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Measurement of Renal Blood Flow in the Rat (38829)

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A radioactive microsphere technique has been developed for the measurement of renal blood flow and intrarenal blood flow distribution in the dog (1, 2). Various techniques have been proposed (3–5) for the measurement of renal blood flow (RBF) in the rat in addition to the conventional PAH clearance method. This study presents a simple method for the assessment of RBF in rats using radioactive microspheres.

Method. Eight male Sprague–Dawley rats weighing 200–240 g were fed with Purina Lab Chow and given tap water ad lib. All animals were lightly anesthetized with ether and cannulated with polyethylene tube No. 10 (i.d. 0.011 in., o.d. 0.024 in.) through the femoral artery for blood collection and through the carotid artery into the left ventricle for injection of microspheres. After awakening from anesthesia, animals were placed in restraining cages and allowed to recover for 45–60 min prior to injection. Radioactive microspheres, 15 μm $\pm$ 5 μm diameter, (3M Co., St. Paul, MN) were utilized to measure renal blood flow (RBF) and its intracortical distribution. Two different nuclides were used, ^{85}Sr and ^{141}Ce, for two separate measurements taken 1 hr apart. For each measurement, approximately 0.1 ml of 2 mg/ml concentration of microspheres in 10% dextran was injected through the carotid artery catheter within 5–7 sec. Just prior to injection, the spheres were sonicated for 5–10 min and then vigorously agitated with a vortex mixer for at least 3 min. Immediately upon injection of the spheres into the left ventricle, the femoral catheter was opened and blood was allowed to flow freely into a preweighed tube for exactly 1 min. Approximately 0.2 ml of blood was collected from the femoral artery in each minute. The adequacy of 1-min collection periods, vis à vis complete removal of microspheres from the circulation, was tested in a separate group of five animals. These animals were treated identically except that the femoral catheter was opened 30 sec after injection of the microspheres into the left ventricle. Blood samples were then taken for 30 sec and tested for radioactivity. This procedure was repeated several times in each animal and in no case was any isotope detected in the sample. Thus, a collection period of 1 min assures complete removal of the microspheres from the circulation.

After the second injection of spheres, the animals were sacrificed and the kidneys removed, decapsulated, and immediately counted in a Packard Gamma Counter and then weighed. The blood samples from the ^{85}Sr and ^{141}Ce injections were also counted and weighed. The exact blood volume collected was calculated by dividing the weight of the blood sample by the specific gravity of rat blood, which was previously determined in nine animals to be 1.107 $\pm$ 0.001 g/ml. All ^{141}Ce kidney counts were corrected for ^{85}Sr spillage which was approximately 7–9%. RBF was calculated as:

$$\text{RBF} = \frac{\text{total kidney cpm}}{\text{femoral blood cpm}} \times \text{femoral blood flow rate (ml/min).}$$

The results are corrected to 100 g body weight, or to 1 g kidney weight to normalize the variation between animals of varying sizes.

Intracortical blood flow distribution was determined by counting the outer two-thirds and inner one-third of the cortex according to the anatomical distribution of two different nephron populations (6, 7). A coronal section of the midportion of the kidney (0.3–0.4 cm thick) was sliced into sections less than 1 mm thick on a Stadie-Rigg's microtome. The cortex was separated from the medulla with a scalpel and divided into two

TABLE I. COMPARISON OF TWO DETERMINATIONS OF RENAL BLOOD FLOW AND INTRACORTICAL BLOOD FLOW DISTRIBUTION IN THE SAME RATS.[a]

$N = 8$	RBF (ml/min/100g/BW)	OC RBF (ml/min/g CW)	IC RBF (ml/min/g CW)	OC/IC
^{85}Sr	5.46 ± .31	12.89 ± .70	5.07 ± .54	2.52 ± .26
^{141}Ce	4.90 ± .41	11.32 ± 1.23	5.76 ± .60	2.13 ± .25
	P = NS	P = NS	P = NS	P = NS

[a] N = number of rats; RBF = Renal Blood Flow; BW = Body Weight; OC = Outer Cortex; IC = Inner Cortex; CW = Weight of Cortex. Results are expressed as mean ± standard error. P = Statistical analysis by paired t test; NS = Not Significant. OC/IC = blood flow ratio of outer cortex to inner cortex.

TABLE II. RENAL BLOOD FLOW AND INTRACORTICAL BLOOD FLOW DISTRIBUTION (AVERAGE OF TWO DETERMINATIONS).[a]

RBF (ml/min/100 g BW)	OC RBF (ml/min/g CW)	IC RBF (ml/min/g CW)	Rt kidney RBF (ml/min/g KW)	Lt kidney RBF (ml/min/g KW)	Rt kidney OC/IC	Lt kidney OC/IC
5.18 ± .29	12.10 ± .79	5.42 ± .52	6.81 ± .63	6.92 ± .41	2.32 ± .23	2.39 ± .21

[a] KW = kidney weight. The other abbreviations are the same as in Table I.

regions, the outer two-thirds (OC) and inner one-third (IC). The regional slices were weighed, suspended in 1 ml normal saline, and counted. Absolute blood flow per g of outer and inner cortical tissue was calculated as follows:

$$\text{rbf} = \frac{\text{cortical tissue cpm}}{\text{total kidney cpm} \times \text{cortical tissue wt}} \times \text{RBF},$$

where rbf is regional blood flow.

Results. The validity of using microspheres for the measurement of renal blood flow depends upon homogeneous mixing of the microspheres in the blood before entering the kidneys. In the present study, the ratio of the isotope counts per g of the outer two-thirds (OC) of the renal cortex to that of the inner one-third (IC) was determined in each animal to test the adequacy of mixing of the microspheres. As shown in Table I, the mean ratios achieved by the two different nuclides in the same kidney were 2.52 ± .26 (^{85}Sr) and 2.13 ± .25 (^{141}Ce), respectively. These were not different by paired statistical analysis. Furthermore, the OC/IC ratio of the right kidney (2.32 ± .23) was not different from that of the left kidney (2.39 ± .21) of the same animal by paired statistical analysis (Table II). This indicates that the results are reproducible and the microspheres are homogeneously mixed in the blood before entering the kidney. The concentration of microspheres is critical in that concentration less than 2 mg/ml may not give consistently reproducible results, as we found in preliminary experiments.

Table I shows that using ^{85}Sr and ^{141}Ce radioactive microspheres, the two determinations of total and regional renal blood flows are similar and reproducible. Table II shows the average of two determinations of renal blood flow and intracortical blood flow distribution. The use of microspheres this size (15 μm ± 5 μm) actually measures glomerular blood flow since the spheres are mainly trapped in the glomerular capillaries (8). Hence, the value of RBF more appropriately reflects total cortical blood flow.

Discussion. The validity of this method for measuring RBF in the rat not only depends on homogeneous mixing of spheres in the blood, but also on the assumption of complete trapping of microspheres inside the kidneys during their first transit. This has been proved to be the case in rats, as only about 1 % or less of the spheres escape from the microcirculation of the kidney (8).

Streaming of the microspheres was not significant in the hydropenic rat; however, after volume expansion of the rat with saline, axial streaming may occur (10). The value of RBF we obtained using microspheres compares favorably with the values reported in the literature. Our RBF of 5.18 ml/min/100 g body weight is similar to Goldman's (9) 4.37 ml/min/100 g (unanesthetized Wistar rats) and almost identical to that of Chedru's (4). Values of intrarenal blood flow distribution, despite some differences in the cortical zonal determination, are also comparable to those of Wallin *et al.* (10). It should be pointed out, that with this microsphere method, both total RBF and cortical blood flow distribution are generally higher than those values determined by xenon wash-out techniques (3, 11). The reason for this difference is not clear. However, the average of 69% distribution of outer cortical flow in the present study is identical to that reported by the xenon wash-out method in the rat (3).

In summary, the radioactive microsphere technique is a simple method for measurement of RBF and intrarenal blood flow distribution in the rat that does not require surgical manipulation of the kidney or general anesthesia. The results are reproducible and compatible with other established techniques.

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