Absence of Aglomerular Blood Flow During Renal Vasodilatation and Hemorrhage in the Dog (37377)

THOMAS M. O'DORISIO, JAY H. STEIN, RICHARD W. OSGOOD, AND THOMAS F. FERRIS
(Introduced by Samuel Saslaw)
Department of Medicine, Ohio State University, Columbus, Ohio 43210

In 1948, Trueta *et al.* (1) suggested that a diversion of renal blood flow from glomerular to an aglomerular pathway may occur in various pathologic settings. Subsequent anatomic and physiologic studies have lent some credence to this hypothesis (2–6). However, in evaluating the radioactive microsphere method to measure regional blood flow in the kidney, McNay and Abe (7), Stein *et al.* (8), and Slotkoff and associates (9) found that 99% of the 15 μm spheres which entered the kidney were trapped within the glomerular circulation. This would strongly suggest that at least in the control setting there is no significant aglomerular blood flow pathway. To determine whether this is also true in other conditions previously thought to be associated with increased shunting (10, 11), we have used the radioactive microsphere method during renal vasodilatation and hemorrhagic hypotension and quantitatively measured renal venous radioactivity in both these states. In both models less than 1% of the microspheres were found in the venous effluent.

Materials and Methods. The studies were carried out on female mongrel dogs weighing 13–21 kg. The dogs were anesthetized with pentobarbitol (30 mg/kg). An endotracheal tube was inserted and the animals were ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, MA). A left flank incision was made and the left kidney was exposed. A 21-gauge hooked needle was placed in the orifice of the left renal artery and the left renal vein was catheterized with PE 160 tubing via the ovarian vein.

Either of two procedures was then done:

a. In six studies, a 23-gauge hooked needle was placed in the left renal artery and kept open with an infusion of Ringer's solution. Acetylcholine 40 μg/min was then given for 15–20 min.

Then 50,000 radioactive microspheres 15 $\pm$ 3 μm labeled with ^{85}Sr were given over 15–30 sec in the left renal artery. As the microspheres were being given, the renal vein was clamped distal to the catheter and renal venous blood was continuously aspirated with 50 ml syringes to which had been added small amounts of heparin. This was continued for 1 to 2 min after completion of the microsphere injection. The removal rate was always kept rapid enough to prevent swelling of the kidney.

Since the extraction of the microspheres was determined by collecting all of the renal venous effluent, the hemodynamic effect of the blood removal might alter intrarenal hemodynamics and the trapping of the indicator. However, there were several points which militate against this possibility. First, since the mean transit time of blood through the kidney is approximately seven seconds, less than 100 ml of blood would have been removed by the time the last wave of spheres would have entered renal venous blood. With shunting, the transit time presumably would have been even shorter. Secondly, the removal rate of blood, which was felt to be a valid index of total renal blood flow, averaged 270 ml/min, a value quite similar to that found previously in this laboratory (8) and indicates that in spite of blood removal, renal blood flow was increased. Third, the removal rate did not change significantly over the period of collection of renal venous blood, demonstrating the persistence of the vasodilatory effect of acetylcholine.

b. In five studies, microsphere recovery

from the renal vein was measured during hemorrhagic hypotension. The animal was bled into a reservoir to a mean arterial pressure between 60 and 70 mm Hg, as previously described (12). Thirty minutes after stabilization of this pressure, an injection of microspheres was given in the renal artery with concomitant withdrawal from the renal vein for 2 min as performed in the first group of studies. Blood removal rate averaged 102 ml/min, a value similar to the value obtained for blood flow in this model (12).

At the conclusion of each study, the kidney was removed and stripped of perirenal fat and sectioned into 0.3 cm coronal slices and placed on the bottom of a plastic container. In 3 studies in each group, the proximal and distal end of the renal vein was also ligated and removed without drainage and counted separately, as was the renal venous catheter. The heparinized renal venous blood was placed in the same type of plastic container and the blood was allowed to stand for 2 hr. This was found to be an adequate period for total settling of microspheres to the bottom of the container. Utilizing this method, the recovery of a known quantity of radioactive spheres injected into 300 ml of heparinized blood was greater than 90%. The blood, kidney slices, and renal vein sections were then counted in a gamma well counter (Packard Model 3002 Tri-Carb scintillation spectrometer) at 0.510 keV with a special well to accommodate the width of the plastic containers. The distance of the plastic container from the crystal was maintained at 30 cm to keep geometry constant. In three studies in each group, histologic sections were also obtained for microsphere localization.

The percentage of microspheres recovered in renal venous blood was calculated by the following formula:

$$\text{recovery (\%) of microspheres} = \frac{\text{renal vein blood}_{cpm}}{\text{kidney}_{cpm} + \text{renal vein blood}_{cpm}},$$

where the numerator is the counts per minute in renal venous blood and the denominator is the sum of the counts per minute in the injected kidney and renal venous blood. It should be noted that calculating recovery in this manner obviates the problem of incomplete injection or the determination of what percentage of the injection remained in the syringe, intravenous tubing, or needle.

Results. In Table I are summarized the results of the six acetylcholine and five hemorrhagic hypotension studies. All cpm are corrected for background. The results of the two groups of studies were quite similar, with per cent recovery in renal venous blood being less than one per cent in every instance. No significant counts were found in the ligat-

TABLE I. Summary of Microsphere Recovery.

Group	Expt.	Kidney (cpm)	Renal vein (cpm)	Recovery (%)
Acetylcholine	1	81,888	310	0.37
	2	26,850	176	0.65
	3	68,800	62	0.09
	4	77,525	103	0.13
	5	27,968	181	0.60
	6	30,876	52	0.16
Mean				0.33
SEM				±0.10
Hemorrhage	1	35,270	140	0.39
	2	42,573	47	0.11
	3	25,062	19	0.07
	4	37,265	133	0.35
	5	47,466	64	0.14
Mean				0.22
SEM				±0.06

ed renal vein or polyethylene catheter in any study. In addition, histologic studies of tissue sections of both experimental models revealed that the microspheres were trapped exclusively in glomeruli or afferent arterioles, with over 95% residing in the former. The spheres were found in all areas of the cortex and in all tissue sections. There was none found in the medulla, nor was there any aggregation of spheres in the smaller arteries sectioned or in the glomeruli. Further subdivision of the tissue sections revealed that virtually all of the radioactivity was localized in the cortex.

Discussion. Several lines of evidence have been utilized to justify the presence of an aglomerular blood flow pathway. First, Ljungqvist (3) reported histologic evidence that juxtamedullary glomeruli of rat, rabbit, and human kidney contained a direct connection between afferent and efferent arterioles. However, in a recent study by Spinelli *et al.* (13) using a silicone rubber injection method, this could not be confirmed in either rat or dog. Munkacsi and associates (4) noted that the smaller renal veins readily filled during hemorrhage but not under normal conditions, which suggested to them the presence of functional arteriovenous anastomoses in the former condition. To further substantiate this view, this same group found stereomicroscopic evidence of well-defined arteriovenous communications as large as 50 μm in the kidney of the dog under the normal conditions as well as during hemorrhage (4). A quantitative evaluation of this anatomic finding was not performed.

Second, Balint and Fekete (2) found in hemorrhagic hypotension that the clearance of para-amino hippurate (C_{PAH}) was markedly lower than indicated by the direct estimation of renal blood flow. This was taken to indicate the presence of renal shunting, assuming that PAH is a reasonable marker of cortical blood flow and that the difference between the C_{PAH} and total renal plasma flow is noncortical flow. However, as recently discussed, this assumption is not valid (14). In addition, Nagy *et al.* (5, 6) found in various experimental circumstances a marked difference between the directly measured renal blood flow and the 86-rubidium extraction which they felt was an index of capillary blood flow. However, the parameter measured by this latter method is not totally clear, nor can it be certain how various experimental procedures may alter the processes affecting the transport of the indicator.

Third, Simkin and associates (15) injected glass microspheres of varying size into the renal circulation of the dog and did recover some in the renal venous effluent. Yet, with the large amount of beads injected and the inability to quantitate this recovery, the significance of these findings remained unclear. In summary, the evidence to date for a significant aglomerular pathway is circumstantial.

Recently, the radioactive microsphere method has been utilized to measure the intrarenal distribution of blood flow. In the course of evaluating the method, it was found in this (8) and other laboratories (7, 9, 16) that the 15 μm spheres are virtually totally extracted in the glomerular capillary circulation of the dog under normal conditions. In the present study, we have extended these findings to two experimental circumstances previously suggested to be associated with vascular shunting, acetylcholine administration and hemorrhagic hypotension (10, 11). As is shown in Table I, there was a trivial recovery of radioactive spheres in renal venous effluent in both experimental circumstances. Since the renal venous effluent was compared with the total counts trapped in the kidney, this serves as a simple quantitative method of measuring renal extraction of the indicator without having to take into account the completeness of the injection and related technical problems. All microspheres trapped were found in the glomerular capillary circulation ($> 95\%$) or in the afferent arteriole, and less than 1% of the renal radioactivity was found in the venous effluent. Possible rheologic differences between microspheres and normal red cells have been considered previously, especially in reference to the possibility of axial streaming of the microspheres (7–9, 12). However, the experimental evidence to date is not in keeping with this view (7–9, 12). Also, it has recently been demonstrated that rigid particles such as micro-

spheres are, if anything, less likely to be subject to axial streaming (17). Further, from the work of Blackshear and associates (18), it would seem that the primary factor affecting the distribution of a particle such as a microsphere markedly diluted among normal red cells would be the rotational and migrational effects of these red cells on the particle. Thus, it is unlikely that rheologic differences between the microspheres and normal red cells would obviate the demonstrating of shunting. Also, it is difficult to imagine quantitatively significant anastamoses smaller than 15 μm. Therefore, these data strongly suggest that no significant aglomerular pathway is operative in either of these experimental models.

Summary. The radioactive microsphere method was used to quantitate aglomerular blood flow during intrarenal acetylcholine administration (6 studies) and hemorrhagic hypotension (5 studies). In each of the 11 studies, less than 1% of the injected radioactivity was recovered in renal venous effluent. These findings strongly suggest that a significant diversion of renal blood flow by a nonglomerular pathway does not occur in these two models.

1. Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J., and Prichard, M. M. L., "Studies of the Renal Circulation," 2nd. ed. Blackwell, Oxford (1948).

2. Balint, P., and Fekete, A., Pfluegers Arch. **270**, 575 (1960).

3. Ljungqvist, A., Nephron **1**, 329 (1964).

4. Munkacsi, S., Nagy, Z., Takacs, L. Kallay, K., and Gomori, P., Rev. Can. Biol. **22**, 353 (1963).

5. Nagy, Z., Bencsath, P., Tornyai K., and Vaslaki, L., Acta Physiol. Acad. Sci. Hung. **40**, 122 (1971).

6. Nagy, Z., Bencsath, P., Tornyai, K., and Vaslaki, L., Acta Physiol. Acad. Sci. Hung. **39**, 21 (1971).

7. McNay, J. L., and Abe, Y., Circ. Res. **27**, 571 (1970).

8. Stein, J. H., Ferris, T. F., Huprich, J. E., Smith, T. C., and Osgood, R. W., J. Clin. Invest. **50**, 1429 (1971).

9. Slotkoff, L. M., Lozar, A., Jose, P., D'Avello, J., and Eisner, G. M., Circ. Res. **28**, 158 (1971).

10. Gomori, P., Munkacsi, S., Nagy, Z., Takacs, L., and Kallay, K., Acta Med. Acad. Sci. Hung. **18**, 119 (1962).

11. Kover, G., Harza, I., Szocs, E., Balint, P., and Tarjan, E., Acta Physiol. Acad. Sci. Hung. **37**, 423 (1970).

12. Rector, J., Stein, J. H., Bay, W. H., Osgood, R. W., and Ferris, T. F., Amer. J. Physiol. **222**, 1125 (1972).

13. Spinelli, F. R., Wirz, H., Brucher, C., and Pehling, G., Nephron **9**, 123 (1972).

14. Stein, J. H., Boonjarern, S., Wilson, C. B., and Ferris, T. F., Circ. Res. in press.

15. Simkin, B., Bergman, H. C., Silver, H., and Prinzmetal, M., Arch. Intern. Med. **81**, 115 (1948).

16. Katz, M. A., Blantz, R. C., Rector, F. C., Jr., and Seldin, D. W., Amer. J. Physiol. **220**, 1903 (1971).

17. Goldsmith, H. L., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **30**, 1578 (1971).

18. Blackshear, P. L., Forstrom, R. J., Dorman, F. D., and Voss, G. O., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **20**, 1600 (1971).

Received Feb. 1, 1973. P.S.E.B.M., 1973, Vol. 143.