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# Compensatory Renal Hypertrophy in Dogs: Single Nephron Glomerular Filtration Rate

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Kidney weight, length of superficial and juxtamedullary proximal tubules, glomerular diameter, kidney filtration rate and PAH clearance, sodium excretion and intrarenal distribution of filtration (with $^{14}$C-ferrocyanide) were measured in the remaining hypertrophic kidneys of dogs 10 days after unilateral nephrectomy. Whereas kidney weight increased to 75 percent of the original total renal mass, proximal tubule length and mean glomerular diameter remained unchanged. PAH and creatinine clearance, and absolute, but not fractional, sodium excretion, rose significantly. The ratio superficial/juxtamedullary filtration rate remained unchanged, indicating parallel increases of filtration in both cortical regions of hypertrophied kidneys.

Removal of a normal kidney in adult mammals rapidly induces morphological changes in the remaining kidney [1,2,3,4]. Functional alterations such as increases in renal blood flow and glomerular filtration rate (GFR) have been observed in man [5,6,7,8,9,10] and in different animal species, particularly in the rat [11,12,13] and in the dog [14,15,16,17,18,19]. Blood flow has been shown to increase uniformly in all regions of the dog renal cortex [20]. The increase in total kidney GFR has not been further characterized as to the relative contribution of the outer cortical or superficial (SUP) and juxtamedullary (JM) glomeruli.

On the other hand, it is generally agreed that renal cell hypertrophy largely accounts for the compensatory renal growth of the remaining kidney [4,21,22,23,24,25]. The number of glomeruli and tubules appears to remain unchanged in adult kidneys during the compensatory growth process [3,26,27]. This, however, remains open to question in very young animals [28,29,30].

With the fixed number of glomeruli in adult animals, it becomes evident that the increased total GFR observed in the remaining kidney can only be attributed to an increase in single nephron GFR (SNGFR). This increase, however, could occur either uniformly in all glomeruli or through a redistribution of the glomerular filtration, favoring either the SUP or JM glomeruli.

The purpose of our study was to determine the ratio of SUP/JM glomerular filtration rate in hypertrophied kidneys.

## METHODS

Two-step experiments were done on mongrel dogs weighing between 7 and 12 kg.

### *Step 1*

The dogs were anesthetized with pentobarbital (30 mg/kg) and given additional doses as required during the experiment. The animals were intubated and allowed to



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breathe room air. Aseptic conditions were observed during this part of the experiment. A femoral artery was cannulated for the continuous monitoring of the arterial blood pressure. The femoral vein was also cannulated for the continuous infusion of 1 ml/min of 0.85 g percent saline. The right ureter was cannulated via a suprapubic incision. A catheter was placed in the bladder for the collection of urine from the left kidney. The right kidney was exposed through a flank incision.

Upon completion of the surgical preparation, priming doses of PAH and creatinine were injected through the femoral vein cannula followed by infusion of maintenance doses of these substances. After a 40 to 60 minute equilibration period, three 20-minute-control clearance periods were initiated. Subsequently, the right kidney was removed, frozen in a mixture of dry ice and acetone, weighed and prepared for microdissection by the method described in Step 2. The catheter and cannulae were removed from the bladder and the femoral artery and vein and all incisions were surgically closed.

The animal was allowed to recover until the second step of the procedure, ten days later.

*Step 2*

The dogs were anesthetized and again the femoral artery and vein were cannulated for the purposes previously described. A cannula was also placed in the left ureter through a suprapubic incision. The left kidney was exposed via a flank incision and loose ligatures were placed around its pedicle. A small polyvinyl catheter (I.D. 0.4 mm; O.D. 0.7 mm) was inserted in the aorta above the left renal artery, by the method of Herd and Barger [31]. Upon completion of the surgical preparation, the same procedures as in Step 1 were carried out. Once the three 20-minute clearance periods were completed, a 0.25 ml bolus containing 500$\mu$Ci $^{14}$C Na-ferrocyanide (specific activity 13.8 Ci/mM: New England Nuclear) plus cold sodium ferrocyanide to obtain a 15 percent solution was rapidly injected into the aortic polyvinyl catheter. Since the catheter dead space was only approximately 10$\mu$L, no flushing was required. Twelve seconds later, the ligatures around the renal pedicle were tightened and the kidney immediately removed and frozen in a dry ice-acetone mixture.

Slices approximately 2 mm thick were cut from the frozen kidneys and prepared for microdissection [32]. The slices were placed in an alcoholic ferric chloride solution previously cooled with dry ice and acetone and were kept in this solution at -18°C for 18 to 20 hours. They were then digested in a 20 percent HCl solution at 37°C for 7 hours, washed in deionized water and placed in a solution of 1 percent acetic acid and 0.2 percent ferric chloride. The proximal tubules were microdissected and transferred to plastic discs where the glomeruli and tubules were outlined with the aid of a camera lucida attached to the dissecting microscope. The largest diameter of the glomeruli was measured with a vernier. The length of the proximal tubule up to the thin segment of the loop of Henle was measured with an odometer. The tubules were then dried and the radioactivity (CPM) contained in the entire proximal tubule measured with a low coincidence, low background planchet counting system.

In order to prevent errors due to a non-homogenous distribution of ferrocyanide within the kidney and to obtain a representative sample of nephrons, five superficial and juxtamedullary glomeruli and tubules of the same pyramid from 7 sections of different regions of the kidney were microdissected. Only the most superficial and deepest glomeruli and attached proximal tubules were dissected and compared as to their radioactivity and morphological configuration [32]. PAH and creatinine were

measured with a Technicon auto-analyzer, sodium by flame photometry and osmolarity by a freezing point depression method.

## RESULTS

### *Morphological Data*

The weights of the dogs prior to Step 1 (8.9 ± 1.2 kg) and before Step 2 (8.6 ± 1.0 kg) did not differ significantly: the animals, thus, had adequately recovered after the first operation.

A marked difference between the weight of the right kidney (28.7 ± 2.8 g) initially removed and that of the left (42.6 ± 3.9 g) removed 10 days later was observed ($p < 0.001$). If the left and right renal masses were initially comparable [20] the weight of the left kidney, at that point, reached 75 percent of the original total renal mass.

As previously demonstrated in normal dogs [32], there was no significant difference between the lengths of SUP and JM proximal tubules in either kidney (Fig. 1). Moreover, the length of SUP and JM proximal tubules in the hypertrophied kidney did not notably differ from that of the excised right kidney.

Figure 2 shows that the mean diameter of SUP glomeruli was slightly smaller than that of JM glomeruli. However, no significant increase in the mean diameter of either group of glomeruli was observed in the hypertrophied kidney.

### *Functional Data*

During the first part of the experiment, on day 1, no significant difference of urine flow, $^{C}PAH$, creatinine clearance ($^{C}creat$) or filtration fraction (FF) between the right and left kidneys was observed (Table 1).

Ten days after right nephrectomy urine flow, $^{C}PAH$ and $^{C}creat$ from the remaining left kidney had significantly increased while FF remained unchanged (Table 1).

Table 2 indicates that absolute ($^{U}Na^{V}$) and fractional ($^{FE}Na$) sodium excretion from the right and left kidneys was comparable on day 1. Ten days after right

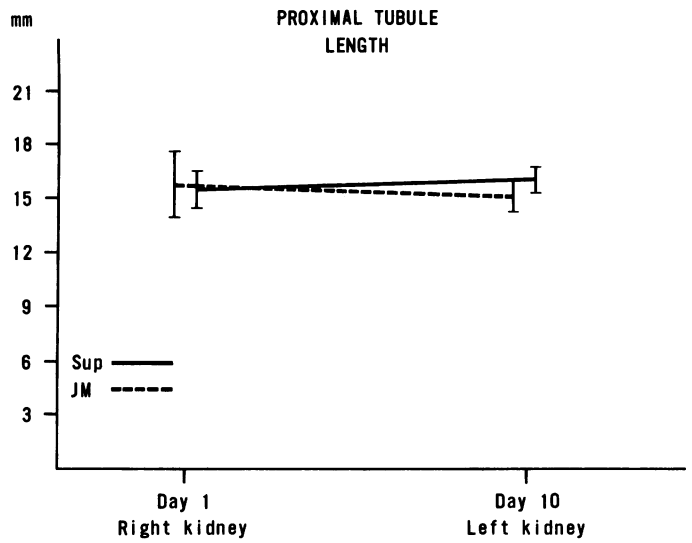


FIG. 1. *Length of superficial and juxtamedullary proximal tubules from normal dog kidneys: (Day 1), right kidney and hypertrophied (Day 10) left kidney.* The data are means ± SEM from 10 dogs (35 superficial and 35 juxtamedullary tubules were dissected and measured for each kidney).

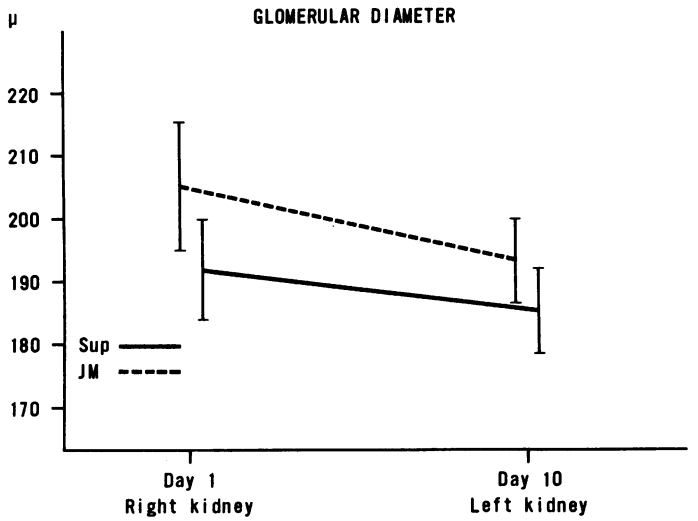


FIG. 2. *Superficial and juxtamedullary glomerular diameters in normal and in hypertrophied dog kidneys.* Explanations as for Fig. 1.

TABLE 1

*Overall functions of both normal kidneys, and of the remaining kidney ten days after unilateral nephrectomy.* Data are means from 10 dogs. The statistical significance of differences shown refers to paired comparisons of values for the left kidney before and after compensatory hypertrophy.

| | Day 1 | | | Day 10 |
|---|---|---|---|---|
| Kidney | Right | Left | | Left |
| V (ml/min) | 0.08 | 0.08 | $p < 0.001$ | 0.41 |
| $C_{PAH}$ (ml/min) | 45.0 | 43.1 | $p < 0.005$ | 60.2 |
| $C_{Creat}$ (ml/min) | 18.0 | 15.3 | $p < 0.01$ | 19.9 |
| ff (%) | 43 | 37 | NS | 35 |

TABLE 2

*Absolute and fractional sodium excretion from both normal kidney and from the remaining kidney ten days after unilateral nephrectomy.* Explanations as for Table 1.

| | Day 1 | | | Day 10 |
|---|---|---|---|---|
| Kidney | Right | Left | | Left |
| $U_{Na}V$ (μEq/min) | 18.1 | 27.2 | $p < 0.05$ | 55.0 |
| $FE_{Na}$ (%) | 0.73 | 1.22 | NS | 1.97 |

TABLE 3

*Ratio of superficial to juxtamedullary glomerular filtration rate in hypertrophied kidneys 10 days after contralateral nephrectomy as compared to the ratio previously [32] observed in normal dog kidneys.* Data are means ± SEM.

| RATIO FOR CPM IN SUP/JM PROXIMAL TUBULES | | |
|---|---|---|
| Control dogs (6) | | Uninephrectomized dogs (9) |
| 0.80 ± 0.03 | NS | 0.90 ± 0.08 |

nephrectomy, $^{U}Na^{V}$ from the left kidney was significantly increased, whereas $^{FE}Na$ had remained unchanged.

The mean ratio of the radioactivity (CPM) measured in the SUP in comparison to the JM nephrons in the left hypertrophied kidney was 0.90 (Table 3). This value was slightly higher but not significantly different from that previously observed in dogs with two intact kidneys [32].

## DISCUSSION

The increase of kidney weight observed in the present experiments was similar to that found by McNay and Miyazaki [20], under comparable conditions. The total increase expected, thus, occurred within 10 days. It was proposed by Arataki in 1926 [3] that the compensatory growth of the kidney occurs through hypertrophy of existing nephrons. This view is still largely supported, even by experiments performed in very young animals [30]. The present study does not broaden the scope of our knowledge on this matter. Indeed, the diameter of the glomeruli and the length of proximal tubules did not differ in hypertrophied versus intact kidneys. However, the maceration of the tubules prior to microdissection might prevent slight changes from being detected. The present method does not permit the evaluation of the diameter of the proximal tubule which has been reported to be increased under similar conditions in rats [11]. We did not attempt to measure the size of other kidney structures.

The functional changes observed were comparable to those reported in dogs under analogous conditions [15,17,19]. As stated by previous investigators [15], the increase in renal plasma flow and glomerular filtration rate observed in the remaining kidney after unilateral nephrectomy was not consistently proportional to the increase of renal mass. For unknown reasons, the blood pressure was slightly lower when the animals underwent anesthesia for the second procedure.

The validity of the sodium-ferrocyanide method for measuring the ratio of superficial to juxtamedullary glomerular filtration rate in dogs has been demonstrated previously [32]. The present results indicate that the increment in the total glomerular filtration rate of the remaining kidney after unilateral nephrectomy can be attributed to parallel increases in superficial and in juxtamedullary glomerular filtration rate. This conclusion concurs with that of Hayslett et al. [11], reached from micropuncture experiments in rats. Using a modified Hanssen ferrocyanide technique, the same group of investigators [11] found a disproportionate increase of

SNGFR in the juxtamedullary glomeruli during compensatory renal growth in rats, which, however, was induced by contralateral hydronephrosis [10].

The present results support the conclusion of McNay and Miyazaki [20], who demonstrated that the perfusion rate of the remaining dog kidney after unilateral nephrectomy increases proportionately in all regions of the cortex. Moreover, these studies on blood flow and on SNGFR distribution in the dog kidney indicate that, whatever the stimulus for compensatory hypertrophy may be, one must look for a factor that affects the cortex in its entirety.

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