Microsphere Measurement of Intrarenal Circulation of the Dog

By Lawrence M. Slotkoff, Alexander Logan, Pedro Jose, John D'Avella, and Gilbert M. Eisner

ABSTRACT

Distribution of cortical blood flow was measured in the dog by a technique based on radionuclide-labeled microspheres. Initially it was necessary to test possible pitfalls of this technique. Completeness of trapping in the kidney, the effect on renal function, and the notion that microsphere distribution reflects blood flow distribution in the kidney cortex were studied. Renal vein blood contained less than 0.2% of the microspheres (16.8μm diameter) found in the renal artery after an aortic injection. No impairment of CrAH (control 167 ± 4; postinjection 179 ± 3 ml/min), CIn (control 39.3 ± 6; postinjection 37.6 ± 2 ml/min), and Tm glucose (control 90.8 ± 13; postinjection 102 ± 24) was found using doses adequate to measure renal blood flow (5 mg/injection X 4 injections). After 4 injections of 50 mg each significant impairment of renal function was observed. Intrarenal blood flow distribution was determined during hemorrhagic hypotension. 39Yb-labeled microspheres were injected into the root of the aorta before, and 85Sr-labeled microspheres after, acute hemorrhage. Radioactivity was measured in the outer two thirds and inner one third of kidney slices. Tissue blood flow was calculated and expressed as the ratio of outer cortex to inner cortex counts. Renal blood flow was redistributed to the inner cortex after hemorrhage (ratio before, 3.00; after 1.30, P<0.01). Finally, the results of this technique were compared to a widely used method of measuring intrarenal blood flow distribution, 133Xe washout. The first component of the washout technique correlated fairly well with total cortical flow but it was not possible to match the second component with any single anatomical area of the kidney. Limitations of the 133Xe washout are discussed.

KEY WORDS shock kidney blood flow radioactive washout renal blood flow distribution radionuclide-labeled microspheres

Interest in the relationship between renal sodium metabolism and intrarenal blood flow distribution has led to the development of a variety of techniques for measuring intrarenal blood flow (1-4). One of the most recent and widely reported methods is based on the principle of compartmental analysis of a tracer (133Xe, 85Kr) washout curve (4). This method has the advantage of being nondestructive and can be performed repeatedly. However, there is a drawback in relating the curves obtained to anatomical zones of the kidney. Since all circulations having the same volume-flow ratio fall into the same component of the washout curve, one is never certain of the anatomic region from which the data are being obtained. This is especially important when performing maneuvers which may alter the intrarenal circulation.

In 1967, Rudolph and Heyman (5) described a method of calculating tissue organ blood flow by the accumulation of radioisotope-labeled microspheres. The present study was designed to apply this method to the determination of intrarenal blood flow distribution. The principle of the method is quite simple. If a bolus of microspheres is injected...
into the left ventricle or root of the aorta and complete mixing occurs before the spheres reach the renal artery, then the amount of the microspheres entering the renal tissue will be a function of the fraction of aortic flow to the tissue. If all of the microspheres are trapped in the capillaries in a single circulation, then the amount entering can be easily computed by direct count. If the aortic or ventricular flow (CO) and the radioactivity injected (Q) are known, the tissue flow (TF) may be calculated by the formula:

$$TF = \frac{CO \times TC}{Q}$$

where tissue radioactivity per gram (TC) is measured. It is also possible to calculate flow to a particular portion of the kidney if flow to the entire kidney (RBF) is determined by some other method. In that case, regional flow = RBF x TC

where now Q = amount of radioactivity in the entire kidney.

The renal blood flow (RBF) may be measured by a flow probe or any other standard technique. This method avoids the difficulty involved in accurately determining the total radioactivity injected.

A number of assumptions must be made before microsphere concentration can be accepted as a measure of intrarenal blood flow distribution. First, all microspheres entering the renal circulation must be trapped in the kidney and distributed in a pattern equivalent to blood flow distribution. Second, the spheres must not cause impairment of renal function on multiple injections. Finally, they must not be separated from the blood in the intrarenal circulation by any mechanism such as plasma skimming.

The present study was undertaken to evaluate the validity of these assumptions. In addition, the method was applied during acute hemorrhage to determine if it was sensitive enough to detect changes in distribution. The technique was also compared to the widely used 133Xe washout method.

**Method**

Plastic microspheres (Minnesota Mining and Manufacturing Corp.), 15 μ ± 5 μ, labeled with 159Yb were suspended in 20% dextran (5 mg/5 ml) to which 2 drops of Tween 90 were added. Just before injection, the suspension was mixed with an ultrasonic probe for 3 minutes to disperse the spheres evenly. Examination of the suspension in a hemocytometer chamber revealed no clumping or shattering of microspheres. Two hundred radioactive (159Yb) and 200 unlabeled microspheres were measured with a Leitz micrometer. The actual size was 16.8 μ ± 3.7 (σ) and 15.4 μ ± 3.3, respectively.

1. **Trapping and Distribution of Microspheres in the Kidney:** Three mongrel dogs weighing 12 to 18 kg were anesthetized with intravenous pentobarbital, 25 mg/kg. A polyethylene catheter was placed at the root of the aorta via a femoral artery. Another catheter was advanced to the level of the renal arteries via the opposite femoral artery. A laparotomy was performed and a catheter inserted manually into the right renal vein via a femoral vein.

A bolus of approximately 5 μ of 159Yb-labeled microspheres was injected into the root of the aorta over a period of 10 seconds. A continuous sample of aortic and renal venous blood was collected, over 30 seconds and analyzed for radioactivity. The kidneys were removed, coronal sections cut, and after fixing in 10% formalin, histologic sections stained with eosin-hematoxylin. The sections were examined for the location and number of spheres per glomerulus in the outer and inner cortex. The outer cortex was taken as the outer two thirds of the entire cortex, which has been shown to contain only glomeruli of short-looped nephrons (6); the juxtamedullary glomeruli appear almost exclusively in the inner third. The size of the spheres in the inner and outer cortex was measured with a Leitz micrometer.

2. **Effects of Microspheres on Renal Function.**—Repeated injections of a quantity of nonradioactive microspheres calculated to be equivalent to the number of radioactive spheres needed to produce good scintillation counting levels (50 μc) were made into the root of the aorta in dogs to test their effect on renal function.

Three mongrel dogs (11 to 12 kg) were anesthetized. Catheters were placed into the root of the aorta for injection of the microspheres, into a brachial vein for infusion of inulin and para-aminobiphrurate, and into a femoral vein for sampling. The right ureter was cannulated for urine collection. Ten percent dextrose in water (with 20 mU/liter vasopressin) was infused into the opposite femoral vein at the rate of 6 to 10 ml/min to determine Tg glucose. After three 10-minute control periods during which blood samples and urine were collected to measure clearance of PAH, inulin, glucose and osmolality, 5 mg of microspheres was injected into the aortic
catheter. Five minutes later, another 10-minute collection was made. After four such injections and collections, the experiment was terminated.

3. Intrarenal Blood Flow Distribution during Control and Acute Hemorrhage.—Six mongrel dogs, weighing 12 to 18 kg, were anesthetized with pentobarbital (35 mg/kg). A polyethylene catheter was advanced to the root of the aorta via a femoral artery. Catheters were inserted into the opposite femoral artery and vein. After monitoring arterial blood pressure by the femoral artery catheter for 30 minutes, 13 μC of 131I-labeled microspheres suspended in 20% dextran (13 μC/ml) was injected over a period of 10 seconds into the root of the aorta. The catheter was flushed with 10 ml of 0.85% saline. The dog was then bled from the aortic catheter until its mean blood pressure was half of its control value. Pre-hemorrhage blood pressures averaged 123 ± 68 mm Hg, falling to 56 ± 5.3 mm Hg after withdrawal of 200 to 500 ml of blood. Fifteen minutes were allowed for equilibration. Then a second injection was made, this time with 13 μC of 85Sr-labeled microspheres.

The kidneys were removed and coronal sections 5 mm thick made. Samples were cut from the outer two thirds and inner one third of the cortex, the red medulla and papilla. Each of the four samples was cut into halves, one part being placed in 10% formalin for histological identification and the other half weighted and counted for radioactivity. Another section was cut through the entire thickness of cortex and handled like the other samples. The concentration of 160Yb and 85Sr were determined by differential counting on the red medulla and papilla. Each of the four collections, the experiment was terminated.

4. Intrarenal Blood Flow Distribution—Comparison of Microsphere and 133Xe Washout Methods.—Four dogs weighing 11 to 15 kg were anesthetized with pentobarbital (35 mg/kg), and a Lebmann ventriculography catheter was advanced into the left ventricle via the right brachial artery. The position of the catheter was confirmed by blood pressure measurements. A polyethylene catheter was introduced through the right external jugular vein and positioned near the right atrium. Another catheter was inserted into the right femoral artery. The left renal artery was exposed by a flank incision, care being taken not to enter the peritoneal cavity or cut the renal nerves. A collimated probe containing a 1-inch sodium iodide crystal and photomultiplier tube was placed over the kidney and connected to a rate meter and recorder. A 27-gauge butterfly needle was introduced into the renal artery near its origin. A bolus of 133Xe (600 μC) dissolved in approximately 0.5 ml of saline was injected into the renal artery and rapidly flushed with an equal volume of 0.85% saline. After the 133Xe washout curve was obtained, a bolus of 13 μC of 85Sr-labeled microspheres was injected into the left ventricle over a 10-second interval. The 133Xe washout curve was analyzed by the method of Thorburn et al. (4).

To measure cardiac output, 1 ml of Cardio- green dye (5 mg/ml) was injected into the renal vein. The catheter was withdrawn at a constant rate through a Gilford densitometer attached to a linear recorder. The curve was replotted on semilogarithmic paper and cardiac output calculated.

The kidney was removed, and sections were cut, weighed and counted as described above.

In the three dogs examined (Table 1), virtually all microspheres entering the kidney remained trapped, with less than 0.2% entering the renal vein. The size and the distribution of microspheres in the inner and outer cortex (Table 2) in these animals did not differ significantly. When examined under the microscope, microspheres were found only in glomerular capillaries (Fig. 1).

The importance of proper dispersion of microspheres in the injectate was illustrated by another case not shown here. In that case the suspension of microspheres was not mixed with the ultrasonic probe but with a vortex mixer. A sample of this suspension exhibited marked clumping with as many as 20 microspheres counted in one lump. When the suspension was injected into a dog's aorta, there were aggregates of microspheres in larger arteries of the kidney.

The effects of the spheres on renal function in one typical dog is shown in Table 3 and in Figures 2 through 4. After four injections of 5 mg of microspheres, there was no significant change in Cr, GFR, or T2 glucose.
shows the effect of these injections on $C_{\text{PAH}}$ and $C_{\text{Hb}}$ in all three animals. The average GFR of the nine control periods in these dogs was $39.3 \pm 6$ ml/min and for the last experimental period $37.6 \pm 2$ ml/min. $C_{\text{PAH}}$ averaged $167 \pm 42$ ml/min during control and $179 \pm 31$ at the end of the experiment. Due to the loss of blood samples, $T_m$ glucose was measured in only two of the three dogs and was initially $90.8 \pm 13$ mg/min (six periods) and at the end of the experiment $102 \pm 24$ mg/min. On the other hand, when the injection was increased to 50 mg of microspheres, $C_{\text{PAH}}$, GFR, and $T_m$ glucose fell progressively. $C_{\text{PAH}}$ for the nine control periods in this series was $163 \pm 53$, falling in the last period to $104 \pm 37$ ($P = .05$). GFR fell from $44 \pm 12$ to $14 \pm 5$ ($P < .01$). There was also a marked drop in

### Table 1

<table>
<thead>
<tr>
<th>Dog</th>
<th>Aorta (counts/min/ml)</th>
<th>Renal vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50,000</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>71,000</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>5,000</td>
<td>10</td>
</tr>
</tbody>
</table>

Distribution and Size of Microspheres

<table>
<thead>
<tr>
<th>Distribution and Size of Microspheres</th>
<th>No.</th>
<th>Per glomerulus</th>
<th>Avg size (um)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer cortex (532)</td>
<td>532</td>
<td>.103</td>
<td>17.1</td>
</tr>
<tr>
<td>Inner cortex (207)</td>
<td>207</td>
<td>.139</td>
<td>17.7</td>
</tr>
<tr>
<td>Outer medulla</td>
<td>9</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>Inner medulla</td>
<td>2</td>
<td>16.6</td>
<td></td>
</tr>
</tbody>
</table>

Number in parentheses is number of glomeruli counted.

![Figure 1](image)

**Figure 1**

Histological section of dog renal cortex. Three microspheres are seen in glomerular capillaries. $\times 200$.  

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Microspheres (6 mg intraaortic)

Microspheres (5 mg intraaortic)

Microspheres (5 mg intraaortic)

Microspheres (5 mg intraaortic)

Tm glucose from 69 ± 16 to 19 ± 5 (P < .01).

Table 3 gives the Tm glucose and Cmin in one dog. There was no significant change, which was typical in both the group receiving 5 mg and the group receiving 50 mg of microspheres. However, since the animal's concentrating mechanism was not maximally challenged, it is not possible to interpret the data on Tm glucose at the relatively low Cmin obtained.

In Table 4 the effect of acute hemorrhage on cortical circulation is tabulated. Since cardiac output and counts injected used to compute tissue blood flow are the same for outer and inner cortex, neither cardiac output nor renal blood flow needed to be measured. The ratio of tissue counts is an index of the fraction of renal blood flow perfusing one zone as compared to the other. Changes in this ratio signify redistribution of the renal...
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Effect of four injections of microspheres (150 g) on \( C_{CPA} \) and \( C_{IN} \) when each injection contained 50 mg of microspheres.

circulation. As shown in Table 4 the ratio of outer cortex to inner cortex counts fell after hemorrhage, indicating that a smaller fraction of cortical blood flow perfused the outer cortex and a greater fraction perfused the inner cortex.

Table 5 compares blood flow determinations using microspheres and \( 133 \)Xe washout. The rate of blood perfusion to the total cortex using both methods was similar to that reported using other methods (1-4). Total flow to the kidney as measured by both techniques was also quite similar. There was good correlation between the two methods. It is apparent, however, that it becomes difficult to find a mate for the first component of the \( 133 \)Xe studies. Autoradiographs done by others indicate that the first component may be either total cortical flow (4) or outer cortical flow (7). Autoradiographs made in this laboratory (8) favor an outer cortical volume of distribution for the first component. However, from the results shown in Table 5 one cannot exclude either possibility. Previous reports (7) also have indicated that the second component of the washout corresponds to inner cortical blood flow but here the results of the present experiments are not in agreement. Inner cortical blood flow is more than twice the flow in the second component. The difference is statistically significant. The flow rates in the first and second component are similar to those reported for this method by previous investigations (4, 7). Outer medullary flow is extremely low and probably represents only that portion of the blood flow in this region which has not previously perfused glomeruli.

Discussion

Recently, a number of investigators have used the rate of microsphere accumulation to measure organ and tissue blood flow (5, 9-  

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of Radioactivity per Gram Tissue Before and After Hemorrhage</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1014</td>
</tr>
<tr>
<td>1015</td>
</tr>
<tr>
<td>1021</td>
</tr>
<tr>
<td>1106</td>
</tr>
<tr>
<td>1107</td>
</tr>
<tr>
<td>1119</td>
</tr>
<tr>
<td>MEAN</td>
</tr>
<tr>
<td>66</td>
</tr>
</tbody>
</table>

\( C = \) total cortex; \( OC = \) outer cortex; \( IC = \) inner cortex.

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TABLE 5
Comparison of Tissue Blood Flow—Microspheres vs $^{133}$Xenon

<table>
<thead>
<tr>
<th>Dog</th>
<th>Cardiac output (ml/kg)</th>
<th>Microsphere blood flow (ml/g)</th>
<th>$^{133}$Xe blood flow (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112</td>
<td>4.61</td>
<td>5.02</td>
</tr>
<tr>
<td>2</td>
<td>107</td>
<td>4.02</td>
<td>4.69</td>
</tr>
<tr>
<td>3*</td>
<td>9.2</td>
<td>7.24</td>
<td>9.08</td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
<td>6.11</td>
<td>8.03</td>
</tr>
<tr>
<td>Avg</td>
<td></td>
<td>3.21</td>
<td>4.85</td>
</tr>
</tbody>
</table>

Abbreviations: C = total cortex; OM = outer medulla; CI and CII = first and second component of $^{133}$Xe washout curve.

*Total kidney. Hematoma around aortic arch found at autopsy.

12). The virtues of the method are that it is simple to perform, it appears theoretically well founded (5, 12), and repeated determinations may be made in the intact animal either acutely or chronically. Studies on large vessels indicate that the rheology of the spheres is similar to red cells occupying the central streams. Phibbs et al. (13) reported that the blood flow measured by each of two injections of microspheres was unchanged, indicating that the first injection did not alter blood flow. Although several investigators have reported on the intrarenal distribution of blood flow (9-11) the effect of the spheres on renal function has not been published nor has there been any study comparing the result of this method with any currently used techniques.

Several conditions must be met before the microsphere accumulation method can be employed. If any significant number of spheres escapes trapping and passes through the kidney into the renal vein, the calculated flow will be in error to the extent of such escape. The degree of escape may be a function of microsphere size. However, if large microspheres (50μ diameter) are employed to measure the flow, they could possibly be excluded from some parts of the renal microcirculation thus limiting their usefulness in measuring total organ flow. A previous report demonstrated that less than 1% of microspheres (15μ) entering were shunted through the kidney (14).

In the present study, microspheres (18 ± 3.7μ) were injected, and as shown in Table 1, an insignificant number was found in the renal vein. In addition, all microspheres in the cortex were trapped in glomeruli, indicating that microspheres gain access to all portions of the microcirculation. Microspheres were not found in other cortical vessels.

If axial streaming and plasma skimming of the injected microspheres occur in the intrarenal circulation, assessment of cortical blood flow distribution by this method would not be possible. If such plasma skimming occurs, the expected result would be a difference in the size and or distribution of microspheres in the outer and inner cortex. No such difference was observed.

One virtue of the technique is that repeated injections may be made with variously labeled microspheres to determine changes in blood flow. However, it is required that the microspheres themselves not interfere with renal function or blood flow. Previous studies cited above have shown that no significant change in blood flow occurs after two injections of microspheres. In the present study $C_{in}$ and $C_{PAH}$ were unchanged after four injections of microspheres, an indication that GFR and cortical blood flow were maintained. In addition, nephron mass as assessed by $T_m$ glucose was unaffected by the microspheres. To test whether microspheres could induce changes in GFR, $C_{PAH}$ and $T_m$ glucose, a massive dose was injected and the expected changes were observed. Interestingly, since there were no effects on $T_m$ glucose under the conditions studied no impairment of concentrating ability occurred. Such changes should occur...
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have resulted from impairment of vasa recta flow caused by the microspheres. It is possible that the maximal concentrating ability was in fact impaired but not challenged by this protocol.

To be useful, the microsphere technique must be sensitive enough to detect changes in blood flow within the range observed in experimental conditions such as acute hemorrhage. Redistribution of intrarenal circulation has been reported previously in this state (7, 15). In the present study, a shift of blood from outer to inner cortex was observed. Since cardiac output was not measured, absolute flows could not be measured. The results were expressed as ratios of outer cortical flow to inner cortical flow. It was observed that a relatively greater fraction of the intrarenal circulation perfused the inner cortex during acute hemorrhage.

The results obtained in determining both RBF and total cortical flow using microspheres are in agreement with previously reported studies using other techniques. Although there was a generally good correlation between the microsphere and 133Xe methods in RBF determinations when performed simultaneously, it was not possible to match the first and second components of the xenon washout with any definite anatomical region. The first component could have been measuring outer cortex or the entire cortex, whereas the second component did not measure inner cortical flow. A major problem, then, in interpreting washout curves (133Xe or 85Kr) is in assigning a specific anatomical zone to a component of the curve. This is so because all regions of the kidney having the same blood flow-volume relationship will fall under one component of the curve. When the conditions of the experiment are changed, as in acute tubular necrosis (16) or hemorrhagic shock (7), both the slope of the components and the anatomical zone they represent change, making interpretation difficult. Although the microsphere method cannot measure medullary flow directly, it offers indirect estimation of changes since the major portion of medullary blood flow probably derives from juxtamedullary glomeruli in the inner cortex.

Functionally distinct nephron populations occupy different zones of the cortex, with short-looped nephrons in the outer cortex and long-looped juxtamedullary nephrons in the inner cortex. To examine shifts in blood flow from one to the other, there is an advantage in using a method which measures the flow to the same anatomical zone before and after an intervention. The microsphere accumulation method has this characteristic.

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References


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