Continuous in vivo Perfusion of the Postglomerular Capillary Network in Superficial Rat Kidney Cortex*

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The experimental design of many micropuncture studies requires changes in the peritubular environment. Classically, these changes are induced by systemic infusion of suitable solutions. This technique has significant shortcomings due to the unavoidable modification in the composition of the glomerular filtrate and to changes in the rate of glomerular filtration. Various approaches are being used to circumvent the effect on glomerular filtration rate (GFR), but nothing can be done, under such circumstances, to maintain constant the composition of the filtrate. Only in Necturus, because of its dual renal blood supply, is it possible to vary independently the composition of the fluid reaching the two sides of the tubular membrane.

The findings suggesting a possible correlation between the transport capacity of the proximal tubular membrane and extratubular events(1,2), stimulated our search for a method which would allow selective monitoring of fluid passing through the peritubular capillaries of the mammalian kidney. We decided, therefore, to attempt to perfuse directly and continuously the postglomerular capillary network on the kidney surface in vivo(3). Frömter(4) and Rumrich and Ullrich(5) developed independently a nearly identical technique. A precedent in this field was established, however, in 1965 by Lechène and Morel(6) who performed single bolus injections of fluid into the peritubular capillaries of hamsters. The anatomy of the vascular bed of the rat kidney seemed suited for our purposes. As shown initially by standard fixation and staining methods(7), confirmed more recently

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by intravascular injections of low-viscosity polymer solutions(8), the unbranched efferent arteriole passes toward the subcapsular region where it divides into several branches (forming the so-called vascular star), which subsequently break up into a fine capillary plexus which surrounds the tubules. It appears that for the superficial cortical nephrons the peritubular capillaries always connect in series with the glomerular capillaries of the same nephron. This is not to say that connections between the peritubular capillary beds of adjacent nephrons do not exist. On the contrary, a rich anastomotic pattern was demonstrated at the level of the cortical postglomerular capillaries(7). It is expected, therefore, that an infusion of fluid into a branch of the efferent arteriole should result in the perfusion of the peritubular capillary network corresponding to that particular nephron as well as perfusion of capillaries corresponding to neighboring nephrons.

The techniques of simultaneous capillary perfusion and micropuncture of tubules is illustrated schematically in Fig. 1. Puncture of the vessels was performed with micropipets with external tip diameters of 6–8 μ. Since the diameter of the capillaries forming the vascular star is somewhere between 8 and 10 μ, short beveling of the pipets was necessary in order to avoid through and through penetration of these vessels. The force needed to push the fluid into the vessel was obtained from a tank of 5% carbon dioxide in oxygen connected to the micropipet through polyethylene tubing. The hydrostatic pressure was controlled by a reduction valve and a stopcock. The rate of peritubular perfusion was considered adequate when a bloodless appearance of the renal cortical surface was obtained over an area about 700 μ in diameter. The magnitude of this area clearly indicates that the vascular network reached by the perfusate was in excess of the number of capillaries originating directly from a single efferent arteriole. As a corollary, any measurement of the outflow from the micropipet cannot be re-
garded as representative of the flow rate of perfusate through the capillaries branching from a single arteriole. This probably explains why, in some of our experiments, adequate capillary perfusion could be achieved only at an estimated infusion rate of 400–800 nl/min, as compared to a normal blood flow of about 200 nl/min through one postglomerular arteriole (calculated on the basis of a single nephron GFR of 30 nl/min, a filtration fraction of 0.3, and a hematocrit of 40%).

Although capillary hydrostatic pressure was not measured, the fact that the stream of blood was kept away from the artificially perfused vessels made it evident that the hydrostatic pressure within these capillaries must have been higher than in those naturally perfused. However, measurements of proximal intratubular pressure made under experimental conditions yielded average values of 13.3 mm Hg (SEM 0.6, 8 obs.) during perfusion with Ringer's solution and 11.7 mm Hg (SEM 0.2, 9 obs.) when 8% Dextran was used instead. These values are not significantly different from the intratubular hydrostatic pressure of 11.9 mm Hg (SEM 0.3, 17 obs.) obtained in the same tubules under control conditions. It seems likely that any major variations in capillary pressure would have been detected by these measurements.

Several possible pitfalls became evident during the development of this technique. First was the high risk of penetrating the tubular lumen and contaminating the tubular fluid. It was, indeed, obvious that, because of the small difference between the diameter of the perfusing pipet and the diameter of the vessel to be perfused, the tip of the pipet could have been partially in the vessel and partially in the neighboring tubule. The location of the entire tip within the tubular lumen is rather easy to detect since in this case the flow of blood through the peritubular capillaries continues undisturbed. In order to minimize the risk of mixing the vascular perfusate with the glomerular filtrate we chose to puncture the tubules at a distance of at least 40μ from the capillary perfusion site and to color the perfusion fluid slightly with lissamine green. Although this certainly allowed detection of significant contamination, it probably (and this statement is entirely speculative) did not avoid this danger completely.

A second serious shortcoming was the impossibility of assessing the fact that the entire length of the tubule chosen for micropuncture had its vascular supply filled with the perfusate. It was, indeed, possible that some of the loops below the surface, or even some of the superficial loops, were beyond the boundaries covered by the perfusion. In order to minimize this danger we tried to cover an area at least twice the diameter of the surface occupied by the superficial tubular segments of a single nephron. Furthermore, we performed similar experiments under “stop-flow” conditions in which the entire tubular segment tested was visualized.

A third important problem refers to the rate of infusion, which might in itself influence reabsorption or might cause changes in capillary hydrostatic pressure, which in turn, might affect tubular transport. This variable was partially controlled in our experiments by using micropipets with no more than 2 μ variation in the outside tip diameter and by aiming for a relatively constant area of per-
fusion. As already mentioned, under these circumstances, using the Landis method, we were unable to detect significant differences in intratubular pressure between control and experimental conditions. However, in vitro testing, using a similar propulsing force and pipet with similar tip resistances, yielded flow rates which varied by as much as a factor of two. How relevant these measurements were to circumstances prevailing in vivo was difficult to assess since factors such as the intracapillary resistance, differences in temperature, and partial obstruction of the tip of the pipet also could influence the flow rate.

Despite these objections, several facts demonstrate that the technique of peritubular capillary perfusion provides reliable information. Of major significance

| TABLE 1 |
| Free Flow Recollections, 8% Dextran Perfusions |
| --- | --- | --- | --- |
| | 8% Dextran (Mean ± SE) | Control (Mean ± SE) | No. of obs. | P |
| TF/P inulin | 1.79 ± 0.13 | 1.59 ± 0.10 | 11 | < 0.20 |
| GFR (nl/min) | 21.8 ± 1.3 | 23.0 ± 1.8 | 11 | < 0.50 |
| Transit time (sec) | 7.9 ± 0.6 | 7.9 ± 0.7 | 11 | < 0.90 |
| Reabsorption rate (nl/min) | 8.8 ± 1.3 | 7.9 ± 1.1 | 11 | < 0.40 |

| TABLE 2 |
| Free Flow Recollections, Ringer's Perfusions |
| --- | --- | --- | --- |
| | Ringer's (Mean ± SE) | Control (Mean ± SE) | No. of obs. | P |
| TF/P inulin | 1.47 ± 0.11 | 1.83 ± 0.14 | 11 | < 0.005 |
| GFR (nl/min) | 22.9 ± 2.8 | 30.5 ± 3.1 | 11 | < 0.10 |
| Transit time | 13.8 ± 2.5 | 7.9 ± 1.0 | 11 | < 0.10 |
| Reabsorption rate (nl/min) | 6.4 ± 1.2 | 13.4 ± 2.2 | 11 | < 0.02 |

| TABLE 3 |
| “Split-Drop” Experiments |
| --- | --- | --- | --- |
| | Radius (μm) (Mean ± SE) | t½ (sec) (Mean ± SE) | Reabsorption rate (nl/sec · mm) (Mean ± SE) | No. of obs. | P |
| Ringer's | 15.7 ± 0.6 | 18.3 ± 0.9 | 0.051 ± 0.02 | 12 | < 0.001 |
| 4% Dextran | 15.2 ± 1.1 | 12.8 ± 0.6 | 0.046 ± 0.04 | 12 | < 0.05 |
| 8% Dextran | 16.1 ± 0.4 | 10.3 ± 0.5 | 0.060 ± 0.05 | 15 | < 0.98 |
| Controls | 16.5 ± 0.3 | 9.7 ± 0.5 | 0.063 ± 0.05 | 21 | < 0.98 |
is the finding that the single nephron glomerular filtration rates observed under control conditions were not significantly different from those obtained when the peritubular capillaries were perfused with a solution of 8% Dextran (Table 1). In the same group of experiments the reabsorptive rate was also similar under control and experimental conditions. A fall in GFR of about 25% was observed, however, when the peritubular capillaries were perfused with a colloid-free solution (Table 2). Under those circumstances the decrease in reabsorption was in excess of 50%. We were unable to find a statistically significant correlation (P>0.1) between changes in nephron GFR and changes in reabsorption of individual nephrons. Nevertheless, the possibility still exists that the decrease in the reabsorptive capacity of the tubule did influence the rate of glomerular filtration.

Another piece of evidence endorsing the reliability of the method is provided by the results obtained in the "split-drop" experiments (Table 3). The t_{1/2} of 10.3 sec (SEM 0.5, 15 obs.) observed under conditions of peritubular capillary perfusion with an 8% Dextran solution in Ringer's was no different from the value of 9.6 sec (SEM 0.5, 21 obs.) observed in the same group of animals while the vessels were normally perfused with blood.

It should be emphasized that the experimenter did not know the composition of the fluid being perfused and that the calculation of the results was done blindly.

In summary, the method of continuous perfusion of the peritubular capillaries is a valuable addition to the investigative arsenal of single nephron function. It has the advantage of circumventing systemic changes and of allowing a more accurate control of the peritubular environment. It has, like other techniques in micropuncture, its dangers and shortcomings. Most of them are, however, predictable and many avoidable, if careful work habits are pursued.

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