Arterial Microsphere Concentrations in Cats Are Not Affected by Changes in Hematocrit

Douglas S. DeWitt, PhD; Donald S. Prough, MD; Dwight D. Deal, BS; Helena M. Hoen, MS

Background and Purpose Acute anemia may lead to erroneously low arterial reference sample concentrations of radioactive microspheres, depending on the sampling rate and the size of the artery from which the reference samples are withdrawn. Because this error would lead to falsely high cerebral blood flow values in studies involving hemodilution caused by hemorrhage and fluid resuscitation, we studied the effects of hematocrit, withdrawal rate, and vessel location and size on arterial microsphere concentrations in anesthetized adult cats.

Methods Cats were anesthetized with ketamine, isoflurane, and nitrous oxide; both brachial arteries were cannulated with polyethylene tubing, as was the abdominal aorta through the femoral artery. Sequential left atrial microsphere injections were made using several doses of each of five isotopes. The rate of reference sample withdrawal from the three sampling catheters was randomized to 1.03 mL • min⁻¹ or 2.06 mL • min⁻¹. We analyzed the ratio of the number of microspheres in paired reference samples using the factors hematocrit, rate of withdrawal, and site. A ratio less than 1 indicates an underestimation of arterial microsphere concentration, which would lead to erroneously high cerebral blood flow values. The procedure was repeated after isovolemic hemodilution with 10% hetastarch to hemoglobin levels approximating 85%, 70%, 55%, and 40% of baseline.

Results No significant effects of hematocrit on ratios of microsphere concentrations existed at any withdrawal rate or site. Ratios of microsphere concentrations in reference samples withdrawn slowly (1.03 mL • min⁻¹) from the aorta and ratios of microsphere concentrations withdrawn either rapidly (2.06 mL • min⁻¹) or slowly from the brachial arteries were significantly (P<.001) less than 1.

Conclusions Hemodilution did not affect microsphere concentrations in arterial reference samples at any withdrawal site or rate and therefore does not affect the accuracy of microsphere blood flow determinations. However, slow withdrawal from a large vessel may underestimate actual microsphere concentrations. (Stroke. 1994;25:1842-1846.)

Key Words • cerebral blood flow • hemodilution • hemorrhage • resuscitation • cats

The requirements for accurate determination of cerebral blood flow (CBF) by means of radioactive microspheres have been carefully enumerated.¹² If the reference sample technique is used, it is essential that the number of microspheres in the reference sample accurately reflect the distribution of microspheres in the arterial circulation.² A variety of factors, including the diameter of the injected microspheres, the rate of withdrawal of the reference arterial sample, and the number of microspheres in the reference arterial and tissue samples,³⁴ have been reported to influence the accuracy of the measurement of reference arterial microsphere content. Moore et al⁵ demonstrated that more rapidly drawn reference samples produced higher counts and therefore lower renal blood flow calculations than did less rapid withdrawal. Rosenberg et al⁷ subsequently reported that in newborn lambs and adult sheep, acute dilutional anemia aggravated the discrepancy between rapidly and slowly withdrawn reference samples.

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The radioactive microsphere method has been used to measure regional CBF after hemodilution by ourselves⁸-¹⁰ and others,¹¹-¹⁴ but the results of Rosenberg et al¹⁵ suggest that changes in hematocrit may invalidate the method. Because hemodilution affected reference sample concentrations in newborn lambs but not in adult sheep or dogs, we considered the possibility that hemodilution might affect the accuracy of the microsphere method only in certain circumstances in one species. To assess the validity of the microsphere method used in studies of acute hemorrhage and hemodilution during fluid resuscitation in cats, we investigated the effects of hematocrit, withdrawal rate, and withdrawal site on reference arterial microsphere concentrations in cats. To assess arterial reference sample microsphere concentrations at different withdrawal rates at different hematocrits, repeated injections of each isotope were necessary, thereby preventing multiple CBF determinations.

Materials and Methods

In a protocol approved by the Institutional Animal Care and Use Committee, six mongrel adult cats of either sex, weighing 3.1±0.3 kg, were anesthetized with ketamine hydrochloride (25 mg • kg⁻¹ IV), tracheotomized, paralyzed with pancuronium bromide (1 mg • kg⁻¹), and mechanically ventilated with 1.6% isoflurane in N₂O/O₂ (70:30). Polyethylene cannulas (PE-90; inner diameter, 0.8 mm) were advanced approximately 5 mm into the proximal brachial arteries. The right femoral artery was cannulated with larger-diameter tubing (PE-190;
inner diameter, 1.2 mm), which was advanced approximately 3 cm into the abdominal aorta. The left femoral artery and vein were cannulated for blood pressure monitoring and drug infusion, respectively. Through a left thoracotomy, a cannula with a slightly flared tip was placed in the left atrium for microsphere injection. After surgical preparation, isoflurane concentration was decreased to approximately 0.8% in N2O/O2 (70:30). The animals were allowed to stabilize for 30 minutes, during which time arterial pH, Pco2, and Po2 were adjusted to normal limits.

At the conclusion of the equilibration interval, baseline CBF was measured by means of radioactive microspheres labeled with 14C. Approximately 1 million microspheres (15 μm) suspended in 0.9% saline and polyoxyethylene sorbitan monololate (Tween 80) were injected into the left atrium. Before injection, the microspheres were agitated for 4 minutes with a vortex mixer. Immediately before and for 90 seconds after injection, an arterial reference sample was withdrawn from each brachial artery at a rate of 1.03 mL · min⁻¹ with a syringe pump (EDCO Scientific). After the baseline injection of microspheres, a total of 10 sequential injections were performed with four other microspheres, labeled with 15Sn, 14Sr, 108Gd, or 98Nb. Because we were comparing only arterial reference sample microsphere concentrations and because microspheres are rapidly cleared from the systemic circulation, repeated injections of spheres labeled with the same radionuclide were performed. However, with the exception of 14C used for baseline CBF calculation, the repeated injections of identically labeled microspheres prevented the determination of CBF other than at baseline. During the first of each of five paired injections, reference samples were withdrawn from one brachial artery at a rate of 1.03 mL · min⁻¹ and from the other brachial artery and the abdominal aorta at a rate of 2.06 mL · min⁻¹. For the second injection, reference samples were withdrawn from one brachial artery at a rate of 2.06 mL · min⁻¹ and from the other brachial artery and the abdominal aorta at a rate of 1.03 mL · min⁻¹. Using this protocol, we compared the number of microspheres in reference samples collected at both slower and faster withdrawal rates from both small and large arteries at each level of hematocrit. This procedure was repeated as the cats were isovolemically hemodiluted with 10% hydroxyethyl starch to hemoglobin levels of 85%, 70%, 55%, and 40% of the baseline hemoglobin concentration.

At the conclusion of the experiment, the cats were euthanized with sodium pentobarbital (100 mg · kg⁻¹); the brains were removed and dissected, and radioactivity was counted (Autogamma 5550, Packard Instruments). The number of microspheres in each reference arterial sample was calculated from the number of counts in each microsphere. Baseline CBF was calculated by means of the reference method described elsewhere.3

CBF was calculated from the first injection only (14C), as described above. For subsequent injections, we analyzed the ratio of the number of microspheres per volume of blood in simultaneously obtained reference samples. Because each injection contained a different, unknown number of microspheres, ratios were used to show the relation between pairs of samples from the same injection. Ratios less than 1 would indicate erroneously low or high microsphere concentrations in the numerator or the denominator, respectively. An erroneously high arterial reference microsphere concentration could result from uneven distribution of spheres, but 15 μm are evenly distributed across the arterial diameter.10 Therefore, ratios less than 1 would likely indicate an underestimation of reference sample microsphere concentration, which would lead to erroneously high CBF values. The ratios were log-transformed to stabilize their variance across the range of hemoglobin levels and to normalize the distribution. Hemoglobin effect on the log-transformed ratios was investigated by simple linear regression analysis of hemoglobin concentration versus ratio of reference sample microsphere concentration. The antilogs of the regression equations were calculated for graphing the regression lines with the untransformed ratios. Individual tests to determine whether ratios differed significantly from 1 were performed with paired t tests on the log-transformed ratios. A significance level of .05 was used for all procedures; however, correction for multiple testing, which would protect against falsely detecting a difference in arterial reference microsphere concentrations, was not performed. We believed that failing to detect a difference would be the more serious error. Because most pairs of reference samples contained fewer than 5000 microspheres, an assumed number of 5000 was used to calculate the confidence limits displayed in Figs 1 and 2 (Buckberg et al.16)
Results

All data in the text and tables are displayed as mean±SEM. With the exception of physiological variables and baseline CBF (Table 1), the data in the text and in Fig 2 are ratios of the number of microspheres per milliliter of blood withdrawn simultaneously from two blood vessels. Thus, a ratio of 1 would indicate that identical numbers of microspheres per milliliter of blood were withdrawn from the two vessels. As described above, the experimental design required repeated injections of spheres labeled with the four radionuclides used to calculate the ratios, and CBF could be calculated only at baseline (ie, when ^46Sc was injected only once).

Baseline hemoglobin concentration was 10.8±0.4 g·100 mL⁻¹. Hemoglobin levels at each of the four subsequent posthemodilution intervals were 9.4±0.4, 7.5±0.3, 6.0±0.2, and 4.4±0.2 g·100 mL⁻¹, respectively. Arterial pH, Pco2, and body temperature were similar at all measurement intervals and were within the normal range for cats (Table 1). Whole brain CBF, calculated only at the first interval, was 61.6±9.4 mL·100 g⁻¹·min⁻¹. Arterial reference sample microsphere numbers varied between 473 and 8353, with 95% of the samples containing more than 650 microspheres (Table 2). In addition, there was a significant correlation (P<.02) between level of hemodilution and arterial microsphere concentrations (ie, the lower the hemoglobin level, the lower the microsphere numbers). A decrease in microsphere concentration may be due to an increase in cardiac output anticipated during hemodilution.

Ratios of sphere concentrations from samples withdrawn rapidly from a larger vessel (aorta, 2.06 mL·min⁻¹) and slowly from a smaller vessel (left brachial artery, 1.03 mL·min⁻¹) were not affected by changes in hematocrit (Fig 1), nor did they differ significantly from 1 at any hemoglobin concentration (Fig 2). Ratios of sphere concentrations from samples withdrawn slowly from the aorta and at either rate from the brachial arteries were significantly lower than 1 (P<.001; Fig 2), but there was no effect of hemoglobin concentration in either case.

Although other ratios were significantly lower or higher than 1 at certain hemoglobin concentrations, they were all within the confidence limits calculated based on number of microspheres withdrawn as described above. In no case was there a relation between hemoglobin concentration and arterial microsphere concentration ratio.

Discussion

Our results indicate that hemodilution does not affect the accuracy of radioactive microsphere CBF determinations. The present study demonstrates that arterial reference sample microsphere concentrations in cats are independent of hemoglobin concentration regardless of the rate of withdrawal of reference sample, vessel size, or location. We also demonstrated that, while withdrawal rate may affect microsphere reference sample concentrations from small vessels (ie, brachial artery), the differences are minor (<10%).

Although approximately 11 million microspheres were injected over the course of these investigations, each injection of more than 1 million 15-μm microspheres in cats blocked 0.032% of gray matter and 0.044% of white matter microvessels, and the 11 injections used in this study should have blocked less than 0.5% of cerebral arterioles. Therefore, the injection sequence employed would have been unlikely to

Table 1. Physiological Variables in Cats at Variable Hemoglobin Concentrations

<table>
<thead>
<tr>
<th>Variable Hemoglobin Concentration, % Baseline</th>
<th>100</th>
<th>85</th>
<th>70</th>
<th>55</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g·100 mL⁻¹</td>
<td>10.8±0.4</td>
<td>9.4±0.4</td>
<td>7.5±0.3</td>
<td>6.0±0.2</td>
<td>4.4±0.2</td>
</tr>
<tr>
<td>CBF, mL·100 g⁻¹·min⁻¹</td>
<td>61.6±9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.40±0.01</td>
<td>7.38±0.02</td>
<td>7.36±0.01</td>
<td>7.35±0.01</td>
<td>7.28±0.02</td>
</tr>
<tr>
<td>Paco2, mm Hg</td>
<td>30.5±0.5</td>
<td>30.1±0.8</td>
<td>30.4±0.6</td>
<td>29.7±0.6</td>
<td>31.8±0.4</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>135±6</td>
<td>139±6</td>
<td>146±5</td>
<td>151±5</td>
<td>153±4</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>116±13</td>
<td>113±8</td>
<td>106.0±9</td>
<td>96±7</td>
<td>85±9</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>195±6</td>
<td>182±7</td>
<td>182±6</td>
<td>210±10</td>
<td>226±12</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>38.1±0.1</td>
<td>38.2±0.1</td>
<td>38.2±0.1</td>
<td>38.3±0.2</td>
<td>38.3±0.2</td>
</tr>
</tbody>
</table>

Hb indicates hemoglobin; CBF, cerebral blood flow (calculated only at first level of Hb); MAP, mean arterial pressure; and bpm, beats per minute. Values are mean±SEM.

Table 2. Arterial Sample Microsphere Concentrations During Progressive Hemodilution

<table>
<thead>
<tr>
<th>% Hb</th>
<th>Rate</th>
<th>RMS (Mean±SD)</th>
<th>n</th>
<th>RMS (Minimum)</th>
<th>RMS (Maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>f</td>
<td>3597±2020</td>
<td>18</td>
<td>1129</td>
<td>8353</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>1605±1083</td>
<td>18</td>
<td>556</td>
<td>4111</td>
</tr>
<tr>
<td>85</td>
<td>f</td>
<td>2730±1357</td>
<td>18</td>
<td>1341</td>
<td>4964</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>1419±826</td>
<td>18</td>
<td>631</td>
<td>3751</td>
</tr>
<tr>
<td>70</td>
<td>f</td>
<td>2655±732</td>
<td>18</td>
<td>1505</td>
<td>3651</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>1277±402</td>
<td>18</td>
<td>720</td>
<td>2089</td>
</tr>
<tr>
<td>55</td>
<td>f</td>
<td>2283±1049</td>
<td>18</td>
<td>982</td>
<td>5427</td>
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<tr>
<td></td>
<td>s</td>
<td>1157±615</td>
<td>18</td>
<td>473</td>
<td>2733</td>
</tr>
<tr>
<td>40</td>
<td>f</td>
<td>1987±668</td>
<td>18</td>
<td>1276</td>
<td>3192</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>953±305</td>
<td>17</td>
<td>562</td>
<td>1519</td>
</tr>
</tbody>
</table>

% Hb indicates percentage of baseline hemoglobin; f, 2.06 mL·min⁻¹ withdrawal rate; s, 1.03 mL·min⁻¹ arterial withdrawal rate; RMS, arterial microsphere numbers; and n, number of arterial samples withdrawn at each rate or level of hemodilution.
have embolized sufficient microvessels to produce significant cerebrovascular or cardiovasucular effects.

Confidence limits were calculated by means of the binomial distribution based on the largest (5000) microsphere concentrations in most sample pairs. We based our confidence limits on the largest number of spheres because that number yielded the most conservative limits, thereby minimizing the likelihood of concluding that hemoglobin concentration had no effect when, in fact, a hemoglobin effect did exist.

Accuracy of measurement of microsphere concentrations in arterial reference samples is essential to the precise measurement of CBF by means of radiolabeled microspheres. Rosenberg et al compared microsphere concentrations in arterial reference samples withdrawn through a small catheter at a rate of 1.3 mL • min⁻¹ with samples withdrawn from a larger catheter in the carotid artery at 7.89 mL • min⁻¹ in newborn lambs. The reference sample microsphere concentrations were equivalent at hematocrit values exceeding approximately 32%, but as hematocrit progressively declined, the sample drawn slowly from the small catheter measured erroneously low concentrations of microspheres in a hematocrit-dependent fashion. Similar comparisons made in samples from adult sheep or dogs or in newborn lambs at different withdrawal rates demonstrated no such relation between microsphere concentration and hematocrit (see Reference 7 and Fig 2). These observations indicate that, with the possible exception of studies in the newborn lamb, hemodilution does not affect the accuracy of microsphere blood flow measurements.

While we observed no significant effect of hematocrit on the ratio of samples withdrawn rapidly from a large vessel or slowly from a small vessel, because the slope of the regression line relating hemoglobin concentration to microsphere concentration ratios was not significantly different from zero (Fig 1). Rosenberg et al suggested that axial streaming, perhaps exacerbated by hemodilution, may have contributed to a paucity of microspheres sampled slowly from a small catheter at the periphery of a vessel. In contrast, we were withdrawing samples from a catheter that completely occluded the artery, which may have prevented any effects of axial streaming on our arterial sampling technique and may account, in part, for the differences between the present study and that of Rosenberg et al.

While we observed no effect of decreasing hemoglobin concentrations, we did observe an apparent effect of withdrawal rate (ie, some of our ratios differed significantly from 1). Ratios of microsphere concentrations from samples withdrawn from the left brachial artery at 1.03 mL • min⁻¹ and from the right brachial artery at 2.06 mL • min⁻¹ were lower than the inverse situation (ie, right brachial artery at 1.03 mL • min⁻¹; left brachial artery at 2.06 mL • min⁻¹; Fig 2). Although the two ratios differed significantly from one another, the difference between the ratios was less than 7%. Likewise, ratios calculated from samples withdrawn from the aorta at 2.06 mL • min⁻¹, while statistically significantly different from 1, were less than 5% higher or lower than 1 (Fig 2). Most laboratories using the radioactive microsphere method accept a 5% to 10% difference between arterial reference sample microsphere concentrations. Thus, the small differences in reference sample sphere concentrations observed in the present study would not introduce an unacceptable error in calculated CBF values.

In contrast, the ratios of sphere concentrations in samples drawn from the aorta at 1.03 mL • min⁻¹ and from the left brachial artery at 2.06 mL • min⁻¹ or the right brachial artery at 1.03 mL • min⁻¹ (0.86±0.01 and 0.87±0.01, respectively) indicated differences greater than 10%. Therefore, our results suggest that slow withdrawal from a large vessel may underestimate actual microsphere concentrations. Moore et al also reported that slow withdrawal may decrease reference sample microsphere concentrations, although they used larger (25 µm) microspheres. While the reason for this underestimation is unknown, a possible explanation is that a small catheter in a relatively large vessel may draw blood from the periphery of the blood column that does not accurately reflect sphere concentration in the entire vessel. Faster withdrawal may have prevented this effect by drawing a greater amount of blood from the entire blood column.

In summary, although we found no effect of hemodilution on arterial reference sample microsphere concentrations, our results and the observations of Rosenberg et al suggest some variability among species, particularly in relation to the size of cannulated arteries relative to the size of cannulas. Therefore, we emphasize that microsphere measurements should be validated in specific species under the conditions of the planned experiments.

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References

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Because hemodilution is of therapeutic interest and continues to be investigated in experimental models, it is important to validate the calibration of blood flow measurements to be made under conditions of hemodilution. The radiolabeled microsphere technique has become one of several standard techniques for the measurement of cerebral blood flow in experimental animals. Microspheres injected into either the left atrium or left ventricle are assumed (1) to be distributed to various organs in proportion to their blood flow relative to cardiac output and (2) to be trapped in small arterioles on their first pass through the microcirculation. To calibrate the technique, most investigators have used radioactive microspheres. Microspheres used for cerebral blood flow studies are typically 15 μm in diameter to minimize cerebral arteriovenous shunting while still approximating the intraorgan distribution of red cell flux. However, because of steric hindrance associated with their large diameter and because of shear stress on these nondeformable spheres, microsphere concentration may be less near the arterial wall, where catheter tips are often situated. Thus, a slow withdrawal rate through a catheter located in a large artery may underestimate the average cross-sectional microsphere concentration. Moreover, decreasing hematocrit may alter the cross-sectional distribution of microspheres because of the fluid mechanical effects of red cell suspensions on the cross-sectional velocity profile.

DeWitt and colleagues demonstrated that the relatively slow withdrawal rate of 1.03 mL · min⁻¹ in cat aorta underestimates microsphere concentration compared with a rate of 2.06 mL · min⁻¹ but that this underestimation was not affected by large changes in hematocrit. In addition, variations in hematocrit did not affect the ratio of microsphere concentrations obtained at different withdrawal rates from two brachial arterial catheters. Therefore, reduced hematocrit does not appear to affect the precision of the microsphere calibration, but low withdrawal rates can affect its accuracy in the cat.

These results differ from those in the lamb observed by Rosenberg et al. Underestimation of microsphere concentration at low withdrawal rates was much greater at low hematocrit versus high hematocrit in the lamb. However, simply increasing the rate from 1.3 to 2.46 mL · min⁻¹ in the lamb eliminated the influence of hematocrit. No influence of hematocrit was observed in the dog, which, together with the present results in the cat, suggests a species-specific effect possibly attributable to the small size of the ovine red blood cell.

Therefore, arterial withdrawal rates of at least 2 mL · min⁻¹ in the cat and 2.4 mL · min⁻¹ in the newborn lamb should be used. Higher rates can be used but are usually constrained by significant blood loss, particularly when multiple labels are used at multiple time points in the same animal.

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Reference
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