TECHNICAL NOTE

A technique for chronic repuncture micropuncture of dog kidney

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The precision of kidney micropuncture for exploring changes in glomerular and tubular function was strikingly enhanced by the introduction of the re-puncture and re-collection technique [1], thus permitting the use of each tubule as its own control. This procedure has allowed detection of changes in function that were not appreciated previously. However, the technical design of re-collection micropuncture is such that it is mainly applicable to the study of rapid and usually massive changes in homeostasis; exploration of the renal response to chronic, more physiologic maneuvers has been limited to clearance and non-repuncture micropuncture studies.

This paper describes a technique for chronic repuncture micropuncture that allows repeat proximal tubule sampling in dogs at an interval of up to 14 days.

Methods. Healthy mongrel dogs of both sexes, weighing 12 to 21 kg, were housed in metabolic balance cages for measurements of daily urinary sodium excretion. They were fed a standard dog food providing 5 mEq of Na/kg of body wt/day for at least nine days before the primary experiment and between studies. Dietary intake was avoided on the day of each micropuncture and clearance experiment. To compensate for surgical fluid and electrolyte losses and lack of dietary intake, the animals received 10 mEq of Na/kg body wt as isotonic saline i.v., upon completion of the initial study, and in some instances the lack of potassium was replaced. Only two of five dogs required minimal oral NaCl supplements to regular dietary intake to maintain sodium balance between studies.

The interval between initial and repuncture micro-puncture-clearance studies varied from 5 to 14 days.

The animals were anesthetized with pentobarbital, 20 mg/kg of body wt, plus small supplements as required. An endotracheal tube was introduced, and respiration was supported by a small-animal respiration pump. Cathlon (Teflon) catheters were introduced percutaneously into a jugular and a foreleg vein, using #14, and #16 catheter placement units, for blood sampling and for inulin and para-aminohippurate (PAH) infusion, respectively. For the first renal micro-puncture sampling, surgical trauma was minimized by exposing only the left kidney and ureter via a flank incision 2 to 3 cm below and parallel to the 12th rib. Overlying muscle fibers were split and separated to provide entry to the retroperitoneal space. The kidney and pedicle were freed from the adjacent peritoneum and perirenal fascia. To avoid entry into the peritoneal cavity, the adherent portion of peritoneum at the superior anterior pole of the kidney was ligated before release of the renal reflection. Hemostasis was achieved with nonreabsorbable synthetic material. The kidney was elevated and the ureter was cannulated through a muscle-splitting incision just beyond the renal pelvis, using polyethylene tubing contoured to minimize ureteral traction. The kidney was immobilized on an aluminum holder and 1 cm² of renal capsule was removed in preparation for micropuncture. The renal surface and retroperitoneal space were repeatedly irrigated with heparinized isotonic saline solution. The decapsulated area was illuminated by a flexible fiber-optic tube and tubules were observed through a stereoscopic microscope. Four to six tubules were injected with latex via micropipet to serve as markers. Eight randomly selected tubules nearby were punctured, using glass micropipets (tip I.D., 8 to 10 μ) filled with liquid silicone (2 centistokes). Care was taken to avoid injection of silicone into sampled tubules, and tubule fluid was allowed to collect spontaneously in the

puncture pipets. To minimize sampling error, collection time exceeded two minutes after intratubular ejection of the initial sample or after the ejection of silicone on the kidney surface immediately before tubule entry. When blockage of the pipet tip on entry into the tubule prevented spontaneous sample collection, gentle aspiration pressure was exerted and the aspirated sample ejected. The puncture sites were recorded by mapping latex-filled and surrounding tubules, for their identification at the time of subsequent repuncture. During micropuncture, urine and plasma collections were obtained simultaneously. After initial loading, the plasma inulin and PAH concentrations were maintained at approximately 80 and 2 mg/100 ml, respectively, by constant infusion. Upon completion of the procedure, the ureteral tubing was removed, its defect repaired by a single layer of 6–0 nonabsorbable suture in the muscle layer, and the kidney returned to the retroperitoneal space. Heparinized saline was instilled to suppress fibrin formation. To minimize injury and distortion of the decapsulated area, a flap of peritoneum was reflected over the lateral surface of the kidney and sutured to the dorsal surface. The incision was closed, in layers where possible, and the animal was returned to its metabolic cage.

At repuncture, preparation and catheterization were the same as for initial studies; in addition, the right ureter was cannulated via a flank incision. The surgical approach to the left kidney was modified to avoid the previous incision. Through a skin incision perpendicular to the original, the aponeurosis formed by the external and internal oblique muscles and the insertion of the transversus abdominus were divided; rarely, small focal collections of hematoma or pus were observed in the retroperitoneal space. The ureter was catheterized and the kidney immobilized as before. At repuncture, the appearance of decapsulated areas varied: some resembled the freshly decapsulated area at initial study, and others were partly obscured by a gelatinous film. The film was easily removed with a #20 hypodermic needle controlled by micromanipulation; in no instance did this prevent successful completion of the study. Micropuncture, urine and plasma collections were obtained as before, and inulin and PAH were infused at the rates used previously.

Plasma and urine inulin determinations were performed by the method of Davidson and Sackner [2] and PAH concentrations were determined by the technique of Bratton and Marshall [3]. Sodium and potassium were measured by flame photometry (IL model), plasma protein was estimated with a refractometer and tubular fluid inulin concentration was measured by fluorometer [4].

Results and discussion. The clearance data obtained

Table 1. Summary[a] of clearance and micropuncture data obtained at initial and repeat experiments in five dogs[b]

Indexes[c]	Initial study, left kidney	Repeat study	
		Left kidney	Right kidney
TF/P_{In} ratio (32 tubules)	1.44±0.02	1.42±0.03	
C_{In}, ml/min	20±2	19±2	21±2
C_{PAH}, ml/min	49±5	51±6	55±6
V, ml/min	0.12±0.01	0.11±0.01	0.10±0.01
$U_{Na}V$, μEq/min	13±2	13±3	18±4

[a] Mean ± SEM.
[b] Clearance data were obtained for only the left (experimental) kidney during the first study but for both during the repeat study.
[c] C_{In}, whole kidney clearance of inulin; C_{PAH}, whole kidney clearance of p-aminohippurate; V, urine volume; $U_{Na}V$, urinary sodium excretion.

at initial study and during repuncture of the left kidney were virtually identical ($P>0.3$) and on both occasions were similar to those obtained by right ureteral catheterization at repuncture (Table 1). The mean change in proximal tubular fluid to plasma inulin ratio (TF/P_{In}) in the 32 tubules repunctured was -0.02 ± 0.017 ($P>0.4$). Comparison of initial and repuncture TF/P_{In} ratios for individual tubules shows that, with few exceptions, they are distributed about the line of identity (Fig. 1), comparing favorably with values reported during control acute micropuncture studies
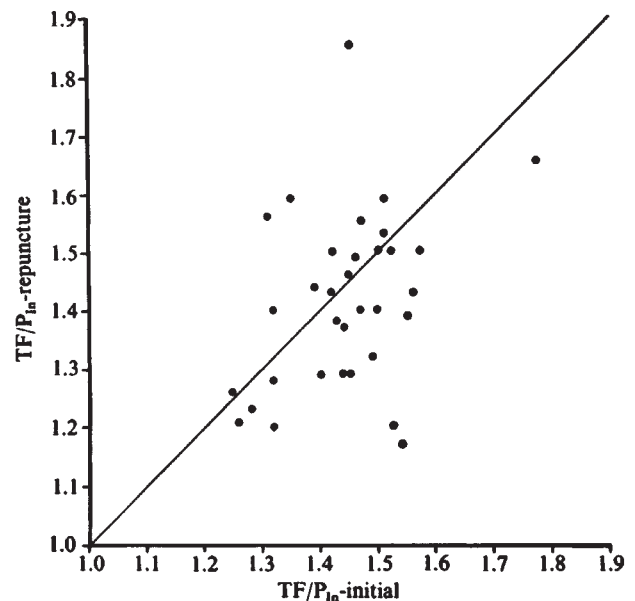

Fig. 1. *Comparison of initial and repuncture proximal TF/P_{In} ratios (mean repuncture ratio, 0.99±0.02). Each point represents one tubule repunctured at the same site 5 to 14 days later.*

Table 2. Data of individual animals

Animal No.	Time between experiments days	Experiment	Weight kg	Plasma K mEq/liter	Plasma Na mEq/liter	Hematocrit %	Plasma protein g/100 ml	No. of tubules repunctured	TF/P_{In}
1	10	Initial	18	3.6	148	43	5.8		1.54
		Repeat	18	3.5	145	43	5.7	7	1.55
2	7	Initial	12	3.0	145	43	5.6		1.51
		Repeat	12	3.0	144	38	5.4	3	1.46
3	14	Initial	13.9	3.1	146	43	5.9		1.38
		Repeat	12.8	3.2	146	38	6.5	8	1.41
4	6	Initial	20.9	3.6	148	46	6.3		1.35
		Repeat	20.2	3.4	148	38	6.1	7	1.35
5	5	Initial	20.3	3.7	148	40	5.6		1.48
		Repeat	19.5	3.7	149	35	5.3	7	1.37
Mean	8	Initial	17 ± 1.8	3.4 ± 0.1	147 ± 0.6	43 ± 0.9	5.8 ± 0.1		1.45 ± 0.04
		Repeat	16.5 ± 1.7	3.4 ± 0.1	146 ± 0.9	38 ± 1.3	5.8 ± 0.2		1.43 ± 0.04
		P	< 0.1	> 0.5	> 0.4	< 0.025	> 0.8		> 0.3

[1, 5]. Analysis of individual animals (Table 2) confirms the similarity between initial and repeat values. The ease of reconstructing the tubule marker map at repuncture varied, but the majority of tubules were recognizable without dissection of the puncture surface. In the absence of a nonaqueous surface coating, sample contamination is possible, particularly in view of the slight capillarity of micropuncture pipets. The precautions outlined, especially the prolonged duration of sample collection, should minimize this potential source of error.

Recovery from the first surgical procedure was rapid and most animals returned to their previous dietary intake on the first postoperative day. This diet maintained sodium balance (based on daily urinary excretion) in three dogs but modest orally administered NaCl supplements were necessary in the other two. Weight loss between studies averaged 0.5 kg, presumably due to postoperative catabolism. Plasma sodium, potassium and protein concentrations were essentially unchanged at repeat study. The fall in plasma hematocrit value (Table 2) probably resulted from blood sampling at the initial procedure and lack of replacement of surgical blood loss. With careful but only partially aseptic surgical technique, local or systemic infection was never severe enough to require termination of a study.

Our results indicate that, despite variations inherent in experiments with animals, proximal tubule reabsorption, renal function and the animal's general condition are reproducible when sodium homeostasis is achieved between these experiments. Thus, it is considered justifiable to utilize the potential enhanced precision of this technique to study the nephron's adaptation to chronic alterations.

References

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