

## The Measurement of Glomerular Filtration Rate in Single Nephrons of the Rat Kidney<sup>1</sup>

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Perhaps nowhere in the broad spectrum of present-day renal physiology studied with micropuncture techniques has there occurred a greater nor more damaging erosion of confidence in the interpretation of results than in the area pertaining to the measurement of glomerular filtration rate (GFR) in single nephrons. In large part, this decline in confidence can be traced to a recent series of reports claiming either that free-flow aspiration techniques may, by inducing large reductions in intratubular pressure, serve to spuriously enhance net filtration pressure and filtration rate(1,2), or that the recollection technique *per se* may induce spurious and often considerable elevations in nephron GFR(3). This report reviews the results of a recent series of experiments in rats undertaken by us to evaluate the extent, if any, to which methodologic influences contribute to systematic alterations in free-flow estimates of single nephron function.

### EFFECTS OF VARIATIONS IN COLLECTION LINE PRESSURES

Using adult Sprague-Dawley rats, we first examined the effects of variations in fluid collection techniques on end-proximal tubule fluid flow rates, trans-tubular inulin concentration ratios, and single nephron (SN)GFR under conditions of normal hydropenia as well as following volume expansion with isotonic Ringer's solution (10% body wt). Three variations in collection technique were employed. For each, we set as a requirement that a freely movable, downstream

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oil column be maintained in order to isolate the end-proximal fluid collection site from more distal fluid-containing sites. During each collection, hydrostatic pressure within the collection line was monitored continuously. Tracings of representative collection line pressures are illustrated in Fig. 1. Surface tension at the micropipette tip was overcome and fluid entry initiated by the momentary application of suction, as shown by the initial negative pressure spikes located under the arrow at the left. As indicated by the flat lower tracing, during so-called *spontaneous collections*, no further suction was applied, since none was required to maintain a collection rate that preserved the constancy of the position of the distal oil column. As shown in the middle tracing, when modest negative pressure (usually less than  $-20$  mm Hg), referred to as *controlled suction*, was required, intermittent aspiration maintained the desired position of the oil marker. As shown in the upper tracing, during so-called *excessive suction*, a deliberate attempt was made to produce marked declines in tubule pressure, using strong, intermittent, collection-line suction. The terminal pressure spike seen in each panel corresponds to the moment immediately after fluid collection when surface mineral oil was aspirated into the collection pipette to prevent evaporation.

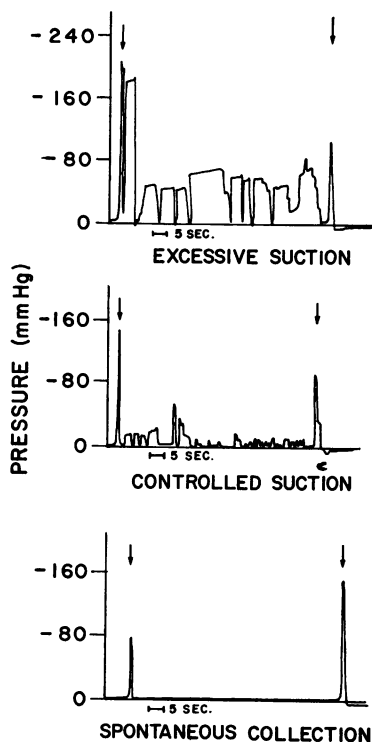


FIG. 1. Representative collection line pressure profiles during collections of tubule fluid by means of spontaneous (lower), controlled suction (middle), and excessive suction (upper) techniques. See text for details.

The effects of these variations in collection technique on several estimates of proximal tubule function measured in 44 tubules in eight hydropenic rats are summarized in Table 1. During spontaneous collections in 15 tubules, fluid to plasma (TF/P) inulin concentration ratios averaged 2.36, flow rates 16.0 nliters/min and SNGFR 36.2 nliters/min. During controlled suction in 15 other tubules, nearly identical values were obtained. It is of interest that even excessive suction failed to exert a significant influence on TF/P inulin ratios and flow rates. However, we calculated an 11% increase in SNGFR which although small was statistically significant ( $P < .05$ ). A similar absence of a large or important effect of these collection techniques on proximal tubule function was observed when identical studies were carried out in these rats after volume expansion with isotonic Ringer's. Especially noteworthy was the finding under these conditions that SNGFR during excessive suction exceeded by only 7% the mean value obtained using more conventional controlled suction techniques. This increase was not significant statistically ( $P > .1$ ).

#### EFFECTS OF VARIATIONS IN INTRATUBULAR PRESSURE

To examine the influence of these variations in collection techniques on proximal intratubular pressure, we repeated these studies using servo-nulling micropipette transducer techniques(4). The arrangement of pipettes used in these experiments is shown in the upper portion of Fig. 2, with the fluid collection pipette (right) in a late proximal convolution and the pressure-sensing pipette (left) at

TABLE 1  
EFFECTS OF VARIATIONS IN COLLECTION TECHNIQUES ON PROXIMAL TUBULE FUNCTION

	Spontaneous	Controlled suction	Excessive suction
Normal hydropenia			
$\left(\frac{\text{TF}}{\text{P}}\right)_{\text{IN}}$	2.36 ± .12 <sup>a</sup> (15)	2.35 ± .16 (15)	2.42 ± .09 (14)
Flow rate (nl/min)	16.0 ± 1.1 (15)	16.7 ± 1.4 (15)	16.9 ± 1.0 (14)
SNGFR (nl/min)	36.2 ± 1.6 (15)	37.5 ± 2.0 (15)	40.4 ± 1.6 (14)
Ringer's loading			
$\left(\frac{\text{TF}}{\text{P}}\right)_{\text{IN}}$	1.40 ± .06 (7)	1.39 ± .06 (7)	1.49 ± .05 (7)
Flow rate (nl/min)	39.3 ± 1.6 (7)	40.0 ± 1.9 (7)	39.6 ± 2.5 (7)
SNGFR (nl/min)	55.4 ± 4.2 (7)	55.4 ± 3.0 (7)	59.3 ± 4.7 (7)

<sup>a</sup> Mean ± SE (*n* tubules).

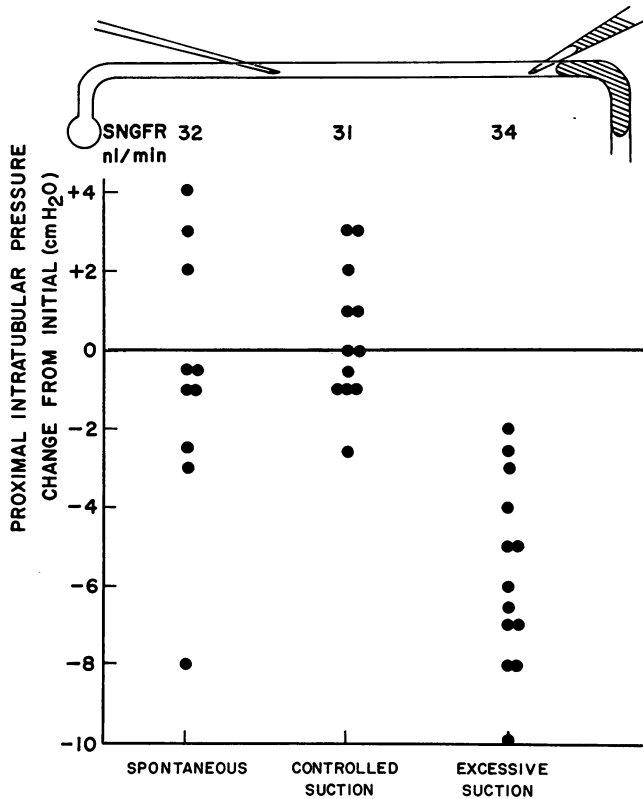


FIG. 2. Effects of variations in fluid sampling techniques on proximal intratubular pressures. Values for SNGFR obtained simultaneously for each technique are also shown.

least two convolutions upstream toward the glomerulus. Shown immediately below are the average values for SNGFR measured in this separate group of eight hydropenic rats. We again found no significant differences among these values measured using spontaneous, controlled suction, and excessive suction techniques (for all,  $P > .1$ ). The individual changes in proximal tubule pressure, relative to precollection values, are grouped according to the collection technique employed. Using either spontaneous or controlled suction, upstream pressures usually ranged within  $\pm 4$  cm H<sub>2</sub>O, the average change being close to zero. In contrast, during excessive suction we uniformly measured significant reductions in intratubular pressure which averaged 6 cm H<sub>2</sub>O ( $P < .001$ ).

At the time these experiments were performed, two explanations seemed plausible as to why reductions in proximal tubule outflow pressure of as much as 10 cm H<sub>2</sub>O failed to increase tubule fluid flow or SNGFR to any large extent. One was that these reductions in pressure were transmitted upstream to Bowman's space but that quantitatively they failed to represent a significant fraction of the effective ultrafiltration pressure. We now know from our recent glomerular pressure measurements(4) that the net driving force for ultrafiltration in the rat

is usually no more than 15 cm H<sub>2</sub>O. All other transglomerular capillary forces remaining constant, a mean reduction in Bowman's space pressure of 6 cm H<sub>2</sub>O would be expected to increase glomerular filtration by some 40 or 50%, a change far greater than the maximum increase of 11% noted in these studies(5). The second, and in retrospect the more likely possibility, is that the fall in collection site pressure is not transmitted upstream to Bowman's space.

Evidence in support of this latter view derives from two sets of experiments(5,6). The initial test was simply to determine whether, at a time when collection site pressures were deliberately reduced, there occurs a quantitatively similar or a significantly lesser decline in pressure at more upstream sites. Such a test was made using a second, separate servo-nulling micropipette transducer system which enabled us to measure pressures simultaneously and continuously at the appropriate two loci in the same proximal tubule. The results of 15 such paired measurements carried out simultaneously at the collection site and at a site located at least two convolutions upstream are shown in Table 2. Prior to excessive suction, upstream and collection site pressures in each tubule were the same or very nearly the same. During excessive suction, collection site pressures were uniformly reduced, the fall averaging 10.4 cm H<sub>2</sub>O. In contrast, pressures at upstream sites always declined less, on average by 4.7 cm H<sub>2</sub>O. The decrease at each site was highly significant ( $P < .001$ ) as were the paired differences between sites ( $P < .001$ ). These results suggest that the fall in collection site pres-

TABLE 2  
EFFECTS OF EXCESSIVE SUCTION ON PROXIMAL TUBULE PRESSURES RECORDED SIMULTANEOUSLY  
AT UPSTREAM AND FLUID COLLECTION SITES

Tubule no.	Upstream ITP <sup>a</sup> (cm H <sub>2</sub> O)			Collection site ITP (cm H <sub>2</sub> O)		
	Control	Excessive suction	ΔITP	Control	Excessive suction	ΔITP
1	11.0	8.5	- 2.5	11.0	4.5	- 6.5
2	20.0	12.5	- 7.5	17.5	4.0	-13.5
3	17.0	15.0	- 2.0	17.0	4.0	-13.0
4	23.0	18.0	- 5.0	23.0	9.0	-14.0
5	10.0	6.0	- 4.0	10.0	1.0	- 9.0
6	15.0	6.0	- 9.0	15.0	1.0	-14.0
7	16.0	15.0	- 1.0	14.0	6.0	- 8.0
8	13.0	9.0	- 4.0	13.0	3.0	-10.0
9	20.5	8.0	-12.5	21.0	4.0	-17.0
10	11.0	9.0	- 2.0	10.0	3.0	- 7.0
11	13.0	8.0	- 5.0	11.0	3.5	- 7.5
12	11.5	4.0	- 7.5	12.0	1.0	-11.0
13	13.0	11.0	- 2.0	12.0	1.0	-11.0
14	14.0	11.0	- 3.0	14.0	8.0	- 6.0
15	12.0	8.0	- 4.0	12.0	4.0	- 8.0
Mean			- 4.7			-10.4
± SE			± 0.8			± 0.8

<sup>a</sup> ITP, Intratubular pressure.

sure was significantly dissipated prior to reaching upstream sites(5). Nevertheless, the fall that was transmitted upstream was by no means trivial but of sufficient magnitude that it might have been expected to increase SNGFR markedly, were it transmitted to Bowman's space. The more definitive study, therefore, required that pressure be measured within, or in close proximity to, Bowman's space.

This requirement could hardly have been met were it not for the generosity of Dr. Klaus Thurau of Munich in providing us with a number of his unique Munich-Wistar rats endowed with glomeruli and Bowman's capsules located directly on the renal cortical surface(6). Table 3 summarizes the results of these experiments. Deliberate application of excessive suction to the collection pipette resulted in marked reductions in collection site pressures in each of eight tubules from eight rats, averaging 10.4 cm H<sub>2</sub>O. Note that despite these large pressure changes at downstream sites, little or no change in pressure was recorded in or within one convolution of Bowman's space of the same nephron, the change for all nephrons averaging less than -0.6 cm H<sub>2</sub>O.

It is important to appreciate that by markedly lowering local intraluminal pressure, excessive suction leads to local segmental collapse of the tubule. When looked for, this phenomenon can readily be recognized to involve the loop of tubule containing the fluid collection pipette as well as one or two loops immediately adjacent to the fluid collection segment. Only about half this fall in pressure can be detected at sites at least two convolutions upstream and, as the Bowman's space measurements indicate, this fall in collection site pressure is completely or nearly completely dissipated by the remaining length of upstream tubule. We presume that this dissipation of the remaining fraction of the reduction in collection site pressure is due to additional zones of segmental narrowing which, although functionally significant, are not visually discernible at magnifications routinely employed. In terms of simple hydrodynamics(6-8), it is likely

TABLE 3  
EFFECTS OF EXCESSIVE SUCTION ON INTRATUBULAR PRESSURES MEASURED SIMULTANEOUSLY IN OR NEAR BOWMAN'S SPACE AND AT LATE PROXIMAL TUBULE FLUID COLLECTION SITES

Tubule no.	Near Bowman's space			Late collection site		
	Control	Exc. suction (cm H <sub>2</sub> O)	Δ ITP	Control	Exc. suction (cm H <sub>2</sub> O)	Δ ITP
1 <sup>a</sup>	8	7	-1	8	0	- 8
2 <sup>a</sup>	10	8	-2	11	0	-11
3	10	10	0	8	-6	-14
4 <sup>a</sup>	14	12	-2	10	0	-10
5	19	19	0	16	2	-14
6	20	20	0	20	6	-14
7 <sup>a</sup>	11	11	0	14	9	- 5
8 <sup>a</sup>	9	9	0	10	3	- 7
Mean ± 1 SE			-0.6 cm H <sub>2</sub> O ±0.3			-10.4 cm H <sub>2</sub> O ±1.2

<sup>a</sup> Pressures measured directly in Bowman's space.

that Bowman's space pressure and filtration rate remain largely independent of the degree of reduction in end-proximal collection site pressure by virtue of the increase in the resistance to downstream flow of tubule fluid offered by the areas of segmental collapse. This increase in downstream resistance, in the presence of a continuous input of fluid from above by filtration, serves to keep relatively constant the level of pressure in segments upstream to the zones of collapse. Consequently, Bowman's space pressure changes little, if at all, and therefore, irrespective of the degree of aspiration employed, effective filtration pressure and filtration rate also change minimally, if at all. It is this fortuitous and very desirable Starling, or more correctly, nonlinear resistor-like property of the proximal tubule cylinder which enables those engaged in micropuncture studies to induce often sizable reductions in collection line and collection site pressures and not emerge with large or important influences on estimates of single nephron function.

Two points of practical concern are raised by these observations. First, the demonstration that Bowman's space pressure remains relatively constant irrespective of the degree of reduction in downstream pressure should not be taken to mean that the opposite is also true, namely that *elevations* in downstream pressures would similarly fail to be reflected at upstream sites. Indeed, we have repeatedly seen (unpublished observations) that increments in late proximal tubule pressures (whether induced inadvertently by temporary obstruction to distal fluid flow by oil droplets, or deliberately, by injection of fluid or oil under pressure) are transmitted promptly and quantitatively to Bowman's space. Under such conditions a fall in net ultrafiltration pressure would be expected. This explanation very likely accounts for the exceedingly low values for SNGFR recently reported by Gertz and co-workers(2) since under the conditions of their experiments, intratubular pressures would have been expected to increase to levels in excess of normal. Second is the possibility that unless a sufficient length of tubule is interposed between the fluid collection site and Bowman's capsule, reductions in collection site pressure might not be effectively dissipated prior to Bowman's space. While we have not examined this possibility experimentally, we think it not unreasonable, based on current evidence, to view with concern the results of studies in both rat and dog in which no effort is made to select predominantly late segments for puncture. Especially vulnerable in this regard are the recent loop microperfusion studies in the rat looking for feedback mechanisms for the control of GFR(9,10) in which the procedure has been to deliberately assess SNGFR from measurements in the earliest accessible proximal tubule segments.

## REPRODUCIBILITY AND ACCURACY OF SNGFR MEASUREMENTS

Let us now turn to a consideration of the degree of reproducibility and accuracy which attends the free-flow measurement of SNGFR. To examine the former, we analyzed the degree of agreement in paired SNGFR measurements

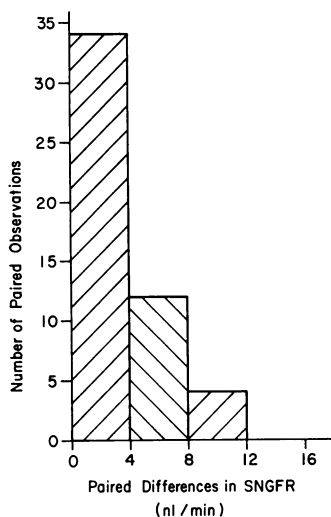


FIG. 3. Frequency histogram indicating the degree of agreement in SNGFR values between two tubules in each of 50 consecutive hydroponic Sprague-Dawley rats.

obtained in each of 50 consecutively studied normally hydroponic Sprague-Dawley rats. Figure 3 is a histogram showing the frequency distribution of the nanoliter per minute differences in SNGFR between each of two tubules in each rat. Using controlled suction techniques filtration rate for the entire population averaged 35 nliter/min. In 34 of 50 rats the agreement between SNGFR pairs was within 4 nliter/min, while in 46 of 50, or 92% of the population, this agreement held to within 8 nliter/min or less. The overall coefficient of variation for these paired SNGFR values, calculated as the standard deviation divided by the mean for each rat, averaged 10.2%.

This value of 35 nliter/min for SNGFR emerges regularly as the average value for normally hydroponic Sprague-Dawley rats in our laboratory. To attempt to determine whether this absolute value is an accurate approximation of the true value, we developed an independent method for estimating SNGFR which does not require the collection of tubule fluid(5). Instead SNGFR was calculated from direct measurements of efferent arteriolar plasma flow (EAPF) and single nephron filtration fraction (SNFF).<sup>4</sup> For these measurements a surface efferent arteriole was identified, punctured, and its branch capillaries blocked with oil. Thereafter, timed quantitative collections of efferent arteriolar blood were made. Hematocrits of efferent arteriolar ( $H_{EA}$ ) and femoral arterial ( $H_{FA}$ ) blood were determined, and from these, EAPF<sup>5</sup> and SNFF<sup>6</sup> were calculated. The

$${}^4 \text{SNGFR} = \frac{\text{EAPF}}{1 - \text{SNFF}} - \text{EAPF}, \text{ where SNGFR and EAPF are in units of nliter/min.}$$

$${}^5 \text{EAPF} = (\text{EA Blood Flow}) (1 - H_{EA}); \text{ where EAPF and EA Blood Flow are in units of nliter/min.}$$

$${}^6 \text{SNFF} = 1 - \frac{(H_{FA})(1 - H_{EA})}{(H_{EA})(1 - H_{FA})}.$$



values for SNGFR calculated in this way were compared with values from these same rats derived from measurements of tubule fluid flow rate and transtubular inulin concentration ratios. SNGFR measured using these conventional free-flow micropuncture techniques averaged  $34.0 \text{ nliter/min} \pm 1.9 \text{ SE}$ . Estimates based on the microvascular measurements yielded a range in each rat similar to that obtained from tubule punctures, and when corrected for a 5% entrapment of plasma water, averaged a remarkably close  $32.3 \text{ nliter/min} \pm 2.0 \text{ SE}$ . This agreement in results obtained by different methods ( $P > .5$ ) gives us additional confidence that the average value for SNGFR derived from conventional free-flow tubule puncture techniques in adult Sprague-Dawley rats is a reasonably close approximation of the true value.

The question arises whether or not values for SNGFR are quantitatively similar in all strains of rats presently studied in micropuncture laboratories. In an attempt to determine if differences in SNGFR exist among various strains, we have compared in retrospective fashion, the absolute and corrected body weight values for SNGFR for a number of consecutively studied normally hydropenic rats of Munich-Wistar and Sprague-Dawley breeds. As shown in Table 4 marked differences in average absolute SNGFR's were noted between strains, which although partially the consequence of modest differences in body weight, persisted to a highly significant degree even when individual values for SNGFR were corrected for these individual differences in body weight. These observations may provide a partial explanation for the often not inconsiderable differences in SNGFR reported from different laboratories.

## THE RECOLLECTION TECHNIQUE

An evaluation of the merits of this approach relative to measurements derived from new tubule punctures has been considered in detail elsewhere(5). In short, we found no evidence that the recollection technique contributes errors of a systematic nature to these measurements of single nephron function. However, since one gains a significant statistical advantage by use of the same tubule as its

TABLE 4  
COMPRISON OF VALUES FOR SNGFR IN MUNICH-WISTAR AND SPRAGUE-DAWLEY RATS

Strain	Body weight (g)	SNGFR (nl/min)	SNGFR/100 g body weight (nl/min)
Munich-Wistar	254.4 (40)	$23.4 \pm 0.8^a$ (76)	$9.3 \pm 0.3$ (76)
Sprague-Dawley	318.1 (22)	$35.6 \pm 1.5$ (49)	$11.2 \pm 0.5$ (49)

$P < .005$

<sup>a</sup> Mean  $\pm$  1 SE. Numbers in parentheses are numbers of observations.

own control, we continue to prefer the use of this method whenever experimentally feasible.

One aspect of these recollection studies deserves comment since it bears on the recent claim by Mandin and co-workers(3) that estimates of SNGFR, when obtained from repunctured tubules of dogs, especially after volume expansion, are apt to be spuriously and systematically higher than estimates obtained from tubules punctured for the first time. To evaluate this possibility in the rat, we compared (Fig. 4) the recollection values for SNGFR from each of 14 tubules from 14 rats (abscissa) with values obtained from 14 newly punctured tubules in these same rats (ordinate). Solid circles denote hydropenia; open circles indicate a 10% body weight infusion of isotonic Ringer's solution. For both states of extracellular volume, values for SNGFR obtained by repuncture and nonrepuncture techniques in the same rat were found to distribute randomly about the line of identity (mean nonrepuncture/repuncture ratio =  $1.05 \pm .05$  SE,  $P > .2$ ). For the rat, then, repuncture of the same tubule appears not to donate the systematic error reported by Mandin and co-workers(3).

## SUMMARY

Studies were performed in rats to determine the influence of variations in fluid-sampling techniques on several measures of proximal tubule function. Fluid/plasma inulin ratios, fluid flow rates and calculated values for single nephron GFR were found to be influenced only to minor degrees, if at all, by graded reductions in collection line and collection site pressures. Evidence is presented to indicate that this failure of reductions in collection site pressure to enhance filtration rate is due to a nonlinear (Starling) resistor-like property of

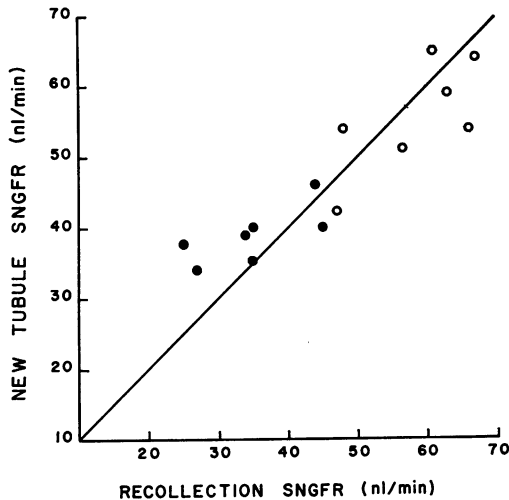


FIG. 4. Comparison of recollection vs new tubule SNGFR values during conditions of normal hydropenia (solid circles) and after acute Ringer's loading (open circles).

the proximal tubule cylinder which serves to maintain relative constancy of Bowman's space pressure.

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