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Direct Measurement of Glomerular Capillary Pressure in Dogs[1] (41191)

GARY R. MARCHAND

Department of Physiology, School of Medicine, University of Minnesota, Duluth, Minnesota 55812 and Department of Physiology and Biophysics, Mayo Medical School, Rochester, Minnesota 55901

Abstract. Direct measurement of glomerular capillary pressure, without removing cortical tissue from the micropuncture site, was the objective of this study. The measurement was accomplished by random micropuncture below the kidney surface with simultaneous recording of pressure at the tip of the micropuncture pipet. Glomerular capillary pressure was identified by a stable, pulsatile tracing synchronous with renal artery pressure or blood flow. In 50 micropunctures in 16 dogs glomerular capillary pressure was 56 ± 3 mm Hg. Mean arterial pressure averaged 127 ± 3 mm Hg. The conclusion that pulsatile pressure recorded during random micropuncture resulted from impalement of glomerular capillaries is supported by experiments in Munich Wistar rats. In 14 rats there was no significant difference between pressure measured either in superficial glomeruli (46 ± 1 mm Hg) or during random micropuncture (47 ± 1 mm Hg).

In dogs, glomerular capillary pressure (P_{GC}) is generally estimated from the sum of single-nephron stop-flow pressure and systemic plasma oncotic pressure. Recently, however, the direct measurement of P_{GC} was reported (1). In this study, glomeruli, which do not occur on the surface of canine kidneys, were exposed by surgical removal of cortical tissue from the micropuncture site. When blood pressure was 130 mm Hg, directly measured P_{GC} was slightly, but significantly less than estimated P_{GC}. It is not known if the local trauma of corticotomy in dogs affects P_{GC} in the exposed glomeruli (2). Therefore, in the present study P_{GC} was measured directly without corticotomy.

To accomplish this it was reasoned that the outermost glomeruli, which lie within 0.5 mm of the surface, would be accessible using a technique of random, blind micropuncture and continuous recording of hydrostatic pressure with a servo-null micropressure system. To validate the technic, pressure measured during random micropuncture was compared to glomerular capillary pressure in superficial glomeruli of Munich Wistar rats.

Methods. All animals were maintained on a standard diet and fasted for 16 hr. Mongrel dogs of either sex were anesthetized (30 mg/kg, iv, pentobarbital) and prepared for micropuncture. Briefly, an endotracheal tube was inserted to allow positive pressure respiration during the experiment. Catheters were placed in a femoral vein, for infusion of inulin, and a femoral artery, for monitoring mean aortic pressure. A left kidney was exposed by a retroperitoneal approach, the surrounding connective tissue removed, and a catheter placed in the ureter. Aortic pressure and either total renal blood flow (RBF) or renal artery pressure (RAP) were continuously recorded in each dog.

A priming injection of inulin in isotonic saline was given and a sustaining infusion (1 ml/min) started 30–60 min before beginning the experiment. The kidney was placed in a plastic holder and illuminated by a fiber optic light source. A small amount of kidney capsule was then removed and the micropuncture surface bathed with isotonic saline.

Munich Wistar rats (131–280 g body wt) were anesthetized with Inactin (120 mg/kg,

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ip) and prepared for micropuncture. An inulin solution was infused throughout the experiment (1.5 ml/hr., iv). Carotid artery blood pressure was recorded continuously.

For micropuncture, sharpened glass pipets of 3- to 5-μm o.d. (pressure recordings) or 6- to 10-μm o.d. (oil injection) were used. To measure hydrostatic pressure in the microvasculature a servo-null apparatus was used (Instrumentation for Physiology and Medicine, San Diego, California). Briefly, the system maintains electrical resistance between a hypertonic-saline-filled micropuncture pipet and physiologic fluid by generating an opposing pressure which is measured with a standard pressure transducer. With this system a linear frequency response (dc to 30 Hz) allows recording of pulsatile pressure in the microcirculation. Stable, pulsatile pressures, synchronous with peripheral pulse, are obtained when the pipet tip is free within the lumen of the impaled structure.

For the direct measurement of P_{GC} in the dog, a random site on the micropuncture surface was selected and the tip of a pressure measuring micropipet advanced progressively into the kidney to a depth of 0.5 to 2.0 mm. At a distance of 2.0 mm from the tip, the outer diameter of the micropipet is 90–100 μm. Impalements were at least 10 tubule diameters apart. Output from the servo-null was continuously recorded. The electrical gain of the micropressure system was set so that the output of the pressure transducer oscillated at high frequency when the micropipet tip entered a tubule or vessel lumen. High-frequency oscillation is absent when the pipet tip is obstructed (e.g., contact with tissue during random micropuncture) and the transducer output fluctuates between zero and the system maximum. Therefore, penetration of structures during random micropuncture was assessed by watching an oscilloscope trace showing the oscillating condition of the servo-null system.

In addition to direct P_{GC}, the stop-flow pressure method for indirectly estimating P_{GC} was applied in the same dogs. A column of Sudan black-stained castor oil was injected into a superficial proximal convolution and the steady-state pressure measured proximal to the oil block. Direct and estimated P_{GC} were recorded in random order from different areas on the micropuncture surface. Identical procedures were used to measure P_{GC} in rats.

Clearance periods were taken during micropuncture. Inulin in urine and plasma was determined by the anthrone method (3). Plasma protein concentration was determined by the Biuret (dogs) or Lowry *et al.* (rats) method (4). Plasma oncotic pressure was calculated from the empiric relationship described for the dog and rat by Navar and Navar (5). Estimated P_{GC} was calculated from the sum of single-nephron stop-flow pressure and plasma oncotic pressure. For each experiment, an average of each parameter was calculated, and the overall mean ± 1 SE reported. Only values obtained during a micropuncture measurement were used to calculate mean arterial pressure. Results of superficial and random micropuncture below the kidney surface were compared by Student's *t* test for paired or unpaired observations, and correlation analysis.

Results. A summary of renal function measured during micropuncture is presented in Table I. Pressure tracings obtained in the dog during random micropuncture below the kidney surface are shown in Fig. 1. As shown in the figure, the pulse rate of directly measured P_{GC} was synchronous with either renal blood flow or renal artery pressure recorded simultaneously.

Glomerular capillary hydrostatic pressure in dogs, obtained by random micropuncture below the kidney surface, is summarized in Table II. The number of micropuncture measurements is reported for each experiment.

Stop-flow and plasma colloid osmotic

TABLE I. SUMMARY OF RENAL FUNCTION MEASURED DURING RANDOM MICROPUNCTURE

Mean arterial pressure, mm Hg	127 ± 3
Glomerular filtration rate, ml/min	29 ± 2
Urine flow, ml/min	0.22 ± 0.03
Proximal tubule pressure, mm Hg	22 ± 2
Stop-flow pressure, mm Hg	41 ± 2
Colloid osmotic pressure, mm Hg	17 ± 1

Note. Results are mean ± SE for 16 dogs.

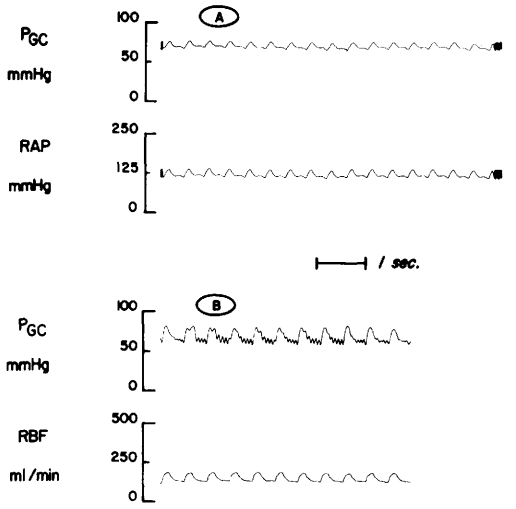

FIG. 1. Comparison between pulsatile tracing of glomerular capillary pressure (P_{GC}), recorded directly during random micropuncture below the kidney surface, and (A) renal artery pressure (RAP) or (B) blood flow (RBF) recorded simultaneously.

pressure averages are shown in Table I. Their sum was 58 ± 2 mm Hg. There was no significant difference between directly measured P_{GC} and estimated P_{GC} (Δ = 1.4 ± 2.0 mm Hg). The correlation between direct and estimated P_{GC} was significant (r = 0.71, $P < 0.05$) and is presented in Fig. 2. Mean arterial pressure, renal blood flow, or renal artery pressure during direct measurement of P_{GC} was not significantly different from that during stop-flow pressure measurement.

TABLE II. P_{GC} MEASURED DURING RANDOM MICROPUNCTURE

Dog no.	P_{GC}[a] (mm Hg)	N	MAP[b] (mm Hg)
1	40	2	138
2	44	2	125
3	56	4	118
4	49	5	134
5	56	1	132
6	68	2	135
7	62	5	129
8	49	2	100
9	72	3	125
10	45	6	130
11	50	4	112
12	56	4	135
13	53	3	110
14	56	4	140
15	79	2	153
16	67	1	117
Mean	56		127
SE	3		3

[a] Directly recorded glomerular capillary pressure measured during random micropuncture. Each value is an average of N determinations.

[b] Mean arterial pressure. Each value is an average of N determinations obtained at the time of micropuncture.

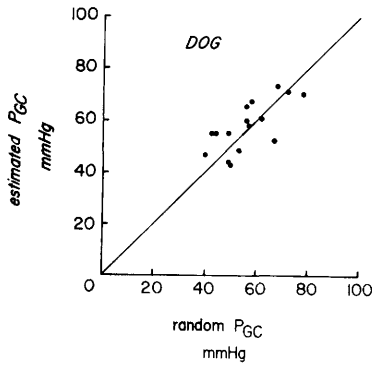

FIG. 2. Correlation between direct and estimated glomerular capillary pressure. Variation of individual experiments about the line of identity is shown. The correlation coefficient and its 95% confidence limits are 0.71 (0.31, 0.90).

Glomerular capillary pressure in Munich Wistar rats recorded in either superficial glomeruli or during random micropuncture is summarized in Table III. There was no significant difference between P_{GC} of superficial glomeruli and that obtained during random micropuncture (Δ = 0.5 ± 0.8 mm Hg). In addition, P_{GC} of superficial glomeruli was significantly correlated with P_{GC} measured during random micropuncture as shown in Fig. 3 (r = 0.83, $P < 0.05$).

In the same rats, the stop-flow method was used to estimate P_{GC} (46 ± 1 mm Hg). There was no significant difference between any of the methods for measuring P_{GC}. In an unpaired comparison of P_{GC} measured during random micropuncture in the dog and rat, a significant difference was observed (56 ± 3 and 47 ± 1 mm Hg), respectively, $P < 0.01$.

Since different technics for measuring P_{GC} were used, the F test of equality of variances was used to compare random P_{GC} to either estimated P_{GC} in the dog, or P_{GC} in superficial glomeruli in the rat. There was

TABLE III. COMPARISON OF P_{GC} MEASURED DURING RANDOM MICROPUNCTURE AND IN SUPERFICIAL GLOMERULI

Rat No.	Random P_{GC}[a] (mm Hg)	Random N	Random MAP[b] (mm Hg)	Superficial P_{GC}[c] (mm Hg)	Superficial N	Superficial MAP (mm Hg)
1	48	5	117	48	4	116
2	52	4	108	46	2	108
3	51	3	117	51	3	118
4	47	4	119	45	4	119
5	50	2	120	52	3	120
6	42	3	120	45	3	122
7	45	3	135	42	1	135
8	48	5	130	50	4	130
9	38	1	130	35	3	123
10	44	2	129	40	1	125
11	48	1	145	53	3	122
12	52	3	127	51	2	132
13	40	3	140	40	3	140
14	48	8	126	48	4	127
Mean	47		126	46		124
SE	1		3	1		2

[a] Directly recorded glomerular capillary pressure measured during random micropuncture. Each value is an average of N determinations.

[b] Mean arterial pressure. Each value is an average of N determinations.

[c] Directly recorded glomerular capillary pressure recorded during micropuncture of superficial, visible glomeruli.

no significant difference in variance of P_{GC} in dogs or rats.

Discussion. In the present study glomerular capillary pressure in the dog was recorded directly. The measurement was obtained by continuously recording hydrostatic pressure at the tip of a micropipet during random micropuncture below the kidney surface. Using this approach, approximately 5–10% of the impalements resulted in the successful recording of glomerular capillary hydrostatic pressure.

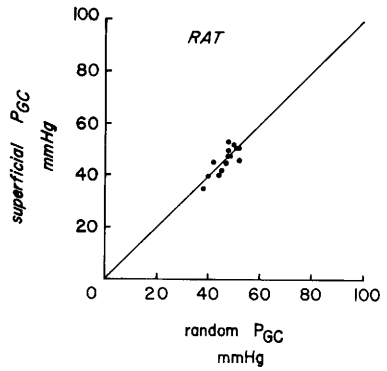

FIG. 3. Correlation between glomerular capillary pressure measured in superficial glomeruli and during random micropuncture. The correlation coefficient and its 95% confidence limits are 0.83 (0.49, 0.95).

During micropuncture, glomerular capillary pressure measurements were identified by a stable, pulsatile waveform synchronous with simultaneously recorded renal artery pressure or blood flow. Similar criteria are used to validate glomerular capillary micropuncture in Munich Wistar rats, since individual capillaries cannot be seen within the surface glomeruli (6, 7).

In addition, in 11 of 50 micropunctures (nine dogs), slow withdrawal of the micropipet, following direct measurement of P_{GC}, caused a rapid drop in recorded pressure, presumably due to moving the pipet from the capillary lumen to Bowman's space. The resultant pressure (20 ± 2 mm Hg) was stable and the pulse amplitude was markedly less than P_{GC}. Qualitatively, the recording was identical to superficial free-flow proximal tubule pressure.

Direct proof of glomerular capillary impalement in dogs is not possible in the present study. Therefore, validation of the technic was sought by comparison between P_{GC} of superficial glomeruli and P_{GC} measured during random micropuncture in Munich Wistar rats. There was no significant difference between the mean pressure recorded by either technique. Accordingly, it is concluded that random micropuncture, as described herein, can be used for the direct measurement of glomerular pressure.

The frequency of arteriole puncture during random micropuncture below the kidney surface is unknown. In the Munich Wistar rat experiments, agreement between superficial and random P_{GC} argues against a significant effect of arteriole puncture. A similar comparison is not possible in dogs, since superficial glomeruli do not occur. On the other hand, indirect evidence suggests that arteriole puncture is negligible in dogs also. For example, if arterioles were systematically impaled during random micropuncture the variance of direct P_{GC} might be different from that of estimated P_{GC}. However, equal variance of direct and es-

timated P_{GC} was observed. In addition, histological evidence suggests that arteriole puncture is unlikely.

In glomeruli exposed by corticotomy, Heller and Horacek recorded glomerular pressures of 60 mm Hg (1). Thomas *et al.* reported similar preliminary results (8). In the former study, reduction of renal artery pressure was accompanied by a significant decline of glomerular pressure. The authors conclude that glomerular pressure and single-nephron function in dogs may not be well autoregulated. However, an effect of corticotomy on glomerular pressure cannot be ruled out.

Estimated P_{GC}, calculated from the sum of single-nephron stop-flow pressure and plasma colloid osmotic pressure, was similar to values previously reported by this laboratory (9, 10). In contrast, Navar observed markedly greater estimated glomerular pressure in dogs with normal blood pressure and concluded that estimated glomerular pressure is an overestimate of true glomerular pressure (11). However, Navar also concluded that direct and estimated P_{GC} are equal when renal perfusion pressure is reduced sufficiently, or autoregulatory efficiency, at normal blood pressures, is low (11). Results of the present study do not resolve this discrepancy since autoregulation was not tested. Therefore, the similarity between direct and estimated P_{GC} should not be taken as evidence for the unconditional application of the stop-flow pressure method. On the other hand, the results do indicate that, in our experiments, glomerular capillary pressure can be estimated in dogs with normal blood pressure.

In two dogs cortical tissue below the micropuncture field was removed and frozen in liquid nitrogen. Sections (5 μm) were stained with hematoxylin and eosin. Serial sections were examined under a light microscope. A section showing evidence of micropuncture is presented in Fig. 4. It is not known whether a pressure recording was obtained from the glomerulus impaled. However Fig. 4 demonstrates that outer cortical glomeruli are accessible to random micropuncture in dogs. In addition, the large area occupied by glomeruli compared to arterioles is apparent. Accordingly, the figure suggests that the random micropuncture of afferent arterioles is likely to be negligible compared to glomerular impalement.

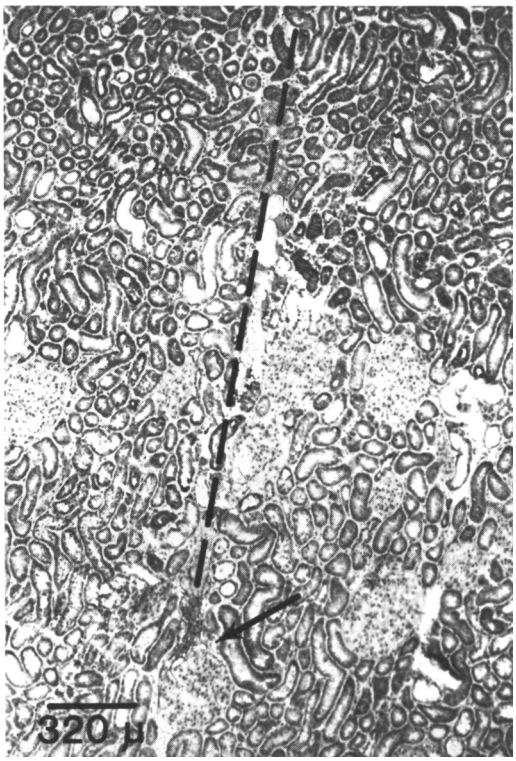

FIG. 4. Section of dog superficial renal cortex. The path of micropuncture is parallel to and to the immediate right of the dashed line. The arrow points to the site of glomerular micropuncture. See text for details.

In summary, during random micropuncture below the kidney surface with a servo-null micropressure system, glomerular pressures were identified by a stable, pulsatile tracing, synchronous with renal artery blood pressure or flow. Mean glomerular capillary pressure averaged 56 mm Hg in 16 dogs. The conclusion that identified pressures were from glomerular capillaries is supported by a significant correlation with estimated glomerular pressure in the same dogs and experiments in Munich Wistar rats, in which there was no difference between glomerular pressure recorded during random micropuncture and glomerular pressure recorded directly in superficial glomeruli.

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