Effects of Sphere Size and Injection Site on Regional Cerebral Blood Flow Measurements

Richard Y. Z. Chen, M.D., Fou-Chung Fan, M.D., George B. Schuessler, Ph.D., Shunichi Usami, M.D., Ph.D., and Shu Chien, M.D., Ph.D.

SUMMARY Regional cerebral blood flows and shunting of microspheres with four different sizes (9, 12, 16 and 25 μm) into the superior sagittal sinus were determined in twelve dogs. Venous blood was collected from the superior sagittal sinus for 120 min after the injection of microspheres, and the dogs were then sacrificed immediately. Results on blood flow measurements and venous shunting determinations were similar between left ventricular and left atrial injections. Blood flows measured by 12, 16 and 25 μm spheres were comparable in various brain tissues, except the choroid plexus. 9 μm spheres underestimated blood flows in all regions studied: by 13-19 percent in the cerebral cortex, midbrain, brain stem and cerebellum, by 34-42 percent in the cortical white matter, corpus callosum and cervical cord, and by 64-81 percent in pituitary gland and choroid plexus. These results probably reflect regional difference in microvascular architecture. Venous shunting of 9, 12, 16 and 25 μm spheres during a 2 hr period were 23.6 ± 2.5, 12.6 ± 1.2, 4.8 ± 1.4, and 4.0 ± 1.2 percent (mean ± SEM), respectively, with respect to the arterial delivery. Although most of the venous shunting occurred during the first 3 min after the injection of microspheres, it continued 3-60 min after the injection. Beyond 60 min, the venous shunting became minimal for 16 and 25 μm spheres, while significant amount of 9 μm spheres continued to appear in sagittal sinus. This time dependent shunting indicates that some microspheres may be transiently trapped in the microcirculation and become gradually dislodged with time. Failure to consider this time dependence may underestimate the shunting of microspheres through the microcirculation. The difference in percent shunting between 9 and 15 μm spheres estimated by cerebral cortex counting agreed well with that by sagittal sinus sampling when both were determined at 120 min after the injection of microspheres.

RADIOACTIVE MICROSPHERES have been utilized to determine the distribution of cardiac output and regional blood flow in many organs, including the brain.1-3 One of the major assumptions made in applying the microsphere technique for blood flow measurements is the complete entrapment of the injected spheres on the first passage. There is, however, evidence indicating the presence of arteriovenous (AV) anastomoses with diameters larger than capillaries in various organs of the body, e.g., the skin, muscle, heart, intestine, liver, spleen, lung, eye and brain.4-6 The use of microspheres smaller than the AV anastomoses would result in a significant shunting of the spheres through the organ and an underestimation of blood flow. The larger spheres, due to their rheological behavior in the blood stream,7 may have the problem of maldistribution, i.e., their distribution is not similar to that of blood flow. Therefore, the choice of a proper size of spheres is important for the accurate measurement of organ blood flow with the microsphere technique.

In addition to their shunting through arteriovenous connections, the trapped microspheres may be slowly propagated in the vessel8 to become dislodged, and increasing amounts may shunt into the venous system with time. It has been reported that sampling venous blood (sagittal sinus) for 2 min results in an incomplete correction for shunting of 9 μm spheres in cerebral blood flow measurement,9 suggesting that the 2 min venous sampling underestimates the degree of shunting. Further studies over a longer time course are needed in order to elucidate the trapping and shunting phenomena and improve the use of microsphere technique in regional blood flow measurements.

Besides the complete entrapment, another requirement for accurate blood flow measurement with the microsphere technique is that the spheres are adequately mixed in the arterial blood prior to distribution to the various regions under study. Inadequate mixing may result in maldistribution of spheres in the arterial system and hence erroneous values for regional blood flows. It has been shown that microspheres are adequately mixed when injected into the left atrium.10 This technique, however, requires a thoracotomy for left atrial cannulation. An alternative method using left ventricular injection in a closed chest preparation has been demonstrated to yield results on renal blood flow comparable to those obtained following left atrial injection.11 There is a lack of quantitative assessments of left ventricular injection of microspheres, as compared to left atrial injection, in regional cerebral blood flow measurements.

The present study was undertaken: (a) to determine the magnitude of venous shunting through the cerebral circulation and the differences in regional blood flow measurements with the use of different sizes of microspheres, (b) to establish the temporal course of the shunting of microspheres through the cerebral circulation over a period of 120 min, and (c) to compare the regional cerebral blood flows measured by left ventricular and left atrial injections of microspheres.

Methods

These experiments were performed on 12 mongrel dogs, weighing between 15 and 25 kg. These dogs...
were initially anesthetized with 30 mg/kg of sodium pentobarbital intravenously and supplemented with approximately 2 mg/kg hourly. Pancuronium bromide (0.2 mg/kg) was given intravenously for muscle relaxation. The trachea was intubated and mechanical ventilation was used to maintain the arterial CO₂ tension (P<sub>CO₂</sub>) between 36 and 42 torr. Oxygen was added to the inspired air at a low flow rate (1 L/min) in order to ensure an arterial oxygen tension (P<sub>O₂</sub>) greater than 100 torr. The P<sub>O₂</sub> and P<sub>CO₂</sub> of arterial blood samples were determined with a blood gas analyzer (Model 213, Instrumentation Laboratory, Lexington, MA). The esophageal temperature was maintained constant between 37 and 38°C with heating pads. A femoral artery was cannulated with a pig-tailed catheter (US catheter and Instrument Corp., Billerica, MA) and the catheter was advanced into the left ventricle as assured by pressure tracing. Another catheter was placed into the lower abdominal aorta through the other femoral artery. The pressure tracings were recorded continuously with the use of Statham transducers and a polygraph recorder (Model 7, Grass Instruments, Quincy, MA). A femoral vein was cannulated for blood replacement to balance the blood loss from sampling. A left thoracotomy was then performed at the fifth intercostal space. A catheter was inserted into the left atrium through a branch of pulmonary vein. The position of the left atrial catheter was ascertained by advancing the catheter into left ventricle and then withdrawing back until the pressure tracing changed to the atrial pattern. A catheter (PE 240 polyethylene tubing) was placed into the superior sagittal sinus through a midline trephination. The dogs were then heparinized (1000 u/kg initially and 500 u/kg hourly).

Four different sizes of microspheres (nominally 9, 10, 15 and 25 μm in diameter) were used in the present study. ¹²⁵I-labelled 9 μm spheres were obtained from 3M Co. (St. Paul, MN), and the other sized spheres labelled with four different radionuclides (¹³¹I, ¹⁰⁵Sn, ¹⁹⁸Ru and ⁹⁶Nb) were purchased from New England Nuclear, Inc. (Boston, MA). All spheres were supplied as suspensions in 10 percent dextran solution (MW 78,000) and were thoroughly examined with respect to the status of aggregation, presence of fragmentation, specificity of radionuclides and specific activity after receiving each shipment. The actual sizes of these spheres were determined with the use of a multi-channel particle size analyzer (Coulter channelizer Model 009 ZB, Coulter Electronics, Inc., Hialeah, FL) and the conversion of spherical volume to diameter. The diameter distributions of these microspheres were found to be 8.8 ± 0.8, 11.5 ± 1.5, 15.6 ± 1.9 and 25.0 ± 2.0 μm (mean ± SD) respectively (fig. 1). Therefore, the spheres used in the present study will be identified as 9, 12, 16 and 25 μm microspheres.

The detailed preparation of the microspheres for injection has been reported elsewhere. Precautions were taken to ensure complete dispersion of spheres with microscopic examination and to count the number of spheres to be injected with a hemacytometer. Approximately 1 × 10⁵ to 2 × 10⁵ spheres per kg body weight were injected for each size of microspheres. More spheres were used for the smaller ones. A total of 6 to 7 × 10⁶ spheres per kg body weight were injected at the same time. Known amounts of 9, 12 and 16 μm spheres were mixed in one syringe and used for left atrial (LA) injection while another syringe containing known amounts of 12 and 16 μm spheres (labeled with different radionuclides than those used in LA injection) was prepared for left ventricular (LV) injection. The mixtures of microspheres were diluted in 10–15 ml of normal saline (0.9 percent NaCl) solution containing 0.05 percent Tween-80 and were injected into the left atrium and left ventricle simultaneously via respective catheters over a period of 30 sec. The catheters were then flushed with 10 ml of normal saline solution over a period of 10 sec. A total of nine dogs were used in this type of experiment. In three other dogs, a mixture consisted of only 16 and 25 μm spheres were injected into the left atrium. The procedures of injection were the same as previously described. A 30-sec period of injection was used in order to ensure adequate mixing and to minimize excessive injection of microspheres during cardiac systole, thus avoiding hemodynamic changes.

Starting 10 sec before the injection of microspheres, the arterial reference flow sample was drawn from the catheter in the abdominal aorta at a constant reference flow rate (Q<sub>R</sub>) of 15.3 ml/min for three minutes with the use of an infusion-withdrawal pump (Harvard Apparatus Co., Millis, MA). Since multiple sampling of arterial reference samples from different locations have been found to yield similar results, a single arterial reference was used in the present study. Simultaneously with the withdrawal of arterial reference sample, venous blood was also sampled continuously through the sagittal sinus catheter with another Harvard pump over a period up to 120 min. The withdrawal rates (Q<sub>v</sub>) for the venous samples were set at 9.89 ml/min for the first 3 min, 3.82 ml/min for the next 57 min, and 3.60 ml/min up to 120 min. The blood loss from arterial and venous sampling was replaced simultaneously with the same amount of donor blood in-
fused into the femoral vein. At 120 min after the injection of microspheres, the dogs were sacrificed with intravenous injection of an overdose of sodium pentobarbital or potassium chloride. The brain and cervical spinal cord were removed and dissected according to anatomical locations. Individual tissue specimens, as well as the blood samples, were placed in separate counting tubes. The radioactivities (in counts per min) of each tube were counted in a gamma counter (Packard Instrument Co., Downers Grove, IL) connected to a multi-channel analyzer (TN-1710, Tracor Northern, Middleton, WI), and the radioactivity attributable to each isotope was resolved for overlapping of energy spectra with the aid of a PDP-11/10 minicomputer (Digital Equipment Corp., Maynard, MA). The cardiac output (CO, in ml/min) and the flow rate in tissues (Qx, in ml-min\(^{-1}\) - 100 gm\(^{-1}\)) were calculated with the use of the following equations:

\[
CO = A_t/(A_v/Q_x)
\]

(1)

\[
Q_x = C_t/(A_v/Q_x)
\]

(2)

where \(A_t\) is the total radioactivity injected, \(A_v\) is the radioactivity of the arterial reference flow sample, and \(C_t\) is the radioactivity per 100 gm of tissue sample.

The percent shunting (S\(_v\)) of 9, 12, 16 and 25 \(\mu\)m spheres into the superior sagittal sinus was calculated as following:

\[
S_v = 100 (A_t(t)/Q_v(t))/(A_v/Q_x)\]

(3)

where \(A_t(t)\) is the radioactivity collected in the venous blood at 3, 10, 60 and 120 min following the injection of microspheres, respectively, and \(Q_v(t)\) is the withdrawal rate. The relative magnitude of non-entrapment (S\(_x\)) of various sizes of microspheres with diameter \(x\) was compared to that of 16 \(\mu\)m spheres by the following equation:

\[
S_x = (1 - Q_x/Q_{16}) \times 100
\]

(4)

where \(Q_x\) is the blood flow for a given tissue determined by using spheres with diameter \(x\) = 9, 12 or 25 \(\mu\)m, and \(Q_{16}\) is blood flow for the same tissue determined by using 16 \(\mu\)m spheres.

The results were tested statistically by two-way analysis of variance and Bonferroni multiple comparisons. The t-ratio for the comparison between two mean values \(M_4\) and \(M_6\) was calculated according to the following equation:

\[
t = (M_4 - M_6) \times N^{1/2}/[\text{RMS} \times (1/n_4 + 1/n_6)]^{1/2}
\]

(5)

where RMS is the residual mean square of all measurements, \(N\) is the number of animals studied, and \(n_4\) and \(n_6\) are the number of conditions (sites of injection or sizes of spheres) included in the calculations of \(M_4\) and \(M_6\), respectively.

Values of the t-ratio greater than 2.46, 2.75 and 3.65 were declared to be significant with 99, 99.5 and 99.95 percent confidence, respectively.

Results

In all dogs studied, the mean values (± SEM) for mean arterial pressure and heart rate before the injection of microspheres were 145 ± 8 mm Hg and 152 ± 16 beats/min, respectively. During the 2 hr duration of experiment, the arterial pressure and heart rate remained stable within 10 percent of the control values. The cardiac output (in ml-min\(^{-1}\)-kg\(^{-1}\)) and regional cerebral blood flows (in ml-min\(^{-1}\)-100 gm\(^{-1}\)) simultaneously determined by left ventricle (LV) and left atrial (LA) injections of various sizes of microspheres are summarized in table 1. The values given are mean ± SEM (\(n = 9\)). The residual mean squares (RMS) of flow values were calculated by two-way analysis of variance. There were considerable variations of blood flow values among different regions of the brain. In each region of the brain, however, comparisons of the flow values measured by LV and LA injections of either 12 or 16 \(\mu\)m spheres show that the t-ratios are considerably less than the value (2.46) required to attain 99 percent confidence. Hence, there was no significant difference of flow values obtained by these two routes of injections among all tissues studied.

Since there was no statistically significant difference between the values obtained from LA and LV injections, the results were grouped. The mean values obtained after combining the two sites of injection were compared between the 12 and 16 \(\mu\)m microspheres. Simultaneous injections of 12 \(\mu\)m and 16 \(\mu\)m spheres yielded similar results of cardiac output and cerebral blood flows among all regions studied, with the exception of the choroid plexus (table 1). In the choroid plexus, the blood flow determined with 12 \(\mu\)m spheres was 339.9 ± 59.9 ml-min\(^{-1}\)-100 gm\(^{-1}\) (mean ± SEM), which was about 30 percent less than the value of 489.8 ± 86.3 ml-min\(^{-1}\)-100 gm\(^{-1}\) obtained with 16 \(\mu\)m spheres. The difference is statistically significant (\(p < 0.001\)).

In comparison with both 12 and 16 \(\mu\)m spheres, 9 \(\mu\)m spheres yield cardiac output values that are not significantly different. The values of regional cerebral blood flows measured with 9 \(\mu\)m spheres, however, were significantly lower than those obtained with 12 and 16 \(\mu\)m spheres in all regions of the brain studied.

The magnitude of non-entrapment of 9 \(\mu\)m spheres in various regions of the brain as compared to 16 \(\mu\)m spheres can be estimated from the percent difference of flow values obtained by 9 and 16 \(\mu\)m spheres (equation 4). These results are shown in figure 2. In most brain regions, including cerebral cortex, midbrain, brain stem, superior colliculus, inferior colliculus and cerebellum, the non-entrapment of 9 \(\mu\)m spheres was 13 to 19 percent more than that of 16 \(\mu\)m spheres. In the cerebral cortex, the gray matter had 13 percent more shunting of 9 \(\mu\)m spheres while the white matter had 42 percent. This additional shunting of 9 \(\mu\)m over 16 \(\mu\)m spheres was 35 percent in the corpus callosum and 34 percent in the cervical cord. The pituitary gland and

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## Table 1  Cardiac Output and Regional Cerebral Blood Flows Determined with Various Sizes of Microspheres

<table>
<thead>
<tr>
<th>Tissues</th>
<th>9 μm (LA)</th>
<th>12 μm (LV)</th>
<th>12 μm (LA)</th>
<th>16 μm (LV)</th>
<th>16 μm (LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cardiac output</td>
<td>121.8 ± 9.0</td>
<td>113.1 ± 7.7</td>
<td>107.2 ± 10.3</td>
<td>110.2 ± 8.0</td>
<td>110.3 ± 11.4</td>
</tr>
<tr>
<td>2. Gray matter</td>
<td>36.9 ± 5.0</td>
<td>43.9 ± 7.2</td>
<td>44.4 ± 6.2</td>
<td>43.5 ± 6.0</td>
<td>41.5 ± 5.9</td>
</tr>
<tr>
<td>3. White matter</td>
<td>13.7 ± 2.0</td>
<td>22.1 ± 3.6</td>
<td>22.2 ± 3.0</td>
<td>24.2 ± 3.0</td>
<td>23.1 ± 3.8</td>
</tr>
<tr>
<td>4. Cerebral cortex</td>
<td>32.4 ± 4.3</td>
<td>40.4 ± 7.5</td>
<td>41.2 ± 3.0</td>
<td>41.1 ± 6.2</td>
<td>39.6 ± 5.4</td>
</tr>
<tr>
<td>5. Caudate nucleus</td>
<td>48.1 ± 9.4</td>
<td>56.8 ± 12.0</td>
<td>54.7 ± 8.8</td>
<td>59.6 ± 10.4</td>
<td>53.5 ± 8.8</td>
</tr>
<tr>
<td>6. Corpus callosum</td>
<td>11.3 ± 1.7</td>
<td>17.1 ± 3.0</td>
<td>16.3 ± 2.5</td>
<td>17.5 ± 2.9</td>
<td>17.1 ± 2.7</td>
</tr>
<tr>
<td>7. Thalamus</td>
<td>31.7 ± 5.0</td>
<td>37.5 ± 7.0</td>
<td>38.7 ± 5.5</td>
<td>38.3 ± 5.9</td>
<td>37.8 ± 5.3</td>
</tr>
<tr>
<td>8. Hypothalamus</td>
<td>32.7 ± 4.8</td>
<td>40.2 ± 7.2</td>
<td>42.0 ± 5.8</td>
<td>39.4 ± 4.8</td>
<td>40.3 ± 4.8</td>
</tr>
<tr>
<td>9. Pons</td>
<td>25.2 ± 4.0</td>
<td>31.6 ± 5.8</td>
<td>32.6 ± 4.6</td>
<td>30.2 ± 5.2</td>
<td>31.0 ± 4.7</td>
</tr>
<tr>
<td>10. Upper medulla</td>
<td>28.5 ± 3.6</td>
<td>35.6 ± 6.2</td>
<td>35.7 ± 4.3</td>
<td>35.6 ± 4.5</td>
<td>35.8 ± 4.1</td>
</tr>
<tr>
<td>11. Lower medulla</td>
<td>21.1 ± 2.9</td>
<td>26.5 ± 4.6</td>
<td>26.3 ± 3.4</td>
<td>25.2 ± 3.0</td>
<td>26.3 ± 3.4</td>
</tr>
<tr>
<td>12. Cervical cord</td>
<td>8.6 ± 0.9</td>
<td>13.0 ± 2.0</td>
<td>13.8 ± 1.5</td>
<td>13.5 ± 1.7</td>
<td>13.2 ± 1.6</td>
</tr>
<tr>
<td>13. Cerebellar hemisphere</td>
<td>32.7 ± 4.6</td>
<td>38.9 ± 6.6</td>
<td>39.6 ± 5.4</td>
<td>39.9 ± 5.7</td>
<td>39.8 ± 5.9</td>
</tr>
<tr>
<td>14. Cerebellar vermix</td>
<td>41.5 ± 5.6</td>
<td>50.0 ± 9.0</td>
<td>50.1 ± 6.9</td>
<td>50.4 ± 8.5</td>
<td>47.9 ± 6.8</td>
</tr>
<tr>
<td>15. Superior colliculus</td>
<td>33.0 ± 6.1</td>
<td>42.7 ± 9.0</td>
<td>42.3 ± 7.3</td>
<td>38.2 ± 6.9</td>
<td>40.2 ± 6.7</td>
</tr>
<tr>
<td>16. Inferior colliculus</td>
<td>58.2 ± 12.6</td>
<td>66.5 ± 16.7</td>
<td>67.4 ± 13.2</td>
<td>66.7 ± 14.3</td>
<td>67.5 ± 12.9</td>
</tr>
<tr>
<td>17. Pituitary gland</td>
<td>100.5 ± 19.3</td>
<td>263.3 ± 42.9</td>
<td>272.0 ± 40.0</td>
<td>298.3 ± 44.1</td>
<td>299.4 ± 41.8</td>
</tr>
<tr>
<td>18. Choroid plexus</td>
<td>77.8 ± 15.1</td>
<td>328.3 ± 59.8</td>
<td>332.5 ± 64.1</td>
<td>523.1 ± 102.1</td>
<td>456.5 ± 72.7</td>
</tr>
</tbody>
</table>

Units: Cardiac output in ml-min⁻¹·kg⁻¹. Regional blood flows in ml-min⁻¹·100 gm⁻¹. LA = left atrial injection; LV = left ventricular injection; RMS = residual mean square. *p < 0.05; tp < 0.01; tp < 0.001.

choroid plexus had the greatest additional shunting of 9 μm spheres among all brain regions, being 64 and 81 percent, respectively. The percent shunting of different sizes of microspheres into the superior sagittal sinus with respect to the arterial delivery was determined by continuous sampling of venous blood up to 120 min. These results are summarized in table 2. There was no statistically significant difference between the LA and LV injections of either 12 or 16 μm spheres, and therefore the data obtained from these two sites of injection were pooled together for each sphere size. The data on shunting of 9, 12 and 16 μm spheres into the sagittal sinus are shown in composite plot of figure 3. During the first 3 min following the injection of microspheres, the percentage of arterial inflow spheres appearing in the sagittal sinus was 2.6 percent for 16 μm spheres, 8.9 percent for 12 μm spheres and 15.7 percent for 9 μm spheres. Within the next 7 min, additional 1.2 percent of 16 μm spheres, 2.2 percent of 12 μm spheres and 2.9 percent of 9 μm spheres appeared in the sagittal sinus; the values for 12 and 9 μm spheres were significantly greater than that of 16 μm spheres. From 10 min up to 120 min, only about 0.8 percent of 16 μm and 1.8 percent of 12 μm spheres passed into the sagittal sinus, while an additional 5.5 percent of 9 μm spheres were collected. The results of percent shunting of 25 μm spheres into the sagittal sinus were similar to those of 16 μm spheres.

The cumulative shunting of microspheres into the sagittal sinus at 120 min after the injection are plotted against the sphere size in figure 4. The total amounts of 16 and 25 μm spheres shunted into sagittal sinus within 120 min following their injections were 4.8 percent and 4.0 percent, respectively. Significantly greater percentages of 9 μm (23.6 percent) and 12 μm (12.9 percent) spheres were found to be shunted into the sagittal sinus as compared to both 16 and 25 μm spheres. The relative non-entrapment of various sizes of spheres estimated by direct tissue counting for the choroid plexus had the greatest additional shunting of 9 μm spheres among all brain regions, being 64 and 81 percent, respectively.

### Figure 2
A schematic diagram of the brain showing regional variations of the relative percent shunting of 9 μm microspheres as compared with 16 μm spheres from using equation 4. The numbers in parentheses indicate the additional shunting of 9 μm spheres for each region. The figure for cerebrum represents the composite of cerebral gray and white. A portion of the cerebral cortex near the central gyrus is drawn to show the values for gray matter and white matter separately.
cerebral cortex (equation 4) is compared with the percentage of shunting determined by sagittal sinus sampling (equation 3). With the use of direct tissue counting, there was no significant difference in non-entrapment (or shunting) among 12, 16 and 25 μm spheres. The magnitude of non-entrapment of 9 μm spheres, however, was 18.3 percent more than that of 16 μm spheres. This difference in percent shunting determined by direct tissue counting between 9 and 16 μm spheres agrees well with the difference in percent shunting measured by venous sampling (23.6 percent for 9 μm spheres and 4.8 percent for 16 μm spheres).

Discussion

In the present study, the experiments were designed in order to minimize variations in experimental conditions. Thus, the simultaneous injections of 12 and 16 μm spheres into the left atrium and left ventricle made possible a comparison of the results of these two routes of injections. The simultaneous injections of 9, 12 and 16 μm spheres allowed a direct comparison of the results obtained from these various sizes of microspheres under the same condition. The sagittal sinus sampling was continued for 120 min after the injection of microspheres, and the dog was sacrificed immediately as the sampling was terminated. Therefore, the relative non-entrapment microspheres estimated by direct tissue counting can be compared with their shunting into the sagittal sinus. Unlike the venous blood sampling, the arterial reference flow was withdrawn for only three minutes after the injection of microspheres. Previously it has been shown that there is essentially no radioactivity detectable in the arterial system 3 min after the injection and all the spheres shunted through the systemic vascular beds eventually trapped in the pulmonary circulation.9,10 It should be emphasized that present experiments were conducted under pentobarbital anesthesia and therefore the measured cerebral blood flow values were all reduced below expected normal values in the awake unanesthetized state.

One of the basic assumptions in using the microsphere technique for blood flow measurements is that the spheres are adequately mixed in the arterial blood prior to distribution. The left atrial (LA) injection is a well accepted technique in providing an adequate mixing of microspheres. However, the adequacy of mixing of microspheres injected into the left ventricle (LV), which can be catheterized in a closed chest preparation, has not been clearly established. Comparing the renal blood flows measured by LA and LV injections of microspheres, Bhattacharya and Belin11 found similar values with these two routes of injection. In the present investigation, the regional cerebral blood flows measured by microspheres injected into the left ventricle (LV), which can be catheterized in a closed chest preparation, has not been clearly established. Comparing the renal blood flows measured by LA and LV injections of microspheres, Bhattacharya and Belin11 found similar values with these two routes of injection. In the present investigation, the regional cerebral blood flows measured by microspheres injected into the LV catheter agreed well with those values determined by the same size of microspheres simultaneously injected into the LA catheter. These results indicate that LV injection provides an adequate mixing of microspheres in the arterial blood before their distribution to the brain and that LV can be an alternative site of injection of microspheres for regional cerebral blood flow determination.

Another assumption in using the microsphere technique for regional blood flow measurement is that the injected spheres are trapped completely in the tissue. The appearance of microspheres in the venous blood would indicate that some of the spheres have shunted through the arteriovenous connections whose diameters are greater than those of capillaries. Various sizes

### Table 1 (Continued)

<table>
<thead>
<tr>
<th>Comparison between injection sites</th>
<th>Comparison of sphere sizes (t-ratios)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMS</td>
<td>12 μm vs 16 μm</td>
</tr>
<tr>
<td>0-3 min</td>
<td>9 μm vs 12 μm</td>
</tr>
<tr>
<td>3-10 min</td>
<td>12 μm vs 16 μm</td>
</tr>
<tr>
<td>10-60 min</td>
<td>16 μm vs 16 μm</td>
</tr>
<tr>
<td>60-120 min</td>
<td>16 μm vs 9 μm</td>
</tr>
</tbody>
</table>

### Table 2 Percent Shunting of Microspheres into the Superior Sagittal Sinus

<table>
<thead>
<tr>
<th>Time periods</th>
<th>Percent shunting into the sagittal sinus (mean ± SEM)</th>
<th>Comparison between injection sites</th>
<th>Comparison of sphere sizes (t-ratios)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3 min</td>
<td>15.7 ± 1.9 9.0 ± 1.5 8.8 ± 0.5 2.6 ± 0.5 3.2 ± 0.9</td>
<td>RMS</td>
<td></td>
</tr>
<tr>
<td>3-10 min</td>
<td>2.9 ± 0.6 2.4 ± 0.6 1.9 ± 0.5 1.3 ± 0.5 1.0 ± 0.3</td>
<td>5.99 -0.17 0.52 -6.81 t -7.35 t -12.81†</td>
<td></td>
</tr>
<tr>
<td>10-60 min</td>
<td>2.8 ± 0.5 1.1 ± 0.4 1.4 ± 0.3 0.5 ± 0.2 0.6 ± 0.3</td>
<td>1.30 -0.93 -0.56 -1.61 -2.63* -3.65†</td>
<td></td>
</tr>
<tr>
<td>60-120 min</td>
<td>2.2 ± 0.1 0.8 ± 0.1 0.4 ± 0.02 0.3 ± 0.02 0.1 ± 0.02</td>
<td>0.83 0.70 0.23 -4.17 t -2.30 -6.05†</td>
<td></td>
</tr>
</tbody>
</table>

LA = left atrial injection; LV = left ventricular injection; RMS = residual mean square.

*p < 0.05; †p < 0.01; ‡p < 0.001.
of arteriovenous anastomoses ranging from 7 to 76 μm in diameter have been found in the dog brain. It also has been reported that arteriovenous shunts up to 200 μm in diameter exist in the pial vessels. With the use of 7–10, 15, 25 and 50 μm spheres, Marcus et al. have suggested that two sizes of arteriovenous shunts present in the brain: small shunts, between 6 and 13 μm in diameter that are relatively numerous; and large shunts, probably greater than 35 μm in diameter that are much lesser in number. The present data support the contention that at least two types of arteriovenous shunts exist in the brain. The large shunts, relatively few in numbers, permit the passage of 12, 16 and 25 μm spheres. Since 12 and 16 μm spheres yield essentially the same flow values, the much more numerous smaller shunts are probably less than 12 μm in diameter; the only exception is the choroid plexus where the data indicate the presence of arteriovenous shunts between 12 and 16 μm in diameter. It is worth noting that while arteriovenous shunts occur in the dog they may not be present in other species such as the human and subhuman primate.

The exact locations of various sizes of cerebral arteriovenous shunts in the dog can not be determined from the present results. However, since the shunting of 9 μm spheres was 13 to 19 percent more than that of 16 μm spheres in most brain regions containing significant amounts of cell bodies (fig. 2), the small arteriovenous shunts are rather evenly distributed in those areas. The present data also indicate that the small arteriovenous shunts are more prevalent in regions of central venous system containing primarily nerve fibers (e.g. cortical white matter, corpus callosum, cervical spinal cord), pituitary gland and choroid plexus. This is in accordance with the findings of Hasegawa et al. that small thoroughfare channels (arteriovenous shunts) exist in relative abundance in the white matter. These small shunts probably allow 9 μm spheres to bypass the capillary beds, leading to an underestimation of the blood flow.

Based on the data of sagittal sinus sampling, about 2.9 percent of 16 μm spheres appeared in the venous blood during the first 3 min after the injection of microspheres (table 2). This value agrees well with the 2 percent shunting of 15 and 25 μm spheres into the sagittal sinus during the first 3 min reported by Marcus et al. Since the sagittal sinus also drains venous blood from extracranial tissues, some of the shunted spheres may be of extracerebral origin. The extracranial contamination is likely to be greater with smaller microspheres and this might be contributing to the apparent shunting through cerebral tissues. The extracerebral contamination of the sagittal sinus, however, has been estimated to be less than 10 percent. Therefore, the extracerebral contamination can not be accounted solely for the difference in venous shunting between the 9 μm and 16 μm spheres (23.6 percent vs 4.8 percent at 120 min) found in the present study. In a study using labelled 15 μm spheres injected into the common carotid arteries, Kaihara et al. found that 7–9 percent of these spheres were detectable in the lung at 10 min after the injection of microspheres. Since the present data showed only about 4.1 percent of 16 μm spheres shunted into the sagittal sinus at 10 min following the injection, most of the shunting found by Kaihara et al. were probably extracerebral.

One of the important findings in the present study is that the shunting of microspheres is time dependent. A total of 4.8 percent of 16 μm spheres was found to shunt through the brain tissue in 120 min. About 2.9 percent (or 60 percent of the total shunted spheres) appeared within the first 3 min after the injection of microspheres (table 2 and fig. 3). Additional 1.9 percent (or 40 percent of the total shunted spheres) shunted with time up to 120 min; most of this additional shunting occurred within the first hour. This time
dependence of shunting is more prominent as the size of microspheres decreases. For 9 μm spheres, 15.7 percent (which represents 67 percent of the total shunting up to 120 min) shunted into the sagittal sinus during the first 3 min, an additional 5.7 percent (or 24 percent of the total shunting) occurred between 3–60 min, and 2.2 percent (about 9 percent of the total shunting) was found between 60–120 min. Such time dependence of shunting of microspheres also has been observed in the coronary vascular bed (unpublished observation).

The time dependence of shunting indicates that some of the microspheres may be transiently trapped in the vessels. The trapped microspheres may distend the vessel lumen to effect their passage in the presence of a sufficient pressure head. Such widening of the capillary lumen would depend upon the viscoelastic properties of the vessel wall as well as the surrounding tissue. Under microscopic examination of the microvasculature in a rabbit ear chamber, Hales and Clifford have observed the slow movement of spheres along vessels approximately 20 percent smaller in diameter than the sphere over a period up to 1 hr. In a previous study, we found that even after correction for 2 min shunting the cerebral blood flows measured with 9 μm spheres was still lower than that determined with 15 μm spheres. Following the correction for 120 min shunting, the present experiments show an excellent agreement of cerebral cortical blood flows measured by 9 and 16 μm spheres. The present investigation indicates that failure to consider this time dependent phenomenon may underestimate the shunting of microspheres. Our data show that the entrapment of 16 μm spheres is essentially stabilized after one hour whereas that of 9 μm spheres is not stabilized even at 120 min after their injection.

The pituitary gland and choroid plexus, which are intracranial extracerebral tissues, deserve further discussion. The blood supplies of these organs have been studied extensively. The blood flows and detailed vascular architecture of these organ, however, are not well defined. Although the tissue masses of pituitary gland and choroid plexus are small (ranging 50 to 80 mg), the extremely high blood flows per unit weight of these tissues permit trapping of between 200 and 600 spheres per tissue sample found in the present study. The blood flows determined with given sizes of microspheres were consistent among all animals studied. The blood flow of the pituitary gland measured with 12 and 16 μm spheres was in agreement with previous reports and the blood flow determined with 9 μm spheres, on the other hand, was about 64 percent lower than that measured by 16 μm spheres (fig. 1). These findings suggest that there are numerous arteriovenous anastomoses in the pituitary gland with diameters smaller than 12 μm. The blood flow of choroid plexus determined by 16 μm spheres was 489.8 ± 86.3 (mean ± SEM) ml-min⁻¹-100 gm⁻¹. By analyzing motion pictures made during the injection of spheres of I-octanol, Welch found that the blood flow of the choroid plexus averaged 286 ml-min⁻¹-100 gm⁻¹. The discrepancy between the present results and that reported by Welch is probably due to the difference in methodology. The present finding that about 80 percent more of 9 μm spheres shunted through the choroid plexus as compared to 16 μm spheres indicates that a large number of arteriovenous vessels in this tissue are between 9 and 16 μm in diameter. Many of these arteriovenous connections are between 12 and 16 μm in diameter, since the 12 μm spheres had an additional shunting in the choroid plexus by 30 percent as compared to 16 μm spheres.

In summary, the present study demonstrated that similar results of regional cerebral blood flows can be obtained with left ventricular and the left atrial injections of microspheres. The shunting of microspheres through arteriovenous anastomoses increases with time, indicating that the microspheres are not permanently trapped in the vessels. The trapped microspheres may become dislodged and thus shunted through the capillary bed. The present data indicate that left ventricular injection of 12–16 μm microspheres is a suitable technique for regional cerebral blood flow measurements except that 12 μm spheres may underestimate the blood flow to the choroid plexus.

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References

Measurement of Cerebral Blood Flow by Washout of Microwave Induced Heating

PABLO M. LAWNER, M.D.,†† WILLIAM H. OLDENDORF, M.D.,††
AND LEON D. BRAUN, B.A.††

SUMMARY A method is described for measurement of cerebral blood flow utilizing the washout of microwave delivered heating. Using a microwave source attenuated to achieve a brain temperature elevation of 0.5-0.75°C after a 2 second exposure in the rat, cerebral blood flow was calculated from the temperature washout curve monitored by a small thermistor implanted in the brain. The results obtained with this method were comparable to those obtained using the [14C] butanol method. To our knowledge this represents the first description of a method to deliver a blood flow "indicator" atraumatically directly into brain tissue.

SINCE 1945 when Kety and Schmidt¹,² developed the inert gas method for the measurement of cerebral blood flow (CBF), many techniques have evolved to measure flow through the brain in animals and man. Almost all "indirect" methods available have in common the fact that an "indicator" or tracer is delivered to the tissue via the circulatory system. Those methods that use a diffusible tracer are based on the Fick principle. Flow is calculated by direct measurement of the arterio-venous difference of tracer concentration; by regional tissue measurement of tracer during desaturation or by determination of the arterial concentration of the tracer during tissue saturation followed by measurement of indicator concentration in the tissue. The methods that use a non-diffusible tracer are based on the principle of the indicator fractionation technique of Sapirstein³ requiring measurement of cardiac output or the utilization of a reference organ of known flow.

In 1933, Gibbs⁴ demonstrated that changes in blood flow could be detected with thermal techniques. Later, Grayson⁵ found a linear relationship between blood flow and thermal conduction of tissues, thus allowing for quantitative measures using thermal washout. Since then, many techniques have been developed to measure blood flow with thermal techniques not only in brain but also in myocardium⁶-⁷ liver,⁸ gastric mucosa⁹ and skin.¹⁰,¹¹ In all these methods heat used as an "indicator" is delivered through a thermistor probe. In most designs, constant heat is applied to one thermistor while measuring temperature differences between the heated and unheated thermistors. Another approach has been to keep the heated thermistor at a constant temperature above that of the tissue (isothermal devices) and measure the power required to maintain the heat; this then is proportional to the thermal conductivity of the tissue and thus blood flow.¹²,¹³

In this study we describe a technique to measure cerebral blood flow using the thermal washout of microwave delivered heat.

**Materials and Methods**

**Thermal Washout Flow**

Adult male Wistar rats (275-325g) were anesthetized with pentobarbital 50 mg/kg i.p. After shaving, a
Effects of sphere size and injection site on regional cerebral blood flow measurements.

R Y Chen, F C Fan, G B Schuessler, S Usami and S Chien

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