Methodological Aspects of Filtrate Determination by the Micropuncture Technique

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The quantitative assessment of the filtration rate of single nephrons (SNGFR) determined from the product of volume flow rate and TF/P inulin at any point along the tubular system has for two reasons become indispensable for the evaluation of renal function. First, disparities in nephron filtration rates in different kidney regions make the inulin clearance a parameter of questionable value for the evaluation of nephron function. Second, measurement of SNGFR is necessary to determine the fluid reabsorption of different tubular segments under normal free flow conditions. Full acceptance of results based on the determination of SNGFR by the micropuncture technique requires previous critical examination of potential errors associated with this method. Without presuming to give a comprehensive review of potential artifacts, the following problems appear worthy of consideration.

1. Some time ago we reported(1) that SNGFR was significantly higher when the tubular fluid was collected under greater negative pressure exerted by the sampling syringe as compared to GFR obtained by spontaneous collections, i.e., without applying an extra negative pressure. Since intratubular pressure during fluid aspiration was found to be somewhat lowered, this was interpreted to indicate an effect of altered intratubular pressure on filtrate formation. In the meantime, however, we and others(2) have obtained evidence indicating that the effect of aspiration on SNGFR is less than previously assumed. In recollection experiments (Fig. 1) we have observed that a pressure fall of about 8 cm water in the early proximal convolution, the maximum we could produce, induced an average GFR increase of only 0.56 nl/min • cm H₂O, a change which is, however, significant when tested by paired statistical analysis (P < 0.001). The reason for this relatively small change, while not clear, is beyond the scope of this discussion. To explain our earlier results we assume that the SNGFR was elevated initially for reasons unknown to us. The high aspiration rate needed to keep the
intratubular pressure low would thus have been the result of the high filtration rate and not the cause as we had proposed earlier. On the other hand, an increase in intratubular pressure depressed SNGFR by 1.3 nl/min. • cm H$_2$O, the change again being statistically significant in a paired analysis ($P < 0.002$). We conclude that it is important to avoid an elevation of tubular pressure during the collection, but that the effect of a pressure fall induced by sample aspiration is practically negligible.

2. Results which we obtained in an attempt to evaluate the reason for rather low filtrates reported by Gertz $et$ $al.$(3) some time ago support this conclusion. These authors collected the tubular fluid against a counter-pressure 3–4 cm H$_2$O lower than the previously measured free-flow pressure. This pressure differential was thought to be great enough to overcome the tip resistance of the sampling pipet. Using this technique we have observed that the actual intratubular pressure during a fluid collection of this type was always higher than the free-flow pressure, on the average by 10 cm H$_2$O. In our opinion this pressure rise explains the low nephron filtrates obtained with this technique. The likely explanation for the pressure elevation arises from our finding that the inflow rate into a glass capillary is quite variable at a given pressure gradient and usually less than the outflow rate at the same pressure difference. This indicates that the resistance to flow of a pipet tip is unpredictably higher than anticipated on the basis of a Poiseuille type of flow when the direction of flow is inward. The reason may be turbulent flow in the narrow part of the sampling pipet.

3. Contamination of tubular fluid by retrograde flow past the injected oil block has been thought to explain unusually high nephron filtration rates(4,5). This hypothesis was tested by perfusing a tubule with a $^{14}$C-inulin-containing solution and collecting the "normal" tubular fluid at the upstream side of an oil block separating collection and perfusion sites. Using this technique we never
detected a significant leakage of fluid around the oil block, even when the perfusion pressure was greatly increased.

4. The validity of the micropuncture method for measuring steady-state GFR's appears doubtful in view of our finding that filtration rates are not identical when collections are made in distal and proximal segments of the same nephron in hydropenic rats. We define hydropenic rats as animals that have had free access to food and water prior to anaesthesia and receive infusions at a rate of 0.4–0.7 cc per hour and 100 g body weight from the time the jugular catheter is inserted. The average GFR obtained by distal collections was 25 nl/min ± 7.5, and that obtained by proximal collections 34.5 nl/min ± 8.4. In saline diuresis this GFR difference largely disappeared. The interpretation of the proximal–distal GFR difference observed under hydropenic conditions is not quite clear. We favour the possibility that institution of the oil block abolishes a flow-dependent signal at a distal sensor site which then leads to an acute elevation of GFR. Thus, the distally measured SNGFR would represent the steady-state value, while the proximally collected volume would be higher than normal. We consider it unlikely that this result was due to leakage of polyfructosan which until now had not been proved an ideal glomerular marker substance. In a preliminary series of experiments we have observed that microinjections of known amounts of polyfructosan into a proximal segment and re-collection at a distal collection site yielded a recovery of about 95%.

The existence of a functional tubuloglomerular coupling has been demonstrated in several earlier experiments(6,7), but nevertheless continues to be a matter of controversy(8). New evidence for such a mechanism was obtained by studying the effect of injecting saline or mannitol solutions into distal tubular segments on the so-called stop-flow pressure which according to Gertz and co-workers(9) can be taken as a measure of glomerular capillary pressure. It was found that injections of saline induced a fall of the stop-flow pressure by 5–8 cm water, while mannitol produced no effect (Fig. 2). In addition, repeated saline injections were associated with a continuous decrease of the stop-flow pressure

![Fig. 2. The effect of injections of Ringer and isotonic mannitol solutions into a distal tubular segment on the so-called stop-flow-pressure (9) of the same nephron.](image)
fall. Further studies must determine whether this finding is due to an adaptation of the feedback mechanism or to a methodological problem.

The conclusion that steady-state GFR's can be measured only by distal collections is, however, not beyond any doubt. We have observed that rapid injection of the distal oil block is usually followed by a pressure fall in the proximal convolution of this nephron (Fig. 3). This might indicate that the filtration pressure head is reduced by this injection thus decreasing the steady-state filtration rate. The distally collected flow rates would then be integrated over a period of increasing SNGFR. Two arguments could be advanced discounting a major influence of such an undefined effect of the distal oil block instillation on SNGFR. First, SNGFR was independent of the length of the stop-flow period (the period between the oil-block injection and the start of the collection), an unexpected finding if distal pressure itself were eliciting the proximal pressure fall. Second, if the oil-block was injected slowly so that it was always positioned downstream from the pipet and if the stop-flow period was short (not longer than 30 sec), the pressure fall was either much smaller or completely absent.

5. Two different protocols appear to be used for the determination of the collection time. The time period is either begun immediately after the injection of the oil block or at the moment of fluid entrance into the capillary. Thus, it is of interest to estimate the possible difference in SNGFR due to either elimination or inclusion of the stop-flow period. Figure 4 shows the influence of the collection time to stop-flow time ratio on the relation between SNGFR's calculated by either of the two methods. It can be seen that the difference between the two methods is reduced to less than 10% at a ratio higher than 9. Thus, at a stop-flow time of 10 sec, the collection time must exceed 90 sec to make SNGFR relatively independent of the timing method. It should be noted that neither procedure results in the true SNGFR, the deviation from the true value being in opposite directions. The safest way to avoid confusion and errors greater than 5% is to make the ratio of collection over stop-flow time large enough. We further recommend adding half of the stop-flow time to the collection time to minimize the remaining error.

![Fig. 3. The change of proximal intratubular pressure during oil-block instillation into three different distal tubular segments. In the first two cases the oil blocks were injected rapidly (at a time indicated by 'O'), while in the third case it was introduced very slowly.](image-url)
### Filtrate Determination by Micropuncture

| GFR Coll Time | GFR Total Time |
|---------------|---------------|
| Collection Time | Stop Flow Time |
| 0 | 5 | 10 | 15 | 20 |
| 2-15 | 0 | 5 | 10 | 15 | 20 |

**Fig. 4.** The ratio of SNGFR's calculated from collection time or total time (collection time plus stop flow time) in relation to the collection time to stop-flow time ratio. Collection time is defined as the time from the moment of fluid entrance into the pipet until the end of the collection, stop-flow time as the time from the instillation of the oil block until the entrance of fluid into the pipet.

6. Results of Berglund et al. (10) showing a higher clearance of polyethylene glycol 1000 compared to the clearance of inulin have led to the suggestion that the permeation of inulin through the glomerular capillary membranes may be restricted because of molecular sieving. In a preliminary series of experiments we have, therefore, determined the concentration of polyfructosan in the Bowman's capsule in comparison to the plasma concentration. The mean concentration ratio of $1.11 \pm 0.1$ SE ($n = 10$) does not support the assumption of a significant concentration difference between plasma and primary urine due to a sieving effect across the glomerular capillary membranes.

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