Evidence for Functional Intrarenal Heterogeneity Obtained by the Micropuncture Technique*

REX L. JAMISON†

Division of Nephrology, Department of Medicine, Stanford University
School of Medicine, Stanford, California 94305

Received for publication 19 November 1971

In this paper, I wish to consider evidence obtained by micropuncture, particularly of the juxtaglomerular nephron, supporting the concept of intrarenal functional nephron heterogeneity. Lassiter, Mylle, and Gottschalk(1) observed that, in young rats, the tip of the papilla protrudes sufficiently beyond the kidney to permit direct access to the papillary tubule segments by simple excision of the ureter. In 1968, Horster and Thurau, using the young rat preparation, demonstrated a striking difference between the filtration rate of the juxtaglomerular nephron and that of the superficial nephron(2). Anatomists, beginning with William Bowman(3), had observed that the glomerulus is larger and the proximal tubule and loop of Henle longer(4) in the juxtaglomerular nephron than in the superficial nephron in the kidneys of many mammalia.

Table 1 summarizes some micropuncture data obtained in our laboratory over the past 3 years on young rats weighing 50–85 g(5–7) (and unpublished data). We have studied two kinds of rats: normal Sprague–Dawley rats and the Brattleboro strain of Long–Evans hooded rats with hereditary hypothalamic diabetes insipidus, discovered by Valtin and Schroeder(8). The normal rats were infused with isotonic saline (Group I) or Tyrode’s solution (Groups II and III) at a rate of 0.03 ml/min; the Brattleboro rats were first infused with half isotonic Tyrode’s solution at a rate of 0.09 ml/min (water diuresis) and then administered isotonic

---

* This work was supported by NIH Grant HE 11806 and grants from Jewish Hospital of St. Louis and Stanford University Hospital.
† Recipient of Research Career Development Award HE 42-685 from the USPHS and Markle Scholar in Academic Medicine.
† The author acknowledges the invaluable help of Dr. John Buerkert, Norman Frey, Betty Henton, Frank Lacy, Robert Mairley, and Daniel Marcus in performing the experiments reported here.

254

Copyright © 1972 by Academic Press, Inc. All rights of reproduction in any form reserved.
Tyrode's solution containing antidiuretic hormone at a rate of 0.045 ml/min (antidiuresis). The fractional excretion of sodium in these rats averaged less than 1%.

Because at this age rats grow rapidly, we have divided the flow rates by the kidney weight to exclude weight as a factor in the comparisons. Although there is some variation in the mean SGFR values of the juxtamedullary and of the superficial nephrons among the groups studied, it is clear that there is consistent and significant difference in SGFR between the two kinds of nephrons.

Since the nephrons accessible to micropuncture are limited to those with tubules on the cortical surface or with loops of Henle reaching the papillary tip, the question arises whether either represents a significant fraction of the total nephron population. A clear cut answer is, unfortunately, not available. One approach to the problem is depicted in Table 2, which presents the sum of SGFR's in the left kidney compared to the total GFR of the right kidney. It was assumed that the kidney has 30,000 nephrons (4,9), of which one-third are juxtamedullary and two-thirds superficial (10). The agreement between the two columns is within 4–8% for Groups II, III, and IV, WD, but off by 21% in Group IV, AD. Calculations assuming a greater proportion of superficial nephrons will lessen the discrepancy for the last group, but impair the similarity for the other three.

Some of the important assumptions and technical problems entailed in this approach deserve to be examined. The calculations assume that the kidneys of rats this size contain their full complement of nephrons. Evidence bearing upon

### TABLE 1
**Summary of Glomerular Filtration Rates in Young Rats***

| Group      | N  | Juxtamedullary | Superficial | Total GFR |
|------------|----|----------------|-------------|-----------|
|            |    | SGFR           | V           | SGFR      |           |
| I          | 12 | 64.8           | 10.1        | 25.1      | —         |
|            |    | (5.99)         | (1.21)      | (3.95)    | —         |
| II         | 7  | 58.0           | 9.43        | 30.1      | 1.235     |
|            |    | (7.22)         | (2.10)      | (2.59)    | (.995)    |
| III        | 9  | 53.1           | 7.35        | 24.3      | 1.697     |
|            |    | (7.39)         | (0.47)      | (2.14)    | (1.05)    |
| IV, WD     | 10 | 62.1           | 19.0        | 29.7      | 1.142     |
|            |    | (7.01)         | (2.09)      | (2.31)    | (.037)    |
| IV, AD     | 10 | 50.9           | 10.3        | 35.4      | 1.011     |
|            |    | (4.72)         | (0.75)      | (1.63)    | (.058)    |

*Groups I–III, Sprague–Dawley rats in hydropenia (Refs. 7,9); Group IV, Brattleboro rats in water diuresis (WD) and vasopressin-induced antidiuresis (AD). N, number of animals for juxtamedullary nephron micropuncture. SGFR, single nephron glomerular filtration rate and V, tubule fluid flow rate, in nanoliters per minute per gram kidney wt. GFR, glomerular filtration rate in milliliters per minute per gram kidney wt. GFR not measured in Group I. Values are means ± SE.
this point is illustrated in Fig. 1. Using the India ink-injection technique of Damadian et al. (11), we counted nephrons in a series of normal rats whose body weight ranged between 40 and 200 g. The technique involves the injection of India ink into the renal artery \textit{in vivo} (12) and unavoidably increases the weight of the kidney artificially. Therefore, the figure compares the number of glomeruli counted (the scale on the left in Fig. 1) with the total body weight rather than directly with the kidney weight. In a second study in which India ink was not injected, kidney weights were compared with the respective body weights (the scale on the right in Fig. 1). The linearity of the latter relationship suggests

\begin{table}
\centering
\caption{Comparison of Sum of SGFR's of Left Kidney with Total GFR of Right Kidney in Young Rats}
\begin{tabular}{lll}
\hline
Group & Sum of SGFR's left kidney & Total GFR right kidney \\
II & 1.182 & 1.235 \\
III & 1.017 & 1.097 \\
IV, WD & 1.215 & 1.142 \\
IV, AD & 1.217 & 1.011 \\
\hline
\end{tabular}
\end{table}

\(^a\) Calculations based on assumption of 10,000 juxtamedullary and 20,000 superficial nephrons (10).
\(^b\) Milliliters per minute per gram kidney wt.
Groups defined in Table 1.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Number of glomeruli per kidney (scale on left, large symbols) as a function of body weight. Since body weight and kidney weight are linearly related (scale on right, small symbols), the total glomerular count is a function of kidney weight also.}
\end{figure}
FUNCTIONAL INTRARENAL HETEROGENEITY

It is reasonable to use body weight as an index of kidney weight(9). It would appear that within the weight range of 50-85 g, the kidneys of smaller rats do not contain the full complement of nephrons; the average number of nephrons per kidney in animals weighing between 50 and 85 g was 25,800 ± 3290 SD; these results are consistent with those from an earlier study by Arataki(9).

This analysis also assumes that only two functional populations of nephrons exist in the rat kidney. It may be that there are more than two: de Rouffignac and his associates(13) have delineated three groups, and there may be a continuum between the smallest and the largest nephron. However, there is some evidence favoring the choice of only two groups: Kriz(10) in a careful histologic study has shown that two thirds of the nephrons in the rat kidney have short loops, all of which turn within the outer medulla, and the remainder have long loops which penetrate the inner medulla.

Another assumption is that the nephrons whose loops are accessible at the papillary tip originate from glomeruli in the juxtamedullary region of the cortex. The only systematic investigation of this point I know of is that by LeChene et al.(14). Their work suggests that while this assumption is basically true, on rare occasions, glomeruli of the long loop nephrons may be located elsewhere than in the juxtamedullary region of the cortex.

Since the ureter is of necessity excised from the left kidney, a simultaneous comparison of the sum of SGFR in the left kidney with the total GFR could not be made. We assumed the GFR of the right kidney was equal to the GFR of the left kidney. This may seem risky since the left kidney is subjected to dissection and manipulation in preparation for micropuncture (see below) and the right kidney is not. In one study of five Brattleboro rats in which the left kidney was treated exactly as it is for micropuncture except that the ureter was left intact(6), we found reasonably good agreement between the total GFR of the left and right kidneys, as illustrated in Fig. 2.

Fig. 2. GFR of right kidney and left kidney of five Brattleboro rats in water diuresis and in vasopressin-induced antidiuresis. The left kidney was prepared for micropuncture; the right kidney was untouched. The mean difference ± SE between the two kidneys for each condition is depicted at the top of the figure.
Perhaps the most important set of assumptions is that our micropuncture techniques do not introduce a serious artifact into the SGFR determinations. At this point, I should like to review our techniques and describe how the exclusion of artifacts is attempted. Figure 3 is a schematic diagram of the left kidney ready for micropuncture of the papilla. Motion, particularly respiratory, is the biggest problem. The entire peritoneal reflection attaching the left kidney to the posterior abdominal wall is cut and, after excision of the ureter, the kidney is placed in a glass cup as shown. In the dissection, the ureteral vessels are ligated to minimize bleeding and to try to avoid traumatizing the inferior branch of the left renal artery, which usually crosses over the papilla as shown in the illustration. Occasionally, the branch constricts during dissection and the lower pole of the kidney becomes ischemic. If that happens, 2–3 μl of 2% procaine hydrochloride is applied to the branch. Usually the vessel promptly reopens and the inferior pole regains its normal appearance. If not, the preparation is discarded. There was no evidence that this variable affected the results: the procaine was applied more than 2 hr before micropuncture and inspection of the data revealed no detectable difference between experiments in which the drug was used and those in which it was not.

The position of the kidney is similar to the normal predissection in situ position, with the papilla pointing toward the midline. Others rotate the kidney so that the papilla points caudally(15) or approach the papilla from behind the kidney(2). The advantage of our technique is that minimum physical constraints are placed upon the kidney; a disadvantage is that limited access is provided for micropuncture, since the approach must be made from the other side of the animal. A second disadvantage is that a wide abdominal incision is required with displacement of the intestines cephalad and to the right. The exposed

---

**Fig. 3.** Left kidney of young rat prepared for micropuncture of papilla or cortex. The animal is supine; the view is from above. For further description, see text.
viscera are wrapped with sponges soaked in warm saline and covered with Saran Wrap (Dow) to minimize evaporation.

If this procedure is followed properly, the countercurrent circulation in the papilla appears to be vigorous, rarely is stasis seen in any capillary, and after the intravenous injection of lissamine green, the vasa recta will blush 3–4 sec after the cortical vessels blush, the loops of Henle will fill transiently approximately 30 sec later, and the collecting ducts will fill between 1 and 1½ min later and empty shortly thereafter.

The loops are narrower in the young rat kidney than in the adult kidney; their diameter is between 10 and 15 μ. We use a sharpened pipet with a rapidly tapering shank and a tip diameter between 5 and 6 μ. Smaller tips may clog or not permit tubule fluid to enter fast enough to prevent a drop of oil injected into a loop from flowing downstream: micropuncture with pipets having larger tips may cause a leak around the tip or transfix the tubule and create a fistula between the loop and an adjacent structure. The latter is either a vas rectum, in which case contamination of the loop fluid is revealed by red cells entering the pipet or by a sticky yellow sample, or it is a collecting tubule. Contamination by fluid from a collecting tubule is not always obvious. Table 3 presents five loop samples contaminated by fluid from an adjacent collecting tubule.(5). Horster and Thurau(2) suggested a criterion for detecting contamination of loop collections by collection of duct fluid. On a plot of the tubule fluid flow rate, V, against the TF/P inulin ratio, each datum should fall on or near the hyperbolic curve representing the product of TF/P inulin × V = juxtamedullary SGFR. If not, contamination by nonloop fluid is implied. In the five instances of clear-cut contamination of loop fluid by collecting duct fluid (Table 3), three would have been eliminated by this criterion. However, the SGFR of the other two collections was sufficiently near the SGFR curve so as not to justify their exclusion on this basis. Yet the high potassium concentration and the very low fraction of filtered sodium remaining (TF/P Na/inulin) resembled more closely the composition of the collecting duct fluid than that of the loop fluid. In contrast, loop fluid TF/P K averaged 9.6 ± .79 and the fraction of filtered sodium

### Table 3

| No. | TF/P* inulin | V (nl/min/g kidney wt) | SGFR | Osm* (mOsm) | TF/P Na | TF/P Na/inulin | % Total osmolality due to NaCl* | TF/P K |
|-----|--------------|------------------------|------|-------------|---------|---------------|---------------------------------|--------|
| 1   | 91.6         | 27.76                  | 2543 | 900         | 1.3     | .01           | 39                              | >36    |
| 2   | 25.8         | 3.55                   | 91.6 | 580         | 0.64    | .03           | 31                              | 19     |
| 3   | 28.5         | 3.32                   | 94.6 | 711         | 0.52    | .02           | 21                              | 19     |
| 4   | 49.7         | 7.56                   | 376  | 767         | 0.77    | .02           | 29                              | 22     |
| 5   | 36.9         | 7.26                   | 268  | 935         | 1.1     | .03           | 35                              | 23     |

---

* TF/P, tubule fluid-to-plasma ratio.

* Osm, osmolality.

* (Na × 1.84) ÷ total osmolality, (1.84 = osmotic coefficient).
remaining was 41 ± 3% (N=42)(7). Moreover the percentage of total osmolality due to NaCl in loop fluid was always 50% or more, in contrast to the specimens in Table 3. Fortunately, such instances of collecting duct contamination did not occur often, but it is a hazard which is potentially more serious in papillary puncture than in puncture of the renal cortex.

The rate at which the tubule fluid is collected is another potential source of error. Our controls lack the precision of those employed recently in superficial SGFR measurements(16). It does not appear feasible to puncture the same loop elsewhere simultaneously to measure intratubule pressure during collection. Instead we compared the composition of fluid obtained by puncture in which the fluid was deliberately collected at less than the tubule fluid flow rate (an injected oil drop was permitted to flow downstream) with that of fluid collected at the tubule fluid flow rate (the oil drop was held stationary). The results are shown in Fig. 4. The agreement of the mean values between the two groups suggests that retrograde contamination by fluid downstream from the puncture site was unlikely, the similar values for TF/P inulin ratios being especially important(17).

The possibility of lowering the intratubular pressure and thus increasing net filtration pressure across the juxtamedullary glomerulus must also be considered. We have devised no direct tests to exclude this possibility, but three points militate against it. The first is that the principal resistance to flow within the

![Fig. 4. Composition of fluid in the loop of Henle collected at less than the intratubular flow rate (control) compared to that collected at rate equal to the intratubular flow (quantitative).](image-url)
juxtamedullary nephron, as in the superficial nephron(18), is very likely upstream to the site of puncture, namely, at the junction of the proximal tubule and the thin loop of Henle; according to the work of Brenner and Daugharty, as reported elsewhere in this journal, excessive suction during collection, even within the proximal tubule itself, does not artifically elevate SGFR (provided the puncture site is at least two convolutions downstream from the glomerulus). The second is the similarity in juxtamedullary SGFR within the Brattleboro rats and among the normal young rats despite variations in mean tube fluid flow rates from 7–19 nl/min/g kidney weight (Table 1). The third point which argues against too rapid fluid collection and for the reliability of our determinations is the similarity among the mean values for juxtamedullary SGFR obtained in three different laboratories, summarized in Table 4. The data were obtained from the studies of Horster and Thurau(2), of Stumpe, Lowitz, and Ochwadt(19), and from our own work.

In summary, I have attempted to indicate the assumptions and technical problems involved in micropuncture of the juxtamedullary nephron. Despite these limitations, there is substantial evidence that the filtration rate of a significant number of nephrons deep within the kidney of the young rat differs from that of nephrons lying near the surface. Other evidence suggests differences in tubule reabsorption between deep and superficial nephrons(5,7). It seems clear that the young rat kidney is not composed of a functionally homogeneous population of nephrons. Whether this holds true for mature rats(13) and for other mammals(12) remains to be established.

|       | Juxtamedullary SGFRa | Superficial SGFRa | Laboratory    |
|-------|----------------------|-------------------|---------------|
| 20    | 58.2                 | 23.5              | Horster and   |
|       | (2.8)                | (0.8)             | Thurau(6)     |
| 8     | 59.7                 | 30.5              | Stumpe et al.(17) |
|       | (3.6)                | (1.4)             |               |
| 38    | 60.1                 | 27.1              | Jamison and   |
|       | (3.4)                | (1.4)             | Lacy(7,9)     |

* Nanoliters per minute per gram kidney wt.; abbreviations as in Table 1; values are means ± SE.

REFERENCES

1. Lassiter, W. E., Mylle, M., and Gottschalk, C. W. Micropuncture study of urea transport in rat renal medulla. *Amer. J. Physiol.* 210, 965–970 (1965).

2. Horster, M., and Thurau, K. Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Arch. Ges. Physiol.* 301, 162–181 (1968).

3. Bowman, W. On the structure and use of the Malphighian bodies of the kidney, with observations on the circulation through that gland, in “The Collected Papers of Sir William
Bowman, Bart, FRS” (J. Burdon-Sanderson and J. W. Hulke, Ed.), Vol. 1, p. 59–84. Harrison and Son, London, 1892.

4. Sperber, I. Studies on the mammalian kidney. *Zool. Bidrag Fran Uppsala*, 22, 294–431 (1944).

5. Jamison, R. L. Micropuncture study of superficial and juxtamedullary nephrons in the rat. *Amer. J. Physiol.* 218, 46–55 (1970).

6. Jamison, R. L., Buerekert, J., and Lacy, F. A micropuncture study of collecting tubule function in rats with heredity diabetes insipidus. *J. Clin. Invest.* 50, 2444–2452 (1971).

7. Jamison, R. L., and Lacy, F. B. Effect of saline infusion on superficial and juxtamedullary nephrons in the rat. *Amer. J. Physiol.* 221, 690–697 (1971).

8. Valtin, H., and Schroeder, H. A. Familial diabetes insipidus in rats (Brattleboro strain). *Amer. J. Physiol.* 206, 427–430 (1964).

9. Arataki, M. On the postnatal growth of the kidney with special reference to the number and size of the glomeruli (albino rat). *Amer. J. Anat.* 36, 399–436 (1926).

10. Kriz, W. Der architektonische und funktionelle Aufbau der Rattenniere. *Z. Zellforsch.* 82, 495–535 (1967).

11. Damadian, R. V., Shwayri, E., and Bricker, N. S. On the existence of non-urine forming nephrons in the diseased kidney of the dog. *J. Lab. Clin. Med.* 65, 26–39 (1965).

12. Weisser, F., Lacy, F. B., Weber, H., and Jamison, R. L. Renal function in the chinchilla. *Amer. J. Physiol.* 219, 1706–1713 (1970).

13. Rouffignac, C. de, and Bonvalet, J. P. Étude chez le rat des variations du debit individuel de filtration glomerulaire des nephrons superficiels et profonds en fonction de l'apport sódé. *Arch. Ges. Physiol.* 317, 141–156 (1970).

14. LeChene, C., Corby, C., and Morel, F. Distribution des nephrons accessible à la surface du rein en fonction de la longueur de leur anse de Henle chez le Rat, le Hamster, le Merion et le Psammomys. *C.R. Acad. Sci.* 262, 1126–1129 (1966).

15. Marsh, Donald, J. Solute and water flows in thin limbs of Henle’s loop in the hamster kidney. *Amer. J. Physiol.* 218, 824–831 (1970).

16. Schnermann, J., Horster, M., and Levine, D. Z. The influence of sampling techniques on the micropuncture determination of tubules. *Arch. Ges. Physiol.* 309, 48–58 (1969).

17. Brenner, B. M., Keimowitz, R. I., Wright, F. S., and Berliner, R. W. An inhibitory effect of furosemide on sodium reabsorption by the proximal tubule of the rat nephron. *J. Clin. Invest.* 48, 290–300 (1969).

18. Gottschalk, C., and Mylle, M. Micropuncture study of pressures in proximal and distal tubules and peritubular capillaries of the rat kidney during osmotic diuresis. *Amer. J. Physiol.* 189, 323–328 (1957).

19. Stumpe, K. O., Hans-Dieter, L., and Ochwadt, B. Function of juxtamedullary nephrons in normotensive and chronically hypertensive rats. *Arch. Ges. Physiol.* 313, 43–52 (1969).