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Potential Sources of Error in Measuring Single-Nephron Glomerular Filtration Rate*

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In recent years single-nephron filtration rate has been measured extensively with micropuncture techniques. The values obtained in our own studies, as well as those reported by others, reveal an impressive degree of scatter. Some of the variation can be attributed to differences in the animals and in their physiologic state. Nevertheless, values in the same animal in a constant physiologic state may differ by as much as 100%. Such discrepancies within a single animal could be the consequence of either heterogeneity of nephrons or experimental error.

Although some heterogeneity of superficial nephrons may exist, most of the variability in measured SNGFR probably stems from experimental error. Some of the potential sources of error in the measurement of SNGFR are listed in Table 1. Obvious analytic errors can arise from the measurement of both the volume of the collected sample of tubular fluid and the concentration of inulin. In our experiments, using radioactive inulin, the total analytic error was 7–8%. This included errors in measurement of volume of fluid samples, loss of fluid during transfer into counting vials, and counting errors. If, however, the total timed collection of tubular fluid was transferred directly into the counting vial, the volumetric errors could be eliminated and the total error reduced to the counting error, which is approximately 4%.

More important sources of error are obviously related to the micropuncture techniques used in the collection of tubular fluid. Timing errors can contribute to some extent, depending upon whether the timing of collection begins with the injection of oil into the tubule or the aspiration of the sample. In our experiments the average time between injection of the oil and starting the collection

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TABLE 1

POTENTIAL ERRORS IN MEASUREMENT OF SNGFR

I.	Analytic errors
	A. Volumetric measurements
	B. Inulin determination
II.	Collection errors
	A. Timing
	B. Incomplete collection
	C. Contamination
	1. Surface fluid
	2. Regrograde flow
	2. Retrograde flow
	4. Proximal displacement of oil block
	D. Alterations in intratubular pressure
	E. Recollection artifacts
III.	Systematic errors
	A. Change in physiologic state
	B. Disruption of feedback control of SNGFR in oil-blocked tubule.

of fluid was 6.7 sec in hydropenia and 4.8 sec in saline diuresis. Most micropuncturists begin timing with the initiation of aspiration, rather than with injection of the oil. This, however, assumes complete cessation of filtration in the interval between blockage and initial aspiration. It is more likely, however, that filtration-reabsorption continues during this interval and may contribute some uncertainty as to the correct timing. The magnitude of this error is strictly dependent upon the total collection time. It will be significant with very short collection periods (10–30 sec) and trivial with prolonged collections. For this reason we collect for a minimum of 2 min and preferably longer.

Loss of tubular fluid around the puncture site will falsely lower the measured value for SNGFR, while contamination with inulin containing fluid will artifactually elevate the values. Possible sources of contamination include fluid on the surface of the kidney, retrograde flow of distal fluid around the oil block, and fistulas into adjacent tubules. Drs. Andreucci and his associates(1) while working in our laboratory, critically examined each of the possibilities. In brief, they found that in freshly punctured, but not in re-collected tubules, leakage around the pipet tip and surface contamination was minimal. They also found that retrograde contamination was not significant even in the presence of high distal tubular pressures. More importantly, Dr. Andreucci developed a method that can be easily used to identify retrograde contamination in each collection when the experimental condition, such as ureteral occlusion, increases the danger of such an error. Surprisingly, the most important source of contamination was shown to be fistulas between tubules. When measuring intratubular pressure by the Landis technique during collection periods, green dye could be seen flowing out of the oil-blocked tubule into an adjacent tubule in approximately 10% of punctures. This problem seemed to be much more serious when puncturing the ventral surface with its adherent covering of peritoneum, than the dorsal surface,

which is free of the peritoneal cover. A final source of contamination arises from improper placement of the oil block. Occasionally when the oil is injected with considerable pressure, particularly when intratubular pressure is high, the oil block will remain between the pipet and glomerulus, and the subsequent collection will consist entirely of fluid distal to the oil block, thus giving large volumes and high inulin ratios. This can be detected by paradoxical movement in an apparently upstream direction upon removal of the pipet.

Both Gertz *et al.*(2) and Schnermann *et al.*(3) have suggested that alteration in intratubular pressure (ITP) during collection of tubular fluid alters the effective filtration pressure and SNGFR. In our experiments this has been a very difficult issue to evaluate. We found that, depending on the technique used to collect fluid, significant reductions in ITP could occur. When fluid was collected by an intermittent aspiration technique to keep the oil block stationary, average ITP fell to 6 cm H_2O in hydropenia and 10.6 cm H_2O in saline diuresis. However, when fluid was allowed to flow spontaneously into the pipet without any aspiration, average ITP did not change significantly during hydropenia and fell only 2.3 cm H_2O during saline diuresis. Although the average ITP did not change significantly, in individual spontaneous collections ITP sometimes changes by several centimeters H_2O.

Whether these small alterations in ITP would influence SNGFR, however, is critically dependent upon the magnitude of the effective filtration pressure. If EFP were high the effect of these changes in ITP would be trivial; if EFP were low, these alterations in ITP might significantly influence SNGFR. Recent studies have shown EFP to be much lower than previously suspected. We have found EFP to be 13–14 cm H_2O in hydropenia and 26 cm H_2O in saline diuresis(4). Brenner, Troy and Daugharty(5) have found similar values in hydropenia.

Despite these low values for EFP we have not been able to show any clear relation between the change in ITP and SNGFR (Fig. 1). In hydropenia SNGFR did not correlate well with either ΔITP ($r = 0.33$) or ITP ($r = 0.38$). In saline diuresis SNGFR showed a slight correlation with ΔITP ($r = 0.62$) and a modest correlation with ITP ($r = 0.71$).

One possible explanation for the failure to show a relation between SNGFR and changes in ITP is that any increase in SNGFR as a consequence of a decrease in ITP would be limited by a correspondent increase in glomerular oncotic pressure. This would be particularly true in hydropenia where oncotic pressure increases as an exponential function of protein concentration. We have found that in hydropenia the balance of pressure in the glomerulus reaches an equilibrium at the end of the capillary network; i.e., the EFP at the efferent end of the glomerulus is zero(4). Therefore, the oncotic pressure at the end of the glomerulus is given by the difference between glomerular hydrostatic pressure (P_G) and intratubular pressure (ITP). If we assume that, as the ITP is raised or lowered, (a) the efferent EFP remains zero because any change in SNGFR produces a change in efferent oncotic pressure, (b) the P_G remains constant, and (c) the glomerular perfusion rate remains constant, then the relation between SNGFR and ITP during hydropenia will be curvilinear, with a steep curve in the region near

stop-flow pressures and a flat curve in the more physiologic region (Fig. 1). The slope of the curve in the physiologic range is such that, if the ITP is reduced from 22 to 16 cm H_2O during the collection, the relative increase in SNGFR will be only 3 nl (24–27 nl/min); such a small difference cannot be detected by micropuncture techniques.

In contrast, during saline diuresis arterial protein concentration is markedly reduced and consequently glomerular oncotic pressure rises only slightly as a result of filtration. For this reason the effect of the change in ITP will not be counterbalanced by such a steep rise in glomerular oncotic pressure. The theoretical line describing the relation between SNGFR and ITP in saline diuresis, calculated from value for stop-flow pressure of 57.7 cm H_2O measured in oil-blocked tubules, is linear with a slope such as to suggest that a 5-cm drop in ITP would produce about 7 nl/min rise in SNGFR (Fig. 1). The correlation coefficient of 0.62 between SNGFR and ΔITP is in the equivocal range which does not permit a conclusion one way or another.

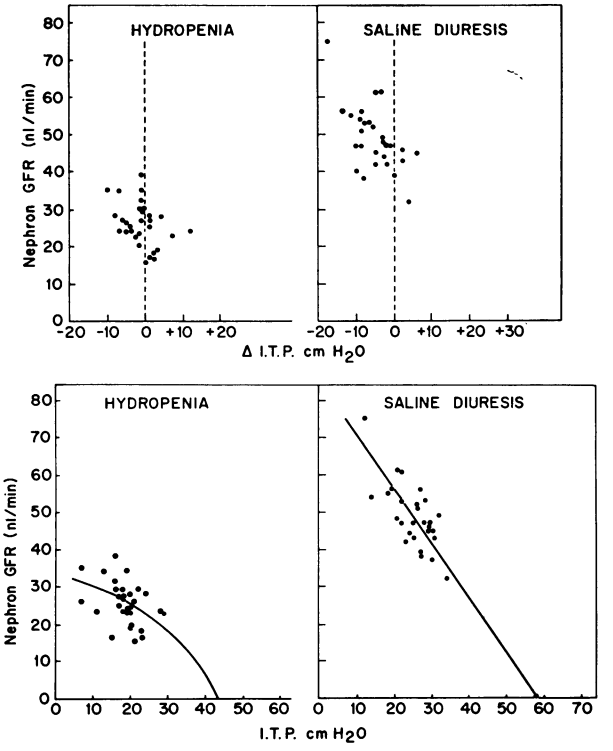

FIG. 1. Relationship between simultaneous measurements of intratubular pressure and SNGFR. Intratubular pressure was measured before and during spontaneous collection of tubular fluid. Change in pressure is plotted in upper panel and the absolute pressure during collection is plotted in lower panel. In hydropenia, correlation coefficient was 0.33 between SNGFR and ΔITP, and 0.38 between SNGFR and ITP. During saline diuresis, correlation coefficient was 0.62 between SNGFR and ΔITP, and 0.71 between SNGFR and ITP. See text for details concerning calculations of two solid theoretical lines in bottom panels.

To examine the possible further effects of ΔITP on SNGFR, recollections were performed during saline diuresis, attempting to lower intratubular pressure during the second collection. In many instances it was difficult to lower the pressure at the site of the pressure-measuring pipet despite aspiration. In four instances (shown in Fig. 2) the ITP was lower during the second collection than in the first, yet there was no difference in SNGFR. Although these results would suggest that decreased ITP during collection of tubular fluid does not significantly alter SNGFR, this conclusion must be qualified because of the possibility recently suggested by Brenner *et al.*(6) that the measured drop in ITP in the convolution near the collecting site is not effectively transmitted to the glomerulus.

An additional issue I would like to discuss is the repeated use of the recollection technique for measurement of SNGFR in the same nephron. To examine the validity of this technique we have performed repeated collections in the same nephron during a constant physiologic state, either continuous hydropenia or continuous saline diuresis. During hydropenia the ratio of the second to the initial SNGFR averaged 1.09 in the rat and 1.08 in the dog. In dogs, during saline diuresis the second collection gave a value that was 27% higher than the first(7). We have not performed similar studies during saline diuresis in the rat.

There are several difficulties with the recollection technique that could contribute to the systematically higher collection values. First, the seal around the pipet tip is very poor during recollection, raising the possibility of surface contamination. A more likely possibility is that, in the dog, ITP in the recollected tubule is significantly lower than in surrounding tubules, and that this ΔITP is effectively transmitted to the glomerulus. During hydropenia, because of the high concentration of glomerular protein, the lower ITP would have only a small effect on SNGFR. However, during saline diuresis, because of the much lower

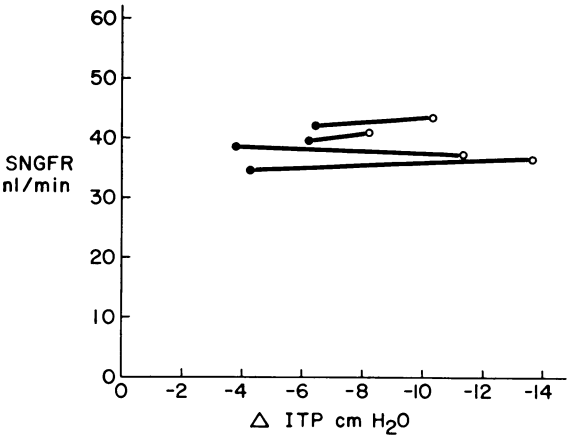

FIG. 2. Effect of lowering intratubular pressure on SNGFR during saline diuresis. The change in intratubular pressure (ΔITP) represents the difference in ITP measured during the collection and that measured in free flow before starting the collection. Values for the first collection are shown by solid dots (●) and those for the second collection are shown by open circles (○). Solid lines connect the two values obtained in the same tubule.

protein concentration, any fall in ITP would have a more significant effect on SNGFR.

In the rat, Dr. Andreucci has found that the recollection technique can be used successfully in both hydropenia and saline diuresis if one simple precaution is taken. Instead of repuncturing at the exact site of the first puncture, he recollects from a site 3–4 tubular diameters more proximal and places the oil block between the two puncture sites. With this technique the ratio of the re-collected and initial SNGFR is not significantly different from 1.0.

Finally, there is the possibility that blocking the tubule disrupts the feedback control of SNGFR by distal Na delivery and that SNGFR measured in this fashion is falsely elevated. Schnermann *et al.*(8) found in perfusion studies of the loop of Henle that SNGFR was approximately 50% higher at very low perfusion rates than at physiologically normal perfusion rates. Although Morgan(9) was unable to confirm these findings, the technical difficulties of this type of experimental design preclude the formation of a firm conclusion on the basis of a single set of experiments, whether they be positive or negative. There are certain indirect observations, however, which raise some question concerning this type of feedback control of SNGFR. First, we have compared TF/P inulin ratios sampled first from free-flowing tubules and then after the sample tubule was completely blocked with oil. There was no significant difference in these values (Table 2). If SNGFR had risen after tubular blockade, one might have anticipated a fall in TF/P inulin. However in all fairness it must be conceded that failure of TF/P inulin to fall does not exclude a rise in GFR, since the factors responsible for increasing SNGFR might also produce proportionate changes in reabsorption. Second, Dr. Blantz in our laboratory has recently measured glomerular hydrostatic pressure in Wistar rats with a servo-null microtransducer before and after tubular blockade. In both normal hydropenic rats and rats maintained on low-salt diets, he found no significant rise in P_G, which might have been anticipated had the feedback system produced either selective efferent vasoconstriction or afferent vasodilatation in response to decreased distal delivery. Since, however, it has been shown(4,5) that filtration attains equilibrium at the efferent end of the glomerulus, an increase in glomerular plasma flow could in-

TABLE 2

EFFECT OF STOPPING DISTAL DELIVERY ON TF/P_{inulin} DURING COLLECTION OF FLUID FROM SUPERFICIAL PROXIMAL TUBULES[a]

	Free flow (TF/P_{In})	Oil block (TF/P_{In})	Mean ratio
Hydropenia	1.80	1.76	0.99
n = 8	(.29)	(.19)	(.07)
Saline diuresis	1.36	1.37	1.01
n = 12	(.21)	(.20)	(.09)
Total	1.53	1.52	1.00
n = 20	(.32)	(.27)	(.08)

[a] Standard deviation in parentheses.

crease SNGFR without necessitating a rise in P_G. Thus, the observations of a constant P_G by Dr. Blantz does not exclude the possibility that the feedback system produced proportionate dilatation of both afferent and efferent arterioles and that SNGFR rose because of an increase in glomerular plasma flow, rather than a rise in P_G. Thus, a systematic error in SNGFR associated with the tubular blockade remains a possibility that cannot be excluded on the basis of presently available data.

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