance technique in anesthetized gerbils without physiological monitoring. Osborne and Halsey reported a CBF in the gerbil of 54 ± 24 ml/100gm/min, and the same laboratory reported a value of 36 ± 10 ml/100gm/min in a later communication. A wide range of flows (17-63 ml/100gm/min) was found by these investigators, possibly reflecting unmonitored variation in Paco2 and depth of anesthesia. Variation in the blood flow values obtained with the present indicator-fractionation method was much less, and the values themselves are considerably higher than those previously reported. The discrepancy between these results may reflect the effects on CBF of the pentobarbital anesthesia used with hydrogen clearance; the present study was performed on unanesthetized, spontaneously breathing gerbils. The lower CBF values obtained by Halsey and coworkers may also be consequent to the use of the hydrogen clearance technique, a method which in small animals tends to yield lower CBF values than other methods.

Several details of the present method deserve further consideration. Indicator extraction by brain during the experimental period was shown to be greater than 95% (fig. 1), thus eliminating the need for extensive surgery to obtain cerebral venous blood for every blood flow determination. Much of the marker that did appear in torcular blood samples may have represented contamination of the samples with arterial blood from the skull which was damaged during drilling of the hole into the torcular. A similarly large extraction of butanol by brain has been reported for the rhesus monkey, rat, and dog. Butanol's low molecular weight, its miscibility with blood plasma, and its passage through both right and left sides of the heart and the pulmonary capillary bed make it likely that butanol is homogeneously dispersed through the blood being ejected into the aorta. Simultaneous arterial indicator concentrations at the brain and the tail could differ, however, if the transit times from heart to these organs differed. In the present method, blood flow computation (equation 4) does not require knowledge of the concentrations of arterial blood at specific points in time but involves instead the total amount of indicator withdrawn into the syringe over the 10 second experimental period (Qs(T)). If the transit time from heart to brain were measurably longer than from heart to tail, the total amount...
of indicator presented to brain could be less than the measured \( Q_s(T) \), and the present method would then underestimate brain blood flow. In the more likely circumstance that the transit time to brain is shorter than to the tail, the measured \( Q_s(T) \) could be too small, resulting in an overestimation of flow. The error, however, would be small for the following reasons: (1) any time delay for \(^4\)C-butanol arriving at the tail would be brief since the marker was detected in tail artery blood 1 second after intravenous administration of the indicator bolus in all animals whose blood was sampled at that time; and (2) the \(^4\)C-butanol reaching the brain in excess of that reaching the tail would be the small quantity represented by the low values of the arterial curve in figure 1 between 10 and 11 seconds (<3% of the integrated arterial content for all 5 gerbils).

The advantages of the present method of measuring brain blood flow in small animals far outweigh these potential errors. Goldman and Sapirstein\(^4\) discussed the practical benefits of the indicator-fractionation technique, but their technique required the determination of the cardiac output and the distribution of a known amount of indicator. The present method simplifies this technique. Use of an "artificial organ" permits direct calculation of regional brain blood flow and thus eliminates the need to calculate cardiac output or measure the amount of indicator injected. Blood flow in any region of the brain is determined simply by comparing the \(^4\)C-butanol accumulated in brain tissue during a 10 second interval to the indicator contained in an "artificial organ" with a known, constant blood flow during the same time period. The need for the rapid withdrawal of multiple blood samples, a difficult task in the small laboratory animal with a limited total blood volume, and the construction of blood indicator concentration vs. time curves for each blood flow determination is thereby obviated. Furthermore, injection of a measured quantity of indicator is unnecessary as the brain blood flow is calculated from the ratio of the indicator present in the brain and the blood and not as a fraction of the total amount of indicator injected.

The present study shows that regional brain blood flow can be measured easily in conscious, spontaneously breathing, small animals by a simplification of the indicator-fractionation technique using \(^4\)C-butanol. The measurement is confined to a 10 second interval during which blood flow that is changing even moderately rapidly may be considered to be constant, and thus the technique can be extended to measure relatively non-steady state flow (e.g., studies of cerebral ischemia). CBF measurements in more discrete brain regions would require use of a less volatile indicator than \(^4\)C-butanol; such a refinement would add even further to the value of this method. The present method gives reproducible results and provides the first non-invasive regional brain blood flow measurements in the gerbil.

Acknowledgment

The authors gratefully acknowledge the expert technical assistance of Catherine L. Pike and the helpful advice of Dr. Fred Plum.

References

8. Raichle ME, Eichling J, Straatmann MG, Welch MJ, Larson KB, Ter-


Regional brain blood flow in the conscious gerbil.
R L Van Uitert and D E Levy

Stroke. 1978;9:67-72
doi: 10.1161/01.STR.9.1.67

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1978 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

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/9/1/67

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/