Effect of Renal Vasodilatation on the Distribution of Cortical Blood Flow in the Kidney of the Dog

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ABSTRACT Studies were performed to evaluate the validity of using the radioactive microsphere technique to measure regional blood flow in the renal cortex. A technique was developed in which the renal cortex was divided into four equal zones, and the fractional and absolute distribution of blood flow in these zones was determined. It was consistently found that approximately 70% of the renal blood flow was distributed to the two outer cortical zones with the remaining 30% going to the two inner cortical zones. In addition, there was a reproducible pattern of distribution of blood flow in different areas of the same kidney after a single injection of microspheres and in the same area of the kidney after multiple injections of microspheres.

Using this method, the distribution of renal blood flow was determined before and during the intrarenal administration of either acetylcholine (40 μg/min) or bradykinin (5 μg/min). Both agents decreased the per cent of blood flow to outer cortical zone 1, caused no change in zone 2, and increased the fractional blood flow in inner cortical zones 3 and 4. When this data was evaluated in terms of total blood flow, there was no change in zone 1, an increase in zone 2 commensurate with the change in total blood flow, and a marked increase in inner cortical zones 3 and 4 which accounted for 60 and 65% of the increase in total blood flow during acetylcholine and bradykinin administration, respectively.

Therefore, the natriuresis of renal vasodilatation is associated with a redistribution to inner cortical nephrons.

INTRODUCTION

It has been suggested that alterations in the distribution of renal blood flow play an important role in the regulation of sodium balance (1). Barger and Herd have found using the ^{85}Kr technique that a redistribution of renal blood flow to the outer cortex occurs in models associated with a natriuresis while the opposite blood flow pattern is seen in salt-retaining states (1, 2). These investigators have suggested that the outer cortical nephrons may have a limited capacity to reabsorb sodium relative to the juxtamedullary nephrons, and a natriuresis would result when the fraction of renal blood flow delivered to these nephrons was increased (redistribution hypothesis).

Since the ^{85}Kr technique depends upon corroboration with radioautography to localize blood flow to a specific area of the cortex, more direct techniques have been utilized. Aukland and Wolgast used a technique in which blood flow is measured with a hydrogen-sensitive electrode placed in different areas of the cortex (3). With this technique these authors found no alteration in the distribution of renal blood flow during hemorrhagic hypotension in sharp contrast with the results of Carriere, Thorburn, O'Morchoe, and Barger using the ^{85}Kr technique (4).

Although the various electrode techniques do precisely localize each area of cortex, the possible local damage from these electrodes is a definite drawback to the method. Recently, the radioactive microsphere technique developed by Rudolph and Heymann (5), and Wagner, Rhodes, Sasaki, and Ryan (6) has been used to measure the distribution of total organ blood flow after various experimental maneuvers. Since this technique will allow precise localization of the blood flow in various areas of cortex without local damage to specific areas of the renal cortex, it seemed ideally suited to be used as a measurement of the distribution of renal blood flow. Recent studies by McNay and Abe have suggested that the microsphere technique could be used as a valid measurement of regional blood flow in the kidney (7).

The present studies were designed to further investigate this method and to determine if there were any

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alteration in the distribution of renal blood flow during renal vasodilatation. The results provide further evidence that this technique is a true indicator of regional blood flow in the renal cortex. In addition, it was found that blood flow was redistributed to inner cortical nephrons during renal vasodilatation.

METHODS

Studies were performed on mongrel dogs weighing between 13 and 23 kg. All animals were deprived of food and water for 18–24 hr before the study. The dogs were anesthetized with pentobarbital (30 mg/kg) and were subsequently given small maintenance doses as necessary. An endotracheal tube was inserted, and the animals were ventilated with a Harvard respirator. Cannulas were inserted in a leg vein for infusions and in the femoral artery for blood pressure measurements and blood collection. A Goodale-Lubin standard wall catheter was placed in the left ventricle by retrograde threading from the left carotid artery. Both ureters were cannulated through a suprapubic incision. In the drug studies, a 23-gauge hooked needle was placed in the orifice of the left renal artery and kept open with an infusion of Ringer's solution at a rate of 0.4 ml/min throughout the study. In some studies, a catheter was placed in the left renal vein for measurement of *p*-aminohippurate (PAH) extraction. An infusion was given to establish and maintain an inulin concentration of 20 mg/100 ml and a PAH concentration of 2 mg/100 ml.

Radioactive microspheres[1] were used to measure regional blood flow in different areas of the renal cortex. Approximately 200,000 microspheres (15–20 μCi), 19 ± 2 μ in diameter, were given with each injection. The nuclides used were ^{85}Sr and ^{141}Ce. The nuclide to be injected was suspended in a 1 ml solution of 10% Dextran and was injected through the left ventricular catheter in approximately 10 sec and then flushed with another 20 ml of saline. There was no change in heart rate, mean pressure, or pulse pressure after a microsphere injection.

At the end of the experiment, the kidney was removed for sectioning. Several sectioning techniques were initially tried, and it was found that the following was the simplest and most reproducible. Three or more tangential sections 1 cm thick were obtained. From each of these sections, a 1 cm cube was removed with a fresh razor blade with the depth of these cuts extending from the outer cortex to the outer medulla. The cortical thickness was measured, and the tissue sample was then placed in a metal kidney cutter. These cutters were of varying lengths with four equidistant grooves on each side of the housing of the apparatus. For example, if the cortical thickness was 8 mm, the grooves were 2 mm apart. The section was placed in the cutter so that the outer cortical zone was juxtaposed to the end of the holder, and the medullary zone was kept under constant pressure by a screw clamp. Four equal sections were then cut with a fresh razor blade placed in each of the four grooves. It was found that the entire cortex was obtained with these four sections and that a small area of medulla was usually also present in the inner portion of the last section. However, it was felt necessary to include this area to be certain that all juxtamedullary nephrons were counted. These sections were called zones 1–4, with zone 1 the most outer cortical zone and zone 4 the most inner cortical zone. In six studies sections of outer and inner medullary tissue were also obtained. Each section was then placed in a preweighed Packard Gamma counting tube,[2] reweighed, and counted in a Packard Auto-Gamma Counter.[2] The sample was placed on the bottom of the counting vial and counted for 5 min. No geometric effect was found by counting the tissue sections in the manner described. Strontium-85 was counted at the 0.510 Mev peak, and cerium-141 was counted at the 0.145 Mev peak. No correction was necessary for the ^{85}Sr counts, but 15% of the ^{85}Sr counts was subtracted to obtain the true ^{141}Ce counts.

The following four types of experiments were performed. (*a*) in six studies after the injection of a single nuclide a comparison was made of the distribution of blood flow to the four cortical zones in two different areas at least 3 cm apart along the long axis of the kidney. (*b*) In six studies a comparison was made of the distribution of cortical blood flow in different zones of the cortex after the injection of two separate nuclides 15 min apart. (*c*) In nine studies the distribution of cortical blood flow was determined before and during the administration of acetylcholine 40 μg/min in the left renal artery. (*d*) In six studies similar measurements were obtained before and during the administration of bradykinin 5 μg/min in the left renal artery. In the last three groups of studies the values presented represent the mean per cent distribution of blood flow in at least three different sections of each zone. Two 15-min clearance periods were obtained before and after the injection of the microspheres in the first two groups of studies, whereas in the studies in which a renal vasodilator was given three 15-min clearance periods were obtained before and during the administration of the drug under study. In the vasodilatation studies the microsphere was given 15 min after the infusion of either acetylcholine or bradykinin had begun.

CALCULATIONS

Glomerular filtration rate was determined from the inulin clearance. Renal blood flow (RBF) was determined by the following formula:

$$\text{RBF(ml/min)} = \frac{C_{PAH}}{E_{PAH}(1 - \text{Hct})} \qquad (1)$$

where C_{PAH} is the clearance of PAH, E_{PAH} is the renal extraction of PAH, and Hct is the arterial hematocrit.

The uncorrected per cent of renal blood flow per cortical zone (P_z), per cent of flow per gram, was determined by dividing the counts per minute per gram of tissue in the respective zone (CPM_z) by the total for all four cortical zones (CPM_T).

The corrected per cent of renal blood flow per cortical zone was determined by the following formula:

$$\text{Corrected per cent zonal blood flow } (P_z') = CPM_z \times Wt_z/CPM_T' \qquad (2)$$

where Wt_z is the weight of the zone and CPM_T' is the total counts per minute per gram times the zonal weight for all four zones.

Wt_z was obtained using the derivation of the volume of the four cortical zones obtained by McNay and Abe in

[1] Minnesota Mining & Manufacturing Co., St. Paul, Minn.

[2] Packard Instrument Co., Inc., Downers Grove, Ill.

which the per cent of renal volume was found to be 27, 22, 17, and 12% in zones 1–4, respectively (7). Therefore:

$$Wt_z = V_z \times Wt_r \quad (3)$$

where V_z equals the volume of the respective zone and Wt_r equals the kidney weight.

Zonal blood flow (BF_z) was estimated by the following formula:

$$BF_z = P_z' \times RBF \quad (4)$$

Zonal perfusion rate was determined by dividing BF_z by Wt_z.

Inulin was determined by the diphenylamine method (8), PAH by the method of Bratton and Marshall (9), and sodium with an IL flame photometer model IL 123. Statistical analysis was performed by standard methods and the data is presented as the mean ±SEM.

RESULTS

Validation studies. In preliminary studies, histological sections were taken at various levels of the cortex, and it was found that the microspheres were trapped exclusively in glomeruli. In six studies, outer and inner medullary tissue sections were obtained, and less than 1% of the counts per minute per gram was found in each study when compared with the total for the four cortical zones. In addition, in four studies no counts were found in the renal vein or ureter after microsphere injection.

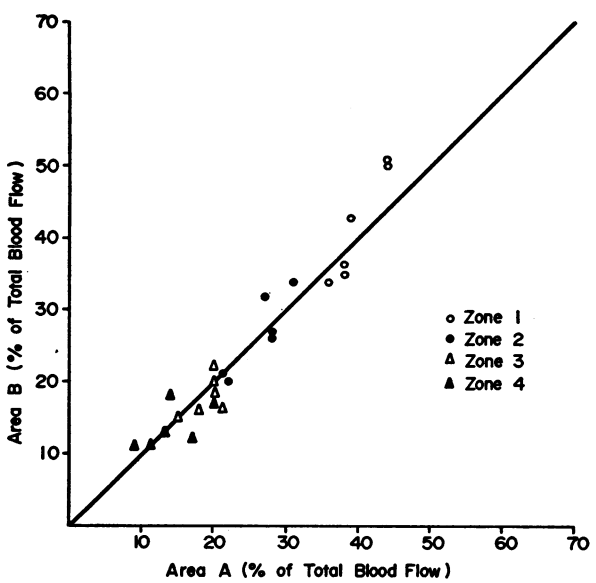

FIGURE 1 Comparison of the fractional distribution of blood flow between two different areas of the renal cortex after a single injection of radioactive microspheres. The corresponding cortical zone for each point is given. The per cent of blood flow to zones 1 and 2 was consistently greater than that to zones 3 and 4.

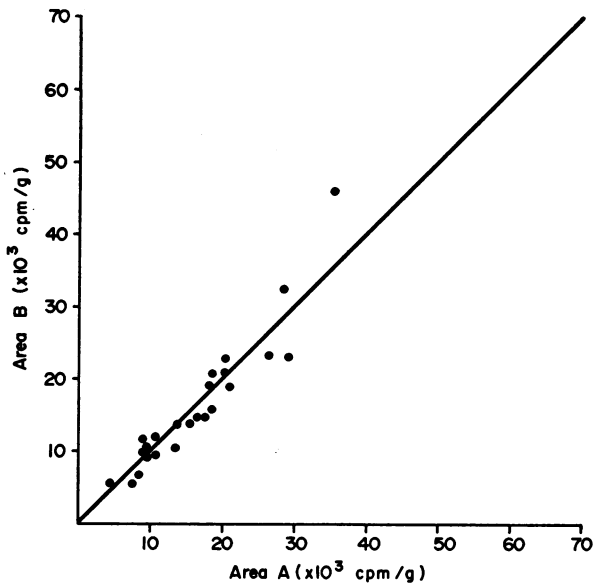

FIGURE 2 Comparison of the absolute distribution of blood flow between two different areas of the renal cortex after a single injection of radioactive microspheres. The radioactive counts per minute per gram in each section were used as an index of absolute blood flow.

Since the radioactive microsphere technique has previously been shown to measure total organ blood flow (5, 10, 11), the main points evaluated were the distribution of blood flow in different portions of the same kidney after the single injection of a radioactive nuclide and a comparison of the distribution of renal blood flow obtained with two different nuclides given 30 min apart.

In Figs. 1 and 2 and Table I are shown the data from six experiments in which blood flow to different areas of the cortex was determined after an injection of ^{141}Ce. Sections of zones 1–4 were obtained from two different areas of the kidney, and a comparison was made of the uncorrected per cent of total blood flow and the counts per minute per gram of tissue in the same zone in the two different portions of the kidney. The former would give a comparison of the fractional distribution of blood flow, whereas the latter is an index of absolute blood flow per zone. The mean per cent of renal blood flow to zone 1 was 40 ±1 and 42 ±3% in the two different areas. The per cent blood flow in zone 2 was 26 ±2% in area A and 27 ±2% in area B, 18 ±1 and 18 ±1% in zone 3, while zone 4 was 14 ±2 and 13 ± 1% of the uncorrected blood flow in the two different areas of this zone.

In Fig. 2 and Table I are presented the data comparing the counts per minute per gram in area A of each zone with area B of the same zone. It is apparent

TABLE I

Comparison of the Distribution of Blood Flow in Different Areas of the Renal Cortex after a Single Injection of Radioactive Microspheres

Exp. no.	Uncorrected per cent distribution to cortical zone								Total radioactivity to cortical zone								Glomerular filtration rate	
	Zone 1		Zone 2		Zone 3		Zone 4		Zone 1		Zone 2		Zone 3		Zone 4			
	A*	B*	A	B	A	B	A	B	A	B	A	B	A	B	A	B	GFR$_1$‡	GFR$_2$§
	%								*cpm/g*								*ml/min*	
1	38	35	28	27	20	20	14	18	29,174	23,190	21,019	18,500	15,400	13,939	10,377	11,801	25	25
2	36	34	31	34	20	22	9	11	18,757	15,823	16,319	15,787	9,176	11,624	4,855	5,376	—	—
3	44	51	21	21	18	16	17	12	20,274	22,628	9,969	9,324	8,166	6,922	7,862	5,109	31	33
4	44	51	22	20	21	16	11	11	35,985	46,745	18,373	18,415	17,380	14,671	9,586	10,564	30	29
5	38	36	27	32	15	15	20	17	26,385	23,157	18,506	20,735	10,537	9,500	13,350	10,430	—	—
6	39	43	28	26	20	18	13	13	28,191	32,724	20,047	20,154	13,991	13,962	9,200	9,850	44	42
Mean	40	42	26	27	18	18	14	13	26,461	27,377	17,372	17,152	12,441	11,769	9,205	8,855	32	32
SEM	1	3	2	2	1	1	2	1	2,616	4,527	1,646	1,745	1,531	1,266	1,165	1,191	4	4
P	NS		NS		NS		NS		NS		NS		NS		NS		NS	

* A and B = different areas of renal cortex.
‡ GFR$_1$ = glomerular filtration rate before microsphere injection.
§ GFR$_2$ = glomerular filtration rate after microsphere injection.

that there is excellent correlation between the counts per minute per gram in areas A and B in the same zone of the renal cortex in the 24 comparative sections. There was no significant difference between the mean counts per minute per gram in areas A and B in any of the four zones. Therefore, the fractional and absolute distribution of blood flow was quite comparable in the same cortical zone in different areas of the same kidney as measured by the microsphere method.

In Table II and Fig. 3 are shown the data comparing the distribution of blood flow obtained with two different nuclides injected 30 min apart. The mean uncorrected distribution of flow with the first nuclide (^{85}Sr) was 38 ±3, 31 ±1, 20 ±2, and 11 ±1% in zones 1–4 respectively, and 37 ±3, 31 ±1, 21 ±2, and 11 ±1% after the injection of the second nuclide (^{141}Ce). There was no significant difference in the per cent of blood flow obtained with the two injections in any of the four cortical zones.

Glomerular filtration rate was measured in 10 studies. As is shown in Tables I and II, no significant change in

TABLE II

*Comparison of the Per Cent Distribution of Blood Flow in the Renal Cortex after Two Separate Injections of Radioactive Microspheres**

Exp. no.	Uncorrected per cent distribution to cortical zone								Glomerular filtration rate	
	Zone 1		Zone 2		Zone 3		Zone 4			
	I_1‡	I_2§	I_1	I_2	I_1	I_2	I_1	I_2	GFR$_1$‖	GFR$_2$¶
	%								*ml/min*	
1	37	32	30	32	21	25	12	12	34	36
2	29	31	35	30	24	28	12	10	37	35
3	48	40	28	30	17	21	7	9	40	38
4	47	47	30	33	13	14	10	6	29	27
5	31	35	33	31	21	22	14	12	32	34
6	35	35	28	28	21	21	16	16	35	36
Mean	38	37	31	31	20	21	11	11	34	34
SEM	3	3	1	1	2	2	1	1	2	2
P	NS		NS		NS		NS		NS	

* All values are the mean of three or more sections in the same kidney.
‡ I_1 = Distribution of renal blood flow determined from first injection of microspheres.
§ I_2 = Distribution of renal blood flow determined from second injection of microspheres.
‖ GFR$_1$ = glomerular filtration rate before first microsphere injection.
¶ GFR$_2$ = glomerular filtration rate after second injection of microspheres.

GFR was found after the injection of either one or both nuclides.

Therefore, these studies indicate consistent functional differences between outer and inner cortical blood flow with approximately 70% of the uncorrected blood flow being distributed to zones 1 and 2 and only 30% to the inner cortex. In addition, the method is reproducible and does not alter renal hemodynamics.

Acetylcholine studies. In the nine acetylcholine studies, sodium excretion increased from 6 ±1 μEq/min in the control period to 101 ±7 μEq/min. The mean control glomerular filtration rate was 36 ±3 ml/min and was 37 ±3 ml/min during acetylcholine. Renal blood flow was measured in four studies and increased from 189 ml/min to 257 ml/min during the administration of acetylcholine.

In Table III and Fig. 4 are shown the data of the effect of acetylcholine on the distribution of renal blood flow. The data is presented for both the corrected and uncorrected per cent of renal blood flow to each cortical zone before and during acetylcholine administration. Although the correction for zonal volume quantitatively increases the per cent of flow in outer cortical zones and decreases the per cent of flow in inner cortical zones in both the control and experimental period, there is no qualitative change in the data. Since this was also true of the bradykinin studies, the remainder of the data will be discussed in terms of the corrected zonal blood flow only. In zone 1 there was a marked decrease in the per cent of renal blood flow from 45 ±2 to 33 ±2% ($P < 0.001$). There was no change in zone 2 whereas both zones 3 and 4 had significant increases. In zone 3 there was a mean increase from 17 ±1 to 23 ±1% of the total blood flow ($P < 0.001$) while in zone 4 there was an increase from 10 ±1 to 16 ±1% of the flow ($P < 0.001$).

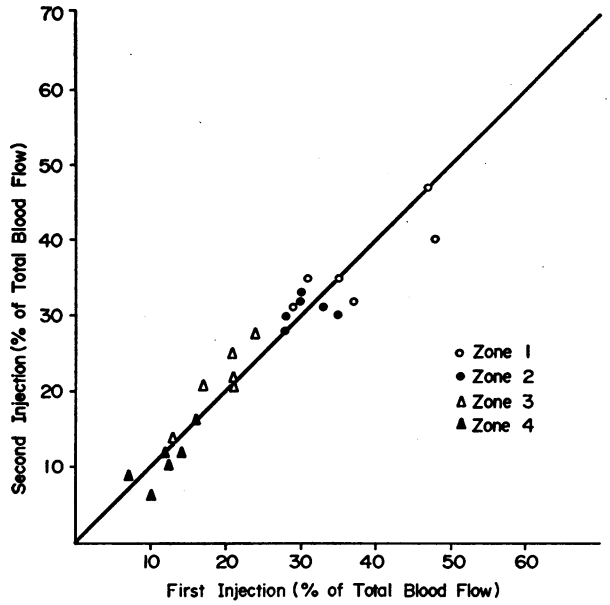

FIGURE 3 Comparison of the fractional distribution of blood flow in the same area of the cortex after the injection of two different radioactive microspheres. The corresponding cortical zone for each point is given. As shown, the per cent of blood flow to zones 1 and 2 was again consistently greater than that to zones 3 and 4.

TABLE III

*Effect of Acetylcholine on the Per Cent Distribution of Blood Flow in the Renal Cortex**

Exp. no.	Experimental kidney: Uncorrected per cent distribution to cortical zone								Experimental kidney: Corrected per cent distribution to cortical zone								Control kidney: corrected per cent distribution to cortical zone							
	Zone 1		Zone 2		Zone 3		Zone 4		Zone 1		Zone 2		Zone 3		Zone 4		Zone 1		Zone 2		Zone 3		Zone 4	
	C‡	Ach§	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach
	%								%								%							
1	37	29	21	18	22	32	22	30	48	29	21	21	18	30	13	20	53	57	24	20	15	15	7	7
2	38	29	32	29	18	23	12	19	41	29	29	29	18	23	12	19	40	40	30	28	20	22	10	10
3	37	27	24	24	20	24	19	25	46	37	26	27	16	20	12	15	49	40	30	34	12	17	9	
4	39	21	39	27	18	27	8	24	45	27	26	27	19	28	10	18	42	41	27	32	20	19	11	7
5	35	26	28	26	22	24	15	24	44	35	30	29	18	21	8	15	—	—	—	—	—	—	—	—
6	33	31	35	29	18	21	14	19	42	40	36	32	14	17	8	11	39	40	31	33	16	18	14	9
7	33	24	27	29	22	24	18	23	42	32	29	33	18	21	11	14	34	35	28	27	22	21	16	17
8	33	20	30	27	18	25	20	28	43	29	30	31	15	22	12	18	47	47	31	33	11	15	11	5
9	47	28	27	23	17	24	9	25	56	38	26	26	13	21	5	15	48	40	28	30	17	22	7	8
Mean	37	25	28	26	19	25	16	24	45	33	28	28	17	23	10	16	44	43	29	30	16	18	11	9
SEM	2	2	2	1	1	1	1	1	2	2	1	1	1	1	1	1	2	2	1	2	2	1	1	1
P	<0.001		NS		<0.001		<0.001		<0.001		NS		<0.001		<0.001		NS		NS		NS		NS	

* All values are the mean of at least three or more sections in the same kidney.
‡ C = control period.
§ Ach = acetylcholine period.

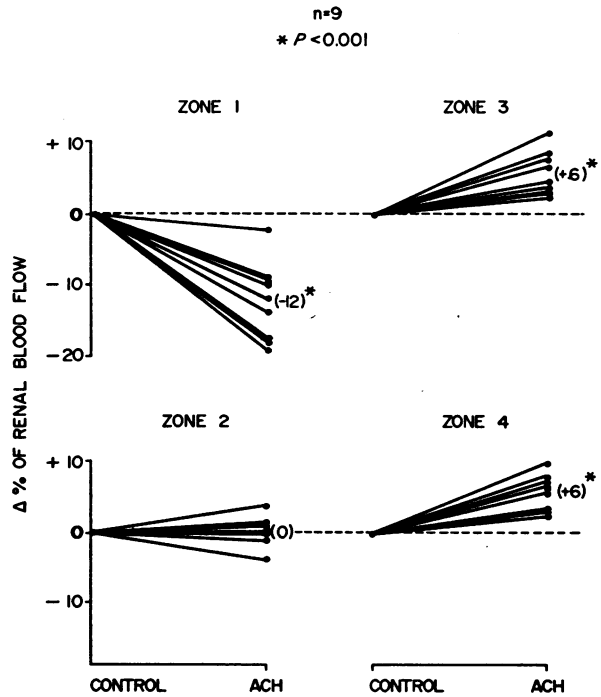

FIGURE 4 The effect of acetylcholine (ACH) on the fractional distribution of renal blood flow. The data are presented in terms of the absolute per cent change in blood flow in each zone, with any point above the zero-line representing an increase in the per cent of blood flow to that zone, whereas a point below the zero-line signifies the converse. The numbers in parentheses are the mean percentage change for each zone.

In eight of the nine studies the distribution of blood flow was determined in the contralateral kidney that was not receiving acetylcholine. This data is shown in Table III. In contrast with the vasodilated kidney, no alteration in the distribution of blood flow was found in any zone.

In four studies, total blood flow to each of the four cortical zones was determined before and during acetylcholine (Table IV and Fig. 5). It should be noted that there was no significant change in total blood flow in outer cortical zone 1 while zonal blood flow increased 20, 21, and 20 ml/min in zones 2–4 respectively during acetylcholine administration. These changes were all statistically significant.

In these same studies zonal perfusion rate in the control period was 7.6, 6.1, 4.1, and 3.0 ml/min per g in zones 1–4 respectively. During acetylcholine administration there was an increase in zones 2–4 of 34, 73, and 130% while the total renal blood flow increased 39%. There was an insignificant increase in zonal perfusion rate of 8% in zone 1.

Bradykinin studies. In the six bradykinin studies sodium excretion increased from a control value of 13 ±1 to 94 ±8 μEq/min. The mean control GFR was 40 ±3 ml/min and 39 ±4 ml/min during bradykinin. Renal blood flow was measured in five studies and increased from 223 ±23 to 325 ±25 ml/min during bradykinin infusion.

In Table V and Fig. 6 are shown the data on the effect of bradykinin on the distribution of renal blood flow. In zone 1, the per cent of blood flow decreased from 49 ±4 to 34 ±3% ($P < 0.001$). There was no change in zone 2. In contrast, in zone 3 flow increased from 15 ±1 to 21 ±2% ($P < 0.005$), and zone 4 increased from 8 ±1 to 16 ±1% ($P < 0.001$). In two studies, the distribution of blood flow in the contralateral kidney was unchanged.

In five studies, total blood flow and zonal perfusion rates to each of the four cortical zones was determined before and during bradykinin administration (Table IV and Fig. 7). There was no change in total blood flow in zone 1 while there was an increase in zone 2 from 60 to 97 ml/min ($P < 0.01$). There was an increase in total blood flow in zone 3 of 32 ml/min ($P < 0.02$) and in zone 4 of 33 ml/min ($P < 0.005$). Therefore, approximately 65% of the increase in total renal blood flow during bradykinin administration was distributed to inner cortical zones 3 and 4. The zonal perfusion rate was unchanged in zone 1 and increased in zones 2–4 in a similar fashion to the zonal blood flow.

DISCUSSION

In the present study, we have evaluated the use of radioactive microspheres to measure regional blood flow

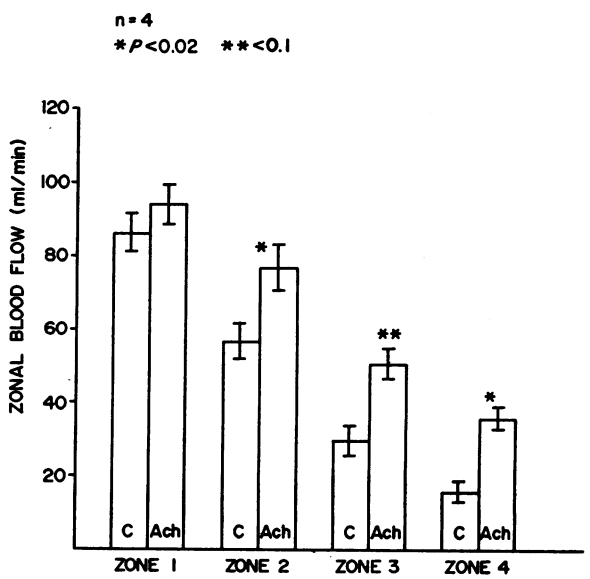

FIGURE 5 The effect of acetylcholine (ACH) on total zonal blood flow. See text for method of calculating zonal blood flow. C = control period.

TABLE IV

Effect of Acetylcholine and Bradykinin on the Distribution of Total Renal Blood Flow

	Zonal blood flow								Total renal blood flow		Zonal perfusion rate								Renal blood flow	
	Zone 1		Zone 2		Zone 3		Zone 4				Zone 1		Zone 2		Zone 3		Zone 4			
Exp. no.	C*	E‡	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
	ml/min								*ml/min*		*ml/min per g*								*ml/min per g*	
Acetylcholine-A_5	81	102	55	85	33	61	16	44	185	292	6.8	8.5	5.5	8.5	4.3	3.9	3.0	8.1	4.1	6.5
-A_6	74	102	64	77	25	42	14	27	177	249	6.8	9.4	7.2	8.8	3.7	6.2	2.9	5.6	4.4	6.2
-A_7	96	84	66	87	41	55	25	37	229	264	8.0	7.0	6.6	8.7	5.3	7.1	4.6	6.8	5.1	5.9
-A_9	92	86	43	59	21	47	8	34	164	227	8.8	8.2	5.0	6.9	3.2	7.1	1.7	7.2	4.2	5.8
Mean	86	94	57	77	30	51	16	36	189	257	7.6	8.2	6.1	8.2	4.1	7.1	3.0	6.9	4.4	6.1
SEM	5	5	5	6	4	4	3	3	14	13	0.6	0.6	0.5	0.5	0.5	0.4	0.7	0.7	0.4	0.2
P	NS		<0.02		<0.01		<0.02		<0.01		NS		<0.01		<0.01		<0.02		<0.02	
Bradykinin -B_1	139	111	59	92	45	70	24	44	267	317	10.2	8.4	5.4	8.4	5.2	8.2	4.0	7.3	5.3	6.3
-B_2	107	122	34	67	18	43	9	32	168	260	9.5	10.8	3.7	7.3	2.5	6.1	1.8	6.4	5.3	6.2
-B_3	84	103	54	87	30	58	19	55	187	303	7.0	8.6	5.4	8.7	3.9	7.5	3.5	11.1	4.1	6.7
-B_4	104	110	62	126	37	99	18	59	221	395	9.2	9.7	6.7	13.7	5.2	14.0	3.6	11.8	5.3	9.4
-B_5	124	114	91	111	39	59	22	63	276	348	9.8	8.9	8.8	10.8	4.9	7.4	3.9	11.2	5.9	7.4
Mean	112	112	60	97	34	66	18	51	223	325	9.1	9.2	6.0	9.8	4.3	8.6	3.4	9.6	4.9	7.2
SEM	9	3	9	10	4	9	3	6	23	25	0.7	0.5	0.9	1.2	1.0	1.6	0.4	0.9	0.4	0.6
P	NS		<0.01		<0.02		<0.005		<0.01		NS		<0.02		<0.02		<0.005		<0.01	

* Control period.

‡ Experimental period.

in the kidney. It has been shown by Rudolph and Heymann (5), Neutze, Wyler, and Rudolph (10), and Kaihara, Rutherford, Schwentker, and Wagner (11) that this technique is a valid measurement of total organ blood flow. However, there are two possible criticisms of the application of the radioactive microsphere technique to measure regional flow in the kidney which must be evaluated before this method can be considered valid. First, the technical problem of measuring regional blood flow in discrete portions of the renal cortex must be considered. Our intention was to divide the cortex into areas that would be representative of the following three groups of nephrons as described by Baines, Baines, and de Rouffignac (12): (*a*) superficial nephrons (zone 1), (*b*) midcortical nephrons (zones 2 and 3), and (*c*) juxtamedullary nephrons (zone 4). Although anatomically this classification is not as totally warranted in the dog as in the rat, it is a useful classification for evalu-

TABLE V

*Effect of Bradykinin on the Per Cent Distribution of Blood Flow in the Renal Cortex**

	Experimental kidney																Control kidney: corrected per cent distribution to cortical zone							
	Uncorrected per cent distribution to cortical zone								Corrected per cent distribution to cortical zone															
	Zone 1		Zone 2		Zone 3		Zone 4		Zone 1		Zone 2		Zone 3		Zone 4		Zone 1		Zone 2		Zone 3		Zone 4	
Exp. no.	C‡	Ach§	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach	C	Ach
	%								%								%							
1	42	26	22	26	22	25	14	23	52	35	22	29	17	22	9	14								
2	53	36	21	23	14	19	10	22	64	47	20	25	11	16	5	12								
3	35	25	28	25	20	22	17	28	45	34	29	29	16	19	10	18	43	43	29	30	16	15	12	12
4	37	20	27	28	21	28	15	24	47	28	28	32	17	25	8	15								
5	36	24	33	29	18	20	13	27	45	33	33	32	14	17	8	18								
6	31	17	31	23	21	27	17	33	40	25	33	28	17	25	10	22	41	42	28	31	21	18	10	9
Mean	39	25	27	26	19	24	14	25	49	34	28	29	15	21	8	16	42	42	29	30	18	17	11	11
SEM	3	3	2	1	1	2	1	2	4	3	2	1	1	2	1	1	1	1	1	1	3	2	1	2
P	<0.001		NS		<0.05		<0.001		<0.001		NS		<0.005		<0.001		NS		NS		NS		NS	

* All values are the mean of three or more sections in the same kidney.

‡ C = control period.

§ B = bradykinin period.

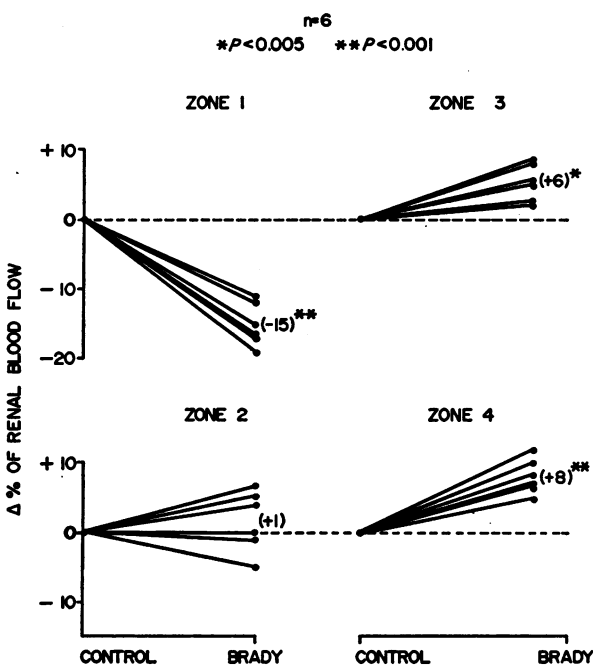

FIGURE 6 The effect of bradykinin (Brady) on the fractional distribution of renal blood flow. The data are presented in the same manner as in Fig. 4.

ation of the data in the context of the redistribution of blood flow and its relationship to salt balance. Therefore, it is of extreme importance that the different zones of the kidney sections be consistently divided from experiment to experiment. That this was the case is shown in Figs. 1 and 3 and Tables I and II in which both the uncorrected per cents of renal blood flow in the different zones are presented. A consistent pattern of blood flow was present, with the most outer cortical zone receiving the greatest per cent of blood flow, zone 4 the lowest, and zones 2 and 3 being intermediate. In addition, this pattern of blood flow was consistent both in different portions of the same kidney after a single injection of spheres (Figs. 1 and 2) and in the same portion of the kidney after separate injections of microspheres (Fig. 3). These findings are indeed evidence that functionally different portions of the renal cortex were being consistently obtained with our method. It should also be noted that even if some variation was present between studies, this should be offset by the control measurements obtained. For example, in the acetylcholine experiments (Table III) although there was a range of 41–56% of the blood flow distributed to zone 1 in the control period in these nine studies, a fall in the per cent of flow to that zone was found in each study during the administration of acetylcholine. Since the same tissue section was used for the control and experimental determination, the identical glomeruli were being evaluated in the measurement of regional blood flow in each zone.

Secondly, Pappenheimer and Kinter suggested that because of "plasma skimming" the hematocrit should progressively increase as the blood flows toward outer cortex (13). If this phenomenon occurs, radioactive microspheres would also be disproportionally concentrated in outer cortical nephrons. Several points militate against this concept. McNay and Abe have studied the distribution of renal blood flow with spheres of different sizes and density (7). If axial streaming did occur in the microvasculature of the renal cortex, it would be expected that the larger and more dense spheres would have a greater per cent distribution to the outer cortical zones. However, no significant differences were found when either variable was evaluated. In addition, studies in our laboratory have demonstrated that the superficial nephron filtration fraction calculated from the systemic hematocrit and the efferent arteriolar hematocrit was 0.30 while the total kidney filtration fraction was 0.36 (14). If significant axial streaming were present, the systemic hematocrit would underestimate the afferent arteriolar hematocrit and an erroneously high filtration fraction would be found in superficial cortical nephrons. Third, the present data itself raises a strong point against axial streaming being a determinant of the distribution of the microspheres. Valasquez, Notargiacomo, and Cohn have found in the dog that acetylcholine markedly decreases the transit time of indocyanine green through the kidney and, therefore, increases the velocity of blood flow in the renal circulation (15).

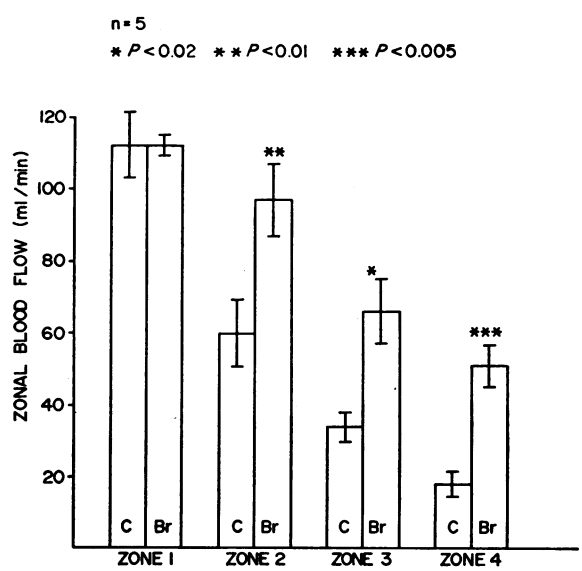

FIGURE 7 The effect of bradykinin (Br) on total zonal blood flow. See text for method of calculating zonal blood flow. C = control period.

Since an increased velocity of flow would increase axial streaming (16), acetylcholine should redistribute blood flow to outer cortical nephrons if streaming had a significant effect on the distribution of microspheres. Since the converse flow pattern was seen, it is quite unlikely that streaming is a significant determinant of the distributional pattern of the microspheres.

Therefore, the data indicates that the microspheres demonstrated a consistent pattern of distribution that is reproducible in different portions of the same kidney and from experiment to experiment. The present work as well as that of McNay and Abe (7) also indicates that axial streaming is not an important variable in the determination of the distribution of blood flow as measured by the microsphere method. It should also be pointed out that the exclusive entrapment of the microspheres in glomerular capillaries indicates that the regional blood flow data obtained are, in fact, an index of glomerular perfusion rate.

There are three possible explanations for the distributional pattern of blood flow seen during renal vasodilatation. First, considering the acetylcholine data alone, it is possible that the alterations in blood flow seen with this agent could be related to a greater concentration of cholinergic receptors in the inner cortical nephrons. Moffat has commented on cholinergic fibers present around efferent arterioles and proximal portions of the vasa recta of juxtamedullary nephrons of the rat (17). No comment was made about the content of these fibers in the remaining nephrons. McKenna and Angelakos noted that cholinesterase-positive nerve fibers were easily demonstrable around various vascular structures of the canine kidney, but presented no quantitative data on the relative distribution of these fibers in different portions of the cortex although the norepinephrine concentration was definitely higher in the juxtamedullary area (18). Therefore, the data present in the literature are not adequate to evaluate completely this possibility. However, since bradykinin has no known cholinergic action and its distributional effect was similar to acetylcholine, it seems more attractive to presume that the distributional changes in blood flow seen during renal vasodilatation with both agents are not due to a difference in cholinergic innervation of superficial and juxtamedullary nephrons.

It has been suggested that alterations in the delivery of sodium to the macula densa may in some manner regulate renin release (19, 20). If the vasodilators increase the delivery of sodium to the macula densa, this could stimulate and subsequently increase the local production of angiotensin. Since renin is present primarily only in outer cortical nephrons (21), this would lead to a selective increase in resistance of superficial nephrons. However, two points tend to militate against this postulate as the mechanism of the redistribution of blood flow found in this study. Tagawa and Vander found no consistent change in renal vein renin concentration during the administration of acetylcholine (22). In addition, we have found that acetylcholine, but not bradykinin, inhibits sodium reabsorption in the proximal tubule and increases delivery to the distal nephron (14). Therefore, unless bradykinin inhibits sodium reabsorption in the ascending limb of superficial nephrons, the delivery of sodium to the macula densa would not be increased by this agent.

The normal vascular resistance of the different groups of glomeruli may be an important determinant of the distributional pattern during renal vasodilatation. When blood flow per glomerulus was calculated from the microsphere data and the number of glomeruli present in each cortical zone,[3] it was consistently found that the blood flow per glomerulus was greatest in zone 1. This finding suggests that these nephrons have a lower total resistance than the remaining nephrons. Since micropuncture studies have shown that the filtration fraction of superficial nephrons of the dog calculated from the efferent arteriolar and systemic hematocrit is the same or slightly lower than that of the whole kidney (14), the diminution in resistance must be primarily preglomerular. If in the control state the preglomerular resistance of superficial nephrons is considerably lower and also relatively fixed relative to the remaining nephrons, a vasodilator such as acetylcholine or bradykinin may have little or no effect on the resistance of the vessels of superficial nephrons and would predominantly dilate and increase flow through the vessels of the more inner cortical nephrons.

Several other techniques have been used to evaluate the distribution of blood flow during renal vasodilatation. Harvey (23) and Pilkington, Binder, de Haas, and Pitts (24) have both noted that the extraction ratio of *p*-aminohippurate was decreased during the administration of acetylcholine. Kövér, Harza, Szőcs, Bálint, and Tarján found similar alterations in PAH extraction during acetylcholine administration (25). These authors also found that the renal extraction of ^{86}Rb and the calculated renal blood flow obtained from the ^{86}Rb dilution technique were decreased during the administration of acetylcholine. These results have been interpreted to indicate that noncortical and presumably medullary blood flow is increased in this experimental model. These results are quite compatible with the present data since an increase in inner cortical nephron blood flow would be likely also to increase medullary blood flow. McNay and Abe have also noted qualitatively similar findings as in the present study using the microsphere technique

[3] Stein, J. H., T. F. Ferris, J. E. Huprich, T. C. Smith, and R. W. Osgood. Unpublished observations.

(7). The only possible contradiction to these results is in work by Barger and Herd (2). However, since the studies were performed in unanesthetized dogs and no tabular data were given, it is not possible to compare those results with the findings using the microsphere technique.

The natriuresis seen during renal vasodilatation could be due at least in part to the increase in inner cortical blood flow found in this study. This could be explained by a model originally suggested by Earley and Friedler (26, 27). The increase in inner cortical blood flow would presumably increase medullary blood flow and therefore decrease the medullary interstitial hypertonicity. This would decrease water abstraction in the descending limb of Henle's loop and lead to the delivery of an increased volume of fluid with a decreased sodium concentration to the ascending limb. If this altered reabsorption in the ascending limb and/or distal nephron, a natriuresis would result.

It should also be noted that the present data indicate that a natriuresis can occur in a circumstance in which blood flow is being redistributed to inner cortical nephrons. Although this could conceivably be an exceptional model, these data would seem to make the redistribution hypothesis as originally proposed less attractive.

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