Serial Measurement of Cerebral Blood Flow Using External Counting of Microspheres

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SUMMARY Described is a modified method of measuring organ blood flow which combines the serial injection of a standard dose of microspheres and the external counting of their gamma activity when they are distributed to the tissues. The method produces similar results to measurements of grey matter blood flow by the clearance of $^{133}$xenon.

USE OF EXTERNAL counting of the clearance of a radioactive inert gas such as $^{133}$xenon to measure blood flow is widespread. However, the technique involves certain assumptions which may not be correct when comparing a control to an experimental measurement. In the study of cerebral blood flow the use of the $^{133}$xenon technique has recently been criticized. The main arguments used were that the extracerebral tissues were not excluded from the counting field, the capillary permeability for xenon may alter and the analysis of the clearance curve is often not as straightforward as a simple biexponential curve. The radio-labelled microsphere technique was proposed as a viable alternative. However, this microsphere technique has certain drawbacks. The number of flow determinations is limited by the number of differently labelled microspheres available. The alterations in flow may not be monitored as an experimental measurement, and the addition of an isotopic, such as $^{95}$Nb, with a large amount of backscatter into low keV ranges, will render inaccurate counting of low keV isotopes ($^{141}$Ce). In addition, it is possible that serial lodging of spheres in the tissue may alter flow.

The present study has resolved some of these disadvantages by external counting of standard injections of microspheres with a single radiolabel.
Methods and Materials

Cerebral blood flow (CBF) was studied in 4 baboons (*Papio ursinus*) by a modified microsphere technique. The CBF at normocapnia was measured and then the alteration of flow was determined with hypercapnia and hypocapnia, and after returning to a normocapnic situation. The baboons were sedated with an intramuscular injection of 0.2 ml phencyclidine (Sernylan, Bio-ceutic) and surgical anesthesia induced by intravenous injection of 50 mg thiotetone (Intraval Sodium M + B). The animals were intubated and ventilated using a Harvard positive pressure ventilator.

A catheter was placed retrograde in the left femoral artery for recording arterial blood pressure using a Statham P23AA transducer and for removal of arterial blood for estimation of PaCO₂, PaO₂, pH, hemoglobin (Hb) and oxygen saturation percentage (HbO₂) prior to each flow determination. A similar catheter was placed in the right brachial artery. Blood was collected from this catheter directly into preweighed Packard scintillation vials for counting of blood microsphere content (C₀) at each injection point. A third catheter was passed via the right femoral artery into the left atrium. This was used for injection of the microspheres. Lastly, a catheter was placed retrograde in the internal jugular vein with its tip in the sigmoid sinus. This was used to collect mixed cerebral venous blood for determination of cerebral venous Hb and HbO₂. The position of the tip of each catheter was confirmed radiologically.

In addition, the skin and temporalis muscle over the parietal area of the brain were removed and a 50 mm diameter sodium iodide crystal scintillation detector was placed over the region. The detector was fitted with a lead collimator to cone the counting field into one of 20 mm in diameter and was further collimated 50 mm into a lead collimation tube to exclude all noncerebral tissue from the counting field. Four leads from a Beckman EEG recorder were screwed into the skull in this region and the area of skull under the detector was carefully marked. The detector was connected to a Nuclear Enterprises Amplifier and Rate-meter system and the analog scintillation signal was displayed, together with pulsatile and mean arterial blood pressure and respiratory % CO₂ (from a capnograph) on a chart recorder. Also, the analog signal was converted into a digital form using appropriate electronics (Nuclear Enterprises).

After surgery the thiotetone was continuously infused (i.v.) at a rate sufficient to maintain anesthesia at a light level (0.5–2.5 mg/min). This level of anesthesia was achieved by maintaining the rhythms of the electroencephalograph at a dominant alpha frequency.

The microspheres used in this study were 15 μm in diameter and were labelled with ⁴⁶Sc. They were supplied as a suspension in saline with Tween 80 as a surfactant (3M). The following protocol was used for each flow determination. Arterial and cerebral venous blood samples were taken. These were used for determination of arterial PCO₂, PO₂, pH, hemoglobin (Hb), hemoglobin-oxygen saturation (HbO₂) and venous Hb and HbO₂. The EEG was monitored and confirmed to be within the alpha frequency range. Rectal temperature was maintained at 37.5 ± 1.5°C (mean ± range). The microspheres were vigorously shaken using a flask shaker for at least 5 minutes. Meanwhile, the background activity of the brain was recorded for at least 2 minutes. A 100 μl quantum of the mixed microspheres was drawn into a syringe. Meanwhile, blood was allowed to flow directly from the brachial artery catheter into a preweighed scintillation vial. The bolus of spheres was injected rapidly into the left atrial catheter and was flushed into the heart using 5 ml of saline. Blood sample collection continued for exactly one minute and at the end the mass of blood collected (M) was determined by reweighing the vial. The volume of this blood and, therefore, the blood sampling rate in ml/min was determined by dividing M by the mass of a calibrated sample of 5 ml of blood and multiplying by 5. During the injection of spheres the radioactivity of the brain was continuously recorded. The arrival of the spheres and their entry into the cerebral circulation was accompanied by a step increase in recorded radioactivity. The amplitude of this step was used to measure blood flow.

After the baseline determination at a PaCO₂ of 35 ± 2.5 and PaO₂ of 80 ± 10 mm Hg (mean ± range, normal blood gases at an altitude of 1600 meters), the procedure was repeated with hypercapnia (55 ± 2.5 mm Hg), hypocapnia (25 ± 2.5 mm Hg) and finally at normocapnia again. At the end of the experiment the animal was killed with an overdose of barbiturate anesthetic and the head dissected to remove, 1) the area of bone underneath the detector, 2) the dura under the detector and 3) the cerebrum under the detector to a depth of 15 mm below the surface of the cortex. These samples were placed in preweighed scintillation vials and were counted together with the reference blood samples from the 4 flow determinations in a well type gamma spectrometer (Packard). The window settings used throughout for the counting of ⁴⁶Sc was 1000–1500 keV.

Computation of Blood Flow

The external counting of the arrival of spheres labelled with ⁴⁶Sc gave a series of step changes in recorded count. The size of each step was measured by calculating the mean background before and after injection of spheres and calculating the difference between these means. The amplitude of each step is proportional to the number of spheres arriving at the time of injection and is therefore an index of blood flow at that time. The relative size of each step was calculated by adding all 4 steps together and calculating the % that each individual step was of the total (table 1).

The postmortem counting of reference blood samples and the brain tissue was then used to determine cerebral blood flow. The total activity counted with the external scintillation detector was the direct result of microspheres remaining in the brain blood.
vessels under the detector. Therefore, when this section of tissue was counted in the well spectrometer the % distribution of the external counting was used to separate the spectrometer total into the 4 component parts representing blood flow with the 4 injections of microspheres. The blood reference samples were also counted in the spectrometer and thus each flow could be calculated using the formula:

\[
\text{Flow (ml/min/100 g)} = \frac{C_T \cdot R \cdot 100}{C_B \cdot T}
\]

- \( C_T \) = Component one of spectrometer tissue count
- \( C_B \) = Blood count at time of component one external counting
- \( R \) = Sampling rate of blood
- \( T \) = Brain tissue mass (g)

This process was repeated for components 2, 3 and 4.

Bone and Dura

The calculation of cerebral blood flow assumes that all of the externally counted amount was lodged in the sample of brain removed for the well spectrometer. The inaccuracy of this assumption was assessed by calculating the percentage that the bone plus dura radioactivity contaminated the brain sample. Thus the role of the tissues between the detector and brain could be estimated. The deep brain tissues would also be counted in the external field. However their involvement was expected to be small as counting efficiency decreases rapidly with the distance away from the crystal detector.

Comparison with Xenon

Lastly, the flow estimations and the responsiveness to alteration in arterial \( P_{\text{CO}} \) was compared between this new technique and with previous work in our laboratory using established \( ^{133} \text{Xe} \) clearance techniques.7

Flow/unit Metabolism

It has become increasingly evident that flow measurements in an organ such as the brain should be accompanied by some estimation of metabolism.8 This is in an attempt to determine if the experimental stimulus (in this case arterial \( P_{\text{CO}} \)) causes the observed alteration in CBF by a direct action on cerebral resistance vessels or by indirectly influencing vascular resistance by changes in cerebral metabolism.

A direct measurement of the oxygen extraction by the brain was made with each injection of microspheres. Using the Hb (g/ml) and HbO\(_2\) values in arterial and cerebral venous blood and assuming that 1.34 ml of O\(_2\) are carried per gram of Hb, the arterio-venous oxygen difference (A-VO\(_2\)) across the brain could be calculated. The cerebral metabolic rate for oxygen (CMR\(_{\text{O}_2}\)) is related to the A-VO\(_2\) and the mean cerebral blood flow (CBF) by the Fick equation as follows:

\[
\text{CMR}_{\text{O}_2} = \frac{\text{CBF} \times \text{A-VO}_2}{12}
\]

Statistics

The measured blood flow and other variables in the normocapnic and experimental situations were routinely compared using the Student’s paired \( t \)-test. In addition, an \( F \) test of the variances was performed. When the variances were found to be significantly different a Wilcoxon rank sum test was used in substitution for the \( t \)-test.

Results

In the present experiments arterial \( P_{\text{CO}} \) was altered from a mean baseline value of 34.2 ± 1.1 mm Hg to 55.1 ± 0.8 mm Hg, then to 25.7 ± 0.4 and finally to 34.4 ± 0.8 mm Hg. The mean cerebral blood flow measured at these different \( P_{\text{CO}} \) values together with the concurrent measurements of \( P_{\text{AO}} \), mean arterial blood pressure (MBP) and the CBF per unit CMR\(_{\text{O}_2}\) are shown in table 2. Hypercapnia was associated with an increased CBF from the baseline value of 53.1 ± 7.3 ml/min per 100 g of tissue to 85.4 ± 13.4 ml/min/100 g (p < 0.05). This flow alteration was not accompanied by a significant alteration in \( P_{\text{AO}} \) or MBP. However, the ratio of CBF to CMR\(_{\text{O}_2}\) was significantly increased (p < 0.025) indicating that the primary action of the increase in arterial \( CO_2 \) was to increase CBF by a direct vasodilator action on cerebral blood vessels.

Hypocapnia by hyperventilation was associated with a decrease in CBF to 31.7 ± 4.2 ml/min/100 g (p < 0.025). This change was due to a direct vasoconstrictor action on cerebral blood vessels as the CBF per unit CMR\(_{\text{O}_2}\) was decreased below baseline.

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**Table 1 Example of the Calculation of the Relative Blood Flow to the Brain by the External Counting of \(^{51}\text{Cr} \) Labelled Microspheres. \( N \) Equals the Number of Determinations of Background.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Radioactivity counts/10 min</th>
<th>Step size</th>
<th>% of total activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocap</td>
<td>10</td>
<td>121.38</td>
<td>21.93</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>359.82</td>
<td></td>
</tr>
<tr>
<td>Hypercap</td>
<td>10</td>
<td>352.75</td>
<td>34.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>722.40</td>
<td></td>
</tr>
<tr>
<td>Hypocap</td>
<td>10</td>
<td>719.80</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>938.20</td>
<td></td>
</tr>
<tr>
<td>Normocap</td>
<td>10</td>
<td>936.20</td>
<td>23.97</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1196.80</td>
<td></td>
</tr>
</tbody>
</table>

The CBF per unit CMR\(_{\text{O}_2}\) may be calculated as follows:

\[
\text{CBF/CMR}_{\text{O}_2} = \frac{\text{CBF}}{\frac{\text{CBF} \times \text{A-VO}_2}{12}} = \frac{1}{\text{A-VO}_2}
\]

Thus, the CBF per unit CMR\(_{\text{O}_2}\) may be assessed by calculating the reciprocal of the A-VO\(_2\) difference.
The present technique may more closely approximate to measurement of cortical gray matter rather than cortical and subcortical white matter.

Bone and Dura

The mean ratio of spectrometer sphere count in bone and dura to the count in the underlying cerebral tissue was found to be only 7.7% of the cerebral count and, therefore, artifacts due to contamination by counts derived from bone or dura blood flow would not significantly alter the measured cerebral flow response to stimuli.

Discussion

This study was stimulated by a need for a method of CBF measurement which would avoid the assumptions of inert gas clearance technique, but preserve its repetitive ease of measurement. The present technique may provide the answer to such a need. In removing some assumptions, however, new ones have taken their place.

The major first assumption is that the gamma count from the postmortem tissue can be correlated to the data generated by the external scintillation detector. The bone and dura under the external detector could only have contributed a small percentage error to the technique. Tissues far away from the detector would also be expected to contribute little to the external count as the signal generated by these deep tissues is proportional to the reciprocal of the distance from the detector. Thus, it is likely that the external count was mainly generated from the first layer of cortex under the detector. The observation that the blood flow value from the present experiment more closely approximates that found in the fast clearing component (gray matter) of the xenon curve further supports this probability. The approximation may, in fact, be even better than reported here if pure gray matter had been sampled instead of the mixed gray-white wedge of tissue. This, however, still remains uncertain.

The responsiveness of the microsphere flow to alteration in CO₂ is also similar to the xenon gray matter changes. The microsphere data showed a change in flow of 3.53% per mm Hg CO₂. This is similar to the CO₂ reactivity generally quoted, but lower than that reported for the microsphere technique in the dog. Comparisons with other identical experiments in our laboratory using ¹³³xenon show the data generated by the external scintillation detector. Tissues far away from the detector would also be expected to contribute little to the external count as the signal generated by these deep tissues is proportional to the reciprocal of the distance from the detector. Thus, it is likely that the external count was mainly generated from the first layer of cortex under the detector. The observation that the blood flow value from the present experiment more closely approximates that found in the fast clearing component (gray matter) of the xenon curve further supports this probability. The approximation may, in fact, be even better than reported here if pure gray matter had been sampled instead of the mixed gray-white wedge of tissue. This, however, still remains uncertain.

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Table 2: CBF with Alteration of Paco₂. Mean Values of CBF, Paco₂, MBP and CBF per Unit CMRO₂ are Shown at Each Level of Paco₂. The Values are Shown ± One SE and the Asterisk Indicates a Significant Difference from the First Normocapnic Baseline.

<table>
<thead>
<tr>
<th>Arterial Paco₂ (mm Hg)</th>
<th>CBF (ml/min/100g)</th>
<th>Paco₂ (mm Hg)</th>
<th>MBP (mm Hg)</th>
<th>CBF/CMRO₂ (ml/min/m10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.2 ± 1.1</td>
<td>55.1 ± 0.8</td>
<td>80.6 ± 3.7</td>
<td>97.0 ± 4.7</td>
<td>28.2 ± 7.5</td>
</tr>
<tr>
<td>53.1 ± 7.3</td>
<td>85.4 ± 13.4*</td>
<td>98.7 ± 8.5</td>
<td>65.9 ± 17.4*</td>
<td>13.6 ± 2.4*</td>
</tr>
<tr>
<td>25.7 ± 0.4</td>
<td>31.7 ± 4.2*</td>
<td>94.4 ± 3.2*</td>
<td>81.2 ± 10.1*</td>
<td>21.8 ± 6.3</td>
</tr>
<tr>
<td>34.4 ± 0.8</td>
<td>47.7 ± 4.9</td>
<td>79.8 ± 4.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison with Xenon

Table 3 shows previously recorded values of mean CBF measured by ¹³³xenon and the responsiveness of this index to the same alteration in Paco₂ as the present study. In addition, the percentage changes from normocapnic baseline were calculated. These ¹³³xenon absolute values and percentage changes are shown together with the absolute values and percentage changes in the present study. The ¹³³xenon results are derived from a study of 5 normal baboons. The comparison shows that the present method tends to overestimate CBF in comparison with our xenon technique and also shows a larger responsiveness to alteration of CO₂. A better correlation was found between xenon derived gray matter flows and the present data. The present technique may provide the answer to such a need. In removing some assumptions, however, new ones have taken their place.

Bone and Dura

The mean ratio of spectrometer sphere count in bone and dura to the count in the underlying cerebral tissue was found to be only 7.7% of the cerebral count and, therefore, artifacts due to contamination by counts derived from bone or dura blood flow would not significantly alter the measured cerebral flow response to stimuli.

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Table 3: Comparison of ¹³³Xenon and the Present Microsphere study. Values of CBF and the Percentage change from Normocapnic Baseline are Shown for Each Level of Paco₂ (mean ± range) and for Each Method of CBF Measurement.

<table>
<thead>
<tr>
<th>Arterial Paco₂ (mm Hg)</th>
<th>Hypocapnia</th>
<th>Normocapnia</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ± 2.5</td>
<td>35 ± 2.5</td>
<td>55 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

¹³³Xenon

mean CBF 26.1 ± 3.4 31.9 ± 1.7 43.3 ± 3.3
% change -18.2 0 +35.7
Grey CBF 38.0 ± 6.0 48.7 ± 3.0 69.9 ± 6.1
% change -21.9 0 +30.3

Microspheres

CBF 31.7 ± 4.2 53.1 ± 7.3 85.4 ± 13.4
% change -40.3 0 +60.8
present CO₂ reactivity to be greater than before. However, in comparison to our previous baboon study using ¹³³xenon and a different anesthetic the present values are low. Therefore, it seems that reported CO₂ reactivity depends on the flow measurement technique, the species studied, and the type of anesthesia used.

Secondly, it may have been expected that sequential blocking of cerebrovascular capillaries would have reduced normocapnic blood flow. The present data show a small decrease between the first normocapnic determination and the final measurement. However, the difference was not significant and thus at least 4 sequential measurements of flow may be made using this technique. In effect, it is probable that many more measurements are possible as the number of spheres per injection is minute in comparison to the billions of capillaries in the systemic cardiovascular system.

The present technique obviates many of the criticisms of the ¹³³xenon clearance method. Extracerebral tissues were excluded from the counting field by removal of skin and muscle with heavy shielding and collimation of the detector. Changes in capillary permeability would not have produced artifacts in the number of spheres lodged in the tissue as the spheres were sufficiently large to lodge in precapillary resistance vessels. In addition, there is no complex clearance curve to analyse, and only simple mathematical calculations to make.

It remains to be seen if this modification of the microsphere technique will prove useful. However, it does seem to combine the best features of inert gas clearance techniques and multilabel microsphere injection.

References

Serial measurement of cerebral blood flow using external counting of microspheres.
T A McCalden

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