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A M Chonko, ... , T F Ferris, J H Stein

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**Research Article**

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## The Measurement of Nephron Filtration Rate and Absolute Reabsorption in the Proximal Tubule of the Rabbit Kidney

ARNOLD M. CHONKO, RICHARD W. OSGOOD, ALLAN E. NICKEL,  
THOMAS F. FERRIS, and JAY H. STEIN

*From the Departments of Medicine, Ohio State University School of Medicine,  
Columbus, Ohio, and the University of Texas Health Science Center,  
San Antonio, Texas 78284*

**ABSTRACT** Micropuncture studies were performed in the rabbit to determine nephron filtration rate and absolute fluid reabsorption in the proximal tubule in order to compare the latter value with data obtained with the *in vitro* microperfusion technique. New Zealand white rabbits, 2–2.8 kg, were studied. Nephron filtration rate was 21 nl/min ( $n = 48$ ) and absolute reabsorption along the length of the accessible portion of the proximal convoluted tubule was 10.3 nl/min. Correcting this value for tubular length gives a fluid reabsorption of approximately 1.9 nl/mm per min. In view of the marked difference between the *in vivo* and *in vitro* techniques and the various sources of error with each, this is reasonably similar to the value of 1.3 nl/mm per min obtained in the isolated proximal convoluted tubule.

perfused proximal tubules was demonstrated by the presence of both active para-aminohippuric acid transport and net fluid absorption. Subsequent to this publication, the technique has been utilized to study a wide variety of questions concerning cellular transport in several segments of the nephron (2–9). Yet, little if any data is available to allow a comparison of the *in vitro* transport capacity of various nephron segments to the *in vivo* setting. This type of comparison would allow a more meaningful interpretation of the physiologic significance of the results obtained with the microperfusion technique. This report presents the first micropuncture data obtained in the rabbit kidney. The results indicate that the *in vivo* and *in vitro* measurements of absolute fluid absorption in the proximal tubule are reasonably similar.

### INTRODUCTION

In 1966, Burg, Grantham, Abramow, and Orloff described a technique for preparation and study of single rabbit nephrons (1). In this study, it was found that isolated proximal tubules of the rabbit were able to maintain large transcellular concentration gradients for sodium, potassium, and chloride. The viability of

### METHODS

Studies were performed on male and female New Zealand white rabbits weighing between 2.0 and 2.8 kg. The rabbits were anesthetized with pentobarbital (30 mg/kg) and subsequently given small maintenance doses as necessary. A polyethylene tube was inserted into the trachea and the animal was then allowed to breathe without a respirator. Polyethylene catheters were inserted into both jugular veins for infusion of inulin and injection of Lissamine green and in the femoral artery for blood pressure measurement and

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TABLE I  
Summary of Rabbit Micropuncture Studies

Exp.	(TF/P) <sub>in</sub>	Nephron GFR	Absolute reabsorption	Kidney GFR
		nl/min	nl/min	ml/min
1	2.01*	12	6.0	4.01
	1.44	20	5.7	
	1.75	19	8.0	
	1.92*	16	7.6	
	1.88*	18	8.5	
		(17)‡		
2	1.36	16	4.5	3.72
	1.52	17	5.8	
	1.81*	27	12.0	
	2.02*	23	11.6	
			(21)‡	
3	1.62	27	10.0	4.04
	1.88*	18	8.4	
	1.99*	27	13.5	
	2.03*	25	12.3	
	2.55*	16	9.8	
		(23)‡		
4	2.01*	37	18.5	4.72
	1.52	34	11.6	
	1.72	30	12.6	
	1.84*	31	14.6	
	1.85*	33	15.2	
		(33)‡		
5	1.93*	20	9.7	3.96
	2.00*	24	12.0	
	1.62	17	6.6	
		(20)‡		
6	1.37	22	6.0	3.78
	1.88*	19	8.9	
	1.54	25	8.8	
	2.03*	18	9.1	
		(21)‡		
7	1.54	16	5.7	3.01
	1.48	13	4.2	
	1.94*	12	5.8	
	1.88*	12	5.6	
		(13)‡		
8	1.93*	22	10.7	3.52
	1.97*	21	10.4	
	1.94*	15	7.2	
	2.25*	18	10.0	
		(19)‡		

\* Late proximal tubular collection.

‡ Mean nephron GFR per experiment.

blood collection. Both ureters were catheterized with PE 90 tubing through a suprapubic incision.

The left kidney was exposed through a small flank incision, the pedicle was gently dissected free, and the kidney

was then placed in a Plexiglas cup. The capsule was left intact, and the surface of the kidney was bathed with mineral oil at 37°C.

An infusion of inulin in Ringer's solution was given at a rate of 0.05 ml/min in order to establish and maintain an inulin concentration of approximately 100 mg/100 ml. Two to three 0.2-ml intravenous injections of 10% Lissamine green were given to measure the transit time through the proximal tubule and to localize the last accessible segment on the surface of the rabbit kidney. At least 30 min elapsed between the last injection of Lissamine green and the collection of tubular fluid samples. Only rabbits with a proximal tubular transit time of 15 s or less that was uniform throughout the superficial cortex were utilized. These criteria were chosen because of previous experience with similar studies in the rat. 27 rabbits were prepared for micropuncture of which 15 met these criteria. The remaining 12 animals were not further studied. Approximately two-thirds of the collections were obtained from end proximal segments. 1 h after the inulin infusion had begun, three to five proximal tubular collections were obtained with sharpened pipettes with tip diameters varying from 7 to 10  $\mu$ m. After puncture, a large column of mineral oil stained with Sudan black was injected into the tubule. After initial gentle aspiration to begin the collection, most of the samples were collected either spontaneously or with only occasional aspiration to maintain the oil column in a constant position. All tubular fluid collections were timed. Eight studies were performed in this manner. In six of these experiments transit times were repeated at the conclusion of all collections and were essentially unchanged. In three further studies, end proximal tubules were localized with Lissamine green, marked, injected with latex, and microdissected by the technique of Cortell (10) in order to measure the length

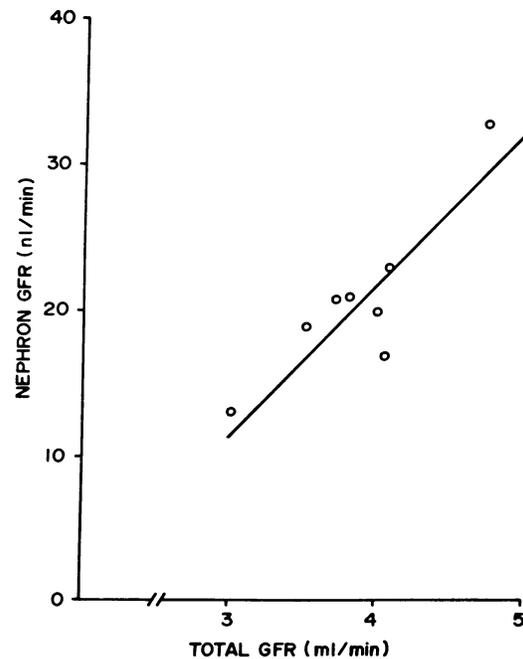


FIGURE 1 Relationship between nephron and total GFR. There was a linear relationship,  $y = 10.5x - 19.4$  ( $r = 0.89$ ,  $P < 0.005$ ). Each nephron GFR point is a mean of 3-5 measurements obtained from a single rabbit.  $n = 8$ .

of the accessible portion of the proximal convoluted tubule of the rabbit. In four additional studies, end proximal tubular collections were obtained followed by microdissection and length measurement of the punctured tubule.

In all studies, two 15-min clearance periods were collected from both kidneys. Plasma and urine inulin concentrations were determined by the anthrone method (11). The concentration of inulin in tubular fluid was measured by the fluorometric method of Vurek and Pegram (12). Tubular fluid volumes were measured in constant-bore quartz capillary tubes of a known internal diameter. Sodium and potassium concentrations in urine and plasma were measured by an Instrumentation Laboratory flame photometer (Instrumentation Laboratory, Inc., Watertown, Mass.).

*Calculations.* Nephron filtration rate ( $V_o$ )<sup>1</sup> = (TF/P)<sub>in</sub> × V<sub>F</sub>, where (TF/P)<sub>in</sub> is the tubular fluid-to-plasma inulin ratio and V<sub>F</sub> is the tubular flow rate in nanoliters per minute. Absolute reabsorption of filtrate in proximal tubule in nanoliters per minute = V<sub>o</sub> - V<sub>F</sub>. The data were analyzed by standard statistical methods and all results are presented as the mean ± SEM.

## RESULTS

Total kidney glomerular filtration rate (GFR) in the micropuncture kidney averaged 3.84 ml/min (range 3.01–4.72 ml/min). This value was slightly but consistently lower than in the untouched control kidney, which averaged 4.21 ml/min ( $P < 0.05$ ). In addition, urinary flow rate and sodium excretion were significantly lower in the micropuncture kidney. Urinary flow rate was 18 and 24 μl/min ( $P < 0.02$ ) while fractional sodium excretion was 0.32 and 0.42% ( $P < 0.02$ ) in the micropuncture and control kidney, respectively. Mean arterial pressure averaged 102 ± 3 mm Hg and was essentially constant throughout each study.

The results of the eight micropuncture studies are summarized in Table I. V<sub>o</sub> was 21.1 nl/min for all 34 punctures and 20.9 nl/min when the mean of each of the eight experiments was averaged. In six of the eight studies, the mean nephron GFR ranged from 17 to 23 nl/min per study while in the remaining two experiments, the means were 33 and 13 nl/min. Yet, in these two studies, the data were quite tight with a range of 30–37 nl/min in exp. 4 and 12–16 nl/min in exp. 7. The mean coefficient of variation of the nephron GFR measurement per experiment in these eight studies was 16%, a value quite similar to previous results from this laboratory in the dog and rat (13, 14). Further evidence of the precision of these determinations is shown in Fig. 1, which compares total kidney and nephron GFR. A strong linear relationship was noted with an  $r$  value of 0.89 ( $P < 0.005$ ). In addition when the total GFR per gram kidney weight is divided by

<sup>1</sup> Abbreviations used in this paper: GFR, glomerular filtration rate; (TF/P)<sub>in</sub>, tubular fluid-to-plasma inulin ratio; V<sub>o</sub>, nephron filtration rate; V<sub>F</sub>, tubular flow rate.

TABLE II  
Direct Measurement of Absolute Fluid Reabsorption  
in Rabbit Proximal Tubule

Exp.	(TF/P) <sub>in</sub>	Nephron GFR	Tubular length	Absolute reabsorption
		nl/min	mm	nl/mm/min
1	1.95	17	4.8	1.73
	1.90	19	5.0	1.80
	2.07	17	5.5	1.60
	1.88	16	4.9	1.53 (1.66)*
2	2.10	27	6.6	2.12
	1.87	25	5.8	1.90
	1.80	23	5.9	1.74
	1.85	30	6.3	2.22 (1.99)*
3	1.89	26	5.2	2.36
	1.89	20	5.0	1.90
	2.11	21	5.4	2.07 (2.11)*
4	2.03	19	5.8	1.66
	1.80	25	5.6	2.05
	1.84	23	5.5	1.90 (1.87)*

\* Mean absolute reabsorption of each experiment.

the nephron GFR, a mean value of 22,300 nephrons/g is obtained, which is quite close to the value of 23,000 nephrons/g kidney measured directly in the rabbit by Bankir, Farman, Grünfeld, de la Tour, and Funck-Brentano (15).

22 of the 34 collections were obtained from end proximal segments. In these 22 collections, the mean (TF/P)<sub>in</sub>, V<sub>o</sub>, and absolute reabsorption were 1.97 ± 0.03, 21.1 ± 1.4, and 10.3 ± 0.07 nl/min, respectively. Although not obtained in these studies, 12 measurements of the accessible length of the proximal tubule were done in three rabbits of the same size as in the studies in Table I. The mean value was 5.4 ± 0.4 mm. By utilizing this measurement and the absolute reabsorptive rate of 10.3 nl/min, a value of approximately 1.9 nl/mm per min is obtained.

Nephron GFR, absolute reabsorption, and tubular length were all determined in 14 end segments in four additional studies. As is shown in Table II mean absolute fluid absorption rates of 1.66, 1.99, 2.11, and 1.87 nl/mm per min were measured in these four experiments.

## DISCUSSION

This study describes the first micropuncture data obtained in the rabbit kidney. As is shown in Table I,

nephron GFR averaged approximately 20 nl/min and half of this amount was reabsorbed by the end of the accessible portion of the proximal tubule. The precision of the measurement of nephron GFR in the rabbit was adequate with a mean coefficient of variation of 16%. In addition, the linear relationship between nephron and kidney GFR (Fig. 1) lends further credence to the nephron GFR measurement. Although the total kidney GFR was reduced 9% in comparison to the untouched contralateral kidney, this small difference would not seemingly have any major effect on the interpretation of these data.

By utilizing the data in Table I and the measured length of the accessible portion of the proximal tubule of 5.4 mm, an absolute reabsorptive rate of approximately 1.9 nl/mm per min was obtained. In addition, in four further studies in which all parameters were measured in the same tubule, a similar value was obtained. In studies utilizing the in vitro microperfusion technique, this value has averaged approximately 1.3 nl/mm per min in tubules from rabbits slightly smaller than those used in the present study (2, 4, 5). In addition, Hamburger, Lawson, and Dennis have suggested that the proximal convolution is quite heterogeneous (16). Absorptive rates in the early part of the microperfused rabbit proximal tubule were closer to the values obtained in vivo averaging 1.6 nl/mm per min, while in late proximal tubules water movement was approximately 0.5 nl/mm per min, a value quite similar to that found in the straight portion of the proximal tubule.

Yet, even with these aspects taken into consideration, the in vivo and in vitro results are surprisingly similar in view of the marked differences in the two procedures and the various sources of error with each. Even if the presence of a capillary circulation, some constituent in the filtrate, or other factors do cause the absorptive rate in the proximal tubule to be higher in vivo, the values obtained with the in vitro perfusion technique are reasonably comparable.

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