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TECHNICAL NOTE

The course of the remnant kidney model in mice

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The course of the remnant kidney model in mice. The remnant kidney model was produced in mice by unilateral nephrectomy and partial infarction of the remaining kidney. Control mice underwent laparotomy only. The mice were studied for up to 44 weeks. No quantitative differences were noted in systolic arterial pressure, proteinuria, or histopathology between control mice and those with a remnant kidney. Glomerular enlargement occurred in the remnant kidney.

The remnant kidney model has been a mainstay of experimental studies of progressive renal disease. Investigators have usually produced this model by unilateral nephrectomy and either partial infarction or amputation of the poles of the remaining kidney [1–3]. Although a simple model of chronic renal disease, many of its features are common to other models and human disease. Also, experimental therapies explored in this model have, in several instances, proven clinically efficacious. The model has been applied mainly in various strains of rats, but has also been employed in baboons, dogs, cats, rabbits, and mice [4–17]. However, the cardinal features of this model (arterial hypertension, proteinuria, and glomerular sclerosis) have not been systematically and quantitatively examined in the mouse. In view of the burgeoning interest in murine physiology, occasioned in a large part by gene knockout and transgenic animals, we investigated the course of this model in a strain of mouse. We chose the inbred strain frequently employed for gene targeting studies: the C57BL/6.

C57BL/6J mice weighing 18 to 26 g and aged approximately four to five weeks were obtained from Charles River Laboratories (Omaha, NE, USA). They were anesthetized by 50 mg/100 g sodium pentabarbital, which was administered intraperitoneally. Preliminary studies with inactin or sodium methohexital (brevital) were complicated by deaths when sufficient drug was given to maintain adequate anesthesia. In experimental animals, the right kidney was removed, and one or two segmental branches of the left kidney were ligated with visible renal ischemia using 8-0 silk suture. The ablation was performed in one procedure. The abdomen was closed with 6-0 silk suture. Control animals underwent the same anesthesia and laparotomy but no ablation. Mice tolerated these procedures as well as rats have in our previous experience. Deaths intraoperatively or in the first two days postsurgery were very rare with pentabarbital anesthesia. The mice were fed standard rodent chow (Teklad, Madison, WI, USA).

Body weights, urine collections, and blood pressure measurements were made at intervals of 2 to 6 weeks following the surgical procedure for up to 44 weeks. Urine collections were obtained in individual cages in which the animals were placed for periods of between two and six hours between 8:30 a.m. and 2 p.m. Urine was measured for protein content by the Coomasie technique and creatinine concentration by the modified Jaffe reaction using a Beckman creatinine analyzer (Beckman Instruments, Fullerton, CA, USA). Plasma creatinine was measured with the same instrument. Systolic blood pressure was measured in awake mice using the tail-cuff method. At the conclusion of the period of observation, animals were again anesthetized with sodium pentabarbital, and their left kidneys were perfusion fixed via the aorta using formaldehyde. The hearts and left kidneys were weighed. The kidneys were imbedded in paraffin, and stained with hematoxylin and eosin. The prevalence of glomerular sclerosis was measured on these sections in a blinded manner. For each mouse, at least 100 glomerular profiles were assessed for the presence or absence of any sclerosis. The results are expressed in the percentage of glomeruli having any sclerotic component. The measurements of mean glomerular volume and percentage of interstitial volume were made as previously described in rats [18]. Data are presented as the mean ± SD. Statistically significant differences in values were determined by the analysis of variance test using the Bon-

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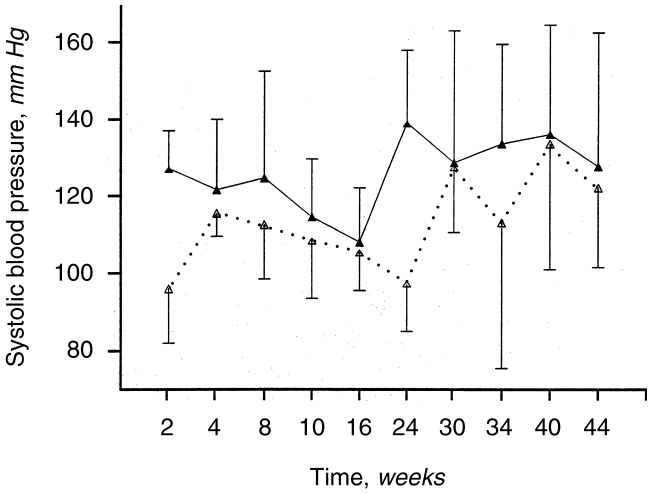

Fig. 1. Systolic blood pressure in control and experimental groups over 44 weeks. Symbols are: (▲) nephrectomized mice; (△) control mice.

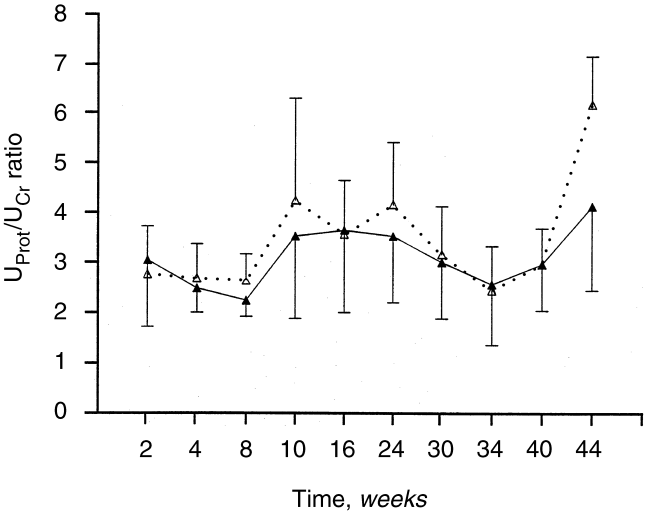

Fig. 2. Urinary protein to creatinine ratio in control and experimental groups over 44 weeks. Symbols are: (▲) nephrectomized mice; (△) control mice.

Table 1. Measurements at 44 weeks after subtotal nephrectomy (experimental) or sham procedure (control)

		Systolic BP *mm Hg*	U_{Prot}/U_{Cr}	Body Wt. *g*	P_{Cr} *mg/dl*	Left kidney Wt. *grams*	Heart Wt. *grams*	V_V interstitial %	Glom V $\mu m^3 \times 10^6$	Glomerulosclerosis %
Experimental	Mean	128	4.1	32	0.3	0.25	0.16	17	0.43[a]	12
($N = 14$)	SD	35	1.7	4	0.1	0.07	0.03	2	0.05	6
Control	Mean	122	6.2	35	0.2	0.24	0.15	18	0.29	7
($N = 6$–7)	SD	20	1.0	1	0.1	0.03	0.02	3	0.03	3

Abbreviations are: SD, standard deviation; U_{Prot}, urinary protein concentration; U_{Cr}, urinary creatinine concentration; P_{Cr}, plasma creatinine concentration; Wt, weight; V_V, interstitium, fractional interstitial volume; Glom V, glomerular volume.

[a] $P < 0.05$

ferroni modification. A P value of less than 0.05 was considered significant.

In the initial studies of six experimental and six control mice, two segmental arteries were ligated in the experimental group, with ischemia comprising an estimated 50 to 70% of the cortex by inspection. Three of the six experimental animals died by two-weeks from postablation, and the plasma creatinine in the three survivors averaged 0.85 mg/dl as opposed to an average of 0.24 mg/dl in the control group. By three weeks post-partial ablation, five of the six experimental animals had died. Thus, for the remaining studies, only one segmental artery was ligated in the experimental group, which resulted in visible ischemia of 20 to 30% by visual estimate.

Within the 22 experimental animals undergoing ligation of one renal artery and followed for at least 16 weeks, three died four days postsurgery and one at eight weeks. None of the controls died. Thus, in this experimental group, death from progressive renal failure did not occur, at least to an appreciable extent. At two weeks postsurgery, plasma creatinine in a subset averaged 0.4 ± 0.1 mg/dl ($N = 5$) compared with 0.2 ± 0.1 mg/dl ($N = 4$) in the control animals ($P < 0.03$). Systolic arterial pressure tended to increase over the course of the study (Fig. 1 and Table 1). However, no differences between the experimental and control mice were notable at any time. The urine protein to urine creatinine concentration ratios were also indistinguishable between the two groups during the entire period of study (Fig. 2 and Table 1). Mean serum creatinines at 44 weeks were not different between the groups being 0.3 ± 0.1 mg/dl ($N = 14$) in the experimental group versus 0.2 ± 0.1 mg/dl ($N = 7$) in the control group. Body weights increased gradually over the period of observation and were not consistently different in the two groups, although they tended to be less in the mice with remnant kidneys (Fig. 3 and Table 1). The left kidney and heart weights were also similar.

Examination of the renal tissue disclosed no readily apparent difference in degree of pathology. Areas of both acellular scar and mononuclear infiltrate were notable in the infarcted region. Discernible glomeruli were also present in the infarcts. This pattern was qualitatively similar to that seen in the infarcted regions in the rat

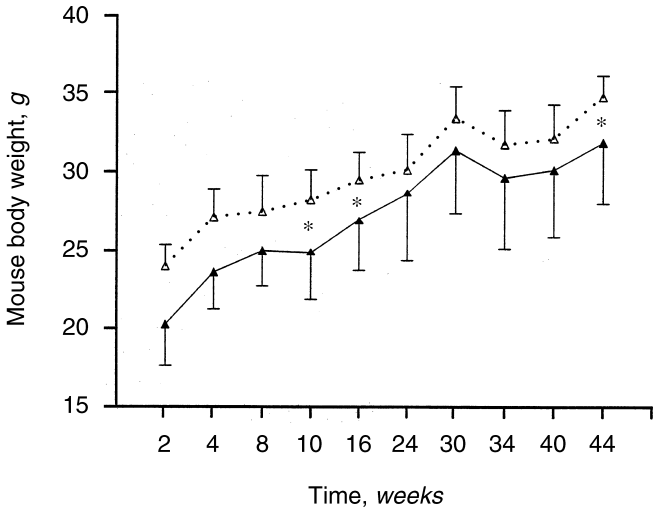

Fig. 3. Body weight in control and experimental groups over 44 weeks. Symbols are: (▲) nephrectomized mice; (△) control mice; *$P < 0.05$.

kidney. The percentage of glomerular sclerosis within the remnant tissue was 12 ± 6% in the experimental group and 7 ± 2% in the control kidneys ($P = 0.08$). Within the experimental group, no relationship could be discerned between the prevalence of sclerosis and systolic pressure, proteinuria, or glomerular enlargement. Fractional interstitial volume was similar at 17 ± 2% in the experimental group and 18 ± 3% in the control group, and interstitial inflammation was not seen. However, glomerular volume was increased in the experimental group at 0.43 ± 0.05 $\mu m^3 \times 10^6$ compared with 0.29 ± 0.03 $\mu m^3 \times 10^6$ in the control group ($P < 0.001$). Thus, interstitial disease in the remnant was absent, and glomerular lesions were slight; however, glomerular enlargement did occur after subtotal ablation.

Murine models of renal disease have been studied for numerous conditions, for example, cystic disease and lupus nephritis [19, 20]. Furthermore, remnant kidney models have been produced in the mouse as well [12–16]. The progressive features of the model (arterial hypertension, proteinuria, and glomerular sclerosis) have not previously been examined comprehensively with any method of partial ablation in this species.

In the earliest studies of mice with subtotal nephrectomy, Inglis and Halliday noted that mice with unilateral nephrectomy and surgical amputation of the poles of the remaining kidney developed what they termed adhesive glomerulitis and hyalinization in the remnant [12]. The sample glomerulus pictured in their publication appears to have sclerotic components as well as capsular adhesions. The quantitative degree of the lesion was not reported. Such lesions were also rarely seen in our experimental group, and they were present in only that group. Enlarged glomeruli were also noted in the subtotally nephrectomized mice of Inglis and Halliday, but the glomerular dimensions were not measured. In that report, the experimental mice did not develop arterial hypertension, and proteinuria was not assessed. More recently, several groups of investigators have induced renal ablation in the mouse by unilateral nephrectomy and electrocoagulation of the cortex of the remaining kidney [13–15]. In these studies, several features of renal insufficiency have been reported, including anemia, azotemia, and reduced somatic growth. However, arterial pressure, urinary protein excretion, and quantitative histology have not been studied with this model. In one study, histological examination was reported. In this instance, periglomerular fibrosis and focal glomerular sclerosis were noted involving an estimated 80 to 90% of the remaining glomeruli [15]. Other changes remarked on included interstitial fibrosis and dilation of the calyceal system. Near absence of histopathology in these studies may result in part from the difference in the methods for producing renal insufficiency. With electrocoagulation, it is conceivable that the residual tissue underlying the cortical burn was damaged to some extent by the ablative procedure. Thus, the presence of histopathology after electrocoagulation may not represent the progressive injury to initially normal renal tissue noted in the rat remnant kidney produced by partial infarction or amputation. Only one prior study has used the arterial ligation technique in mice [16]. Arterial pressure and proteinuria were not assessed, and qualitative histological findings seem modest. Specifically, they reported but did not quantitate mesangial hypercellularity and increased mesangial matrix. The glomeruli appeared enlarged, but no tubulointerstitial or vascular pathology was noted. In this study, pathological changes were very rare, although glomerular hypertrophy was substantial.

The absence in the mice of the major pathological components seen with the remnant kidney model in the rat is somewhat surprising. However, studies of subtotal ablation (achieved by nephrectomy and partial infarction) in other species have not always demonstrated the same degree or even type of abnormalities, as evidenced in most studies using rats. Indeed, in the rabbit, hypercalcemia, hypercalciuria, and urinary stones attend the ablation model and provide the dominant pathology [11]. The remnant kidney model in the dog yields glomerular sclerosis; however, proteinuria is modest, and arterial hypertension is inconsistently noted [5–8]. When imposed in the cat, the model resulted in all features seen in the rat hypertension, proteinuria, and glomerular sclerosis [9, 10]. Baboons with a remnant kidney manifest proteinuria and hypertension relative to baseline values, but the glomerular structure has not been examined [17]. Differences in the degree of ablation do not readily explain the differences between our results in the mouse and those in the rat because infarction of only one seg-

mental artery in rats leads to progressive hypertension, proteinuria, and an increased prevalence of glomerular sclerosis [21]. Thus, species differences in the response to renal ablation do seem to exist, although their bases are unknown.

Glomerular sclerosis can be induced in mice. For example, mineralocorticoid-salt hypertension leads to this lesion, at least when studied in B10.D2NSN mice [22]. This strain of mice is the usual control comparison strain to the B10.D2OSN strain, which is deficient in the fifth complement component, C5. We previously found progressive proteinuria over 30 weeks in this strain after subtotal ablation, but expressed in milligrams per 24 hours, the rates increased by only approximately threefold [23]. The C5-deficient strain also showed a modest but lesser proteinuric response to partial nephrectomy. The same relative resistance in the deficient strain with mineralocorticoid-salt hypertension was observed by Raij et al [22]. We did not in the previous studies of the remnant kidney in B10.D2NSO or OSN measure arterial pressure or examine histology. Perhaps the sufficient strain, B10.D2NSN, is more susceptible to hemodynamic injuries as judged by our earlier study and those of mineralocorticoid salt. However, in both those studies, the increase in proteinuria was modest compared with that found in rats with these experimental diseases, which is typically 5- to 10-fold greater than normal or baseline values.

Differences in the degree of expression of disease seem to exist between strains of mice. For example, the Os mutant gene (for oligosyndactyly) is associated with more glomerular sclerosis when residing in the ROP strain than when in the C57 strain [24]. However, the reason for this difference is unknown. No systematic studies have examined differences in responses to renal ablation between strains of mice. Thus, the two possibilities—that the mouse, as a species, resists hypertension and renal injury after partial ablation or that some strains are sensitive but as yet untested—both remain open.

Differences in response to renal ablation have been noted between rat strains. In at least one strain of rat, the resistance to injury after nephrectomy and partial infarction is associated with the absence of arterial hypertension [25]. This pattern of response was documented in this Wistar-Kyoto strain and parallels the pattern seen in the C55BL/6 mice in these studies, namely glomerular hypertrophy but no arterial hypertension or glomerular pathology. The cause for the resistance of the Wistar-Kyoto is unknown. Another strain of Wistar rat, the Wistar-Furth, also fails to develop renal injury after a reduction of renal mass (abstract; Fitzgibbon et al, *J Am Soc Nephrol* 8:614A, 1997). In this strain, hypertension developed to a level comparable to that in control Wistar rats, but structural damage and proteinuria were markedly attenuated. Relative resistance to aldosterone in the Wistar-Furth has been proposed, but not proven, as an underlying mechanism.

The absence of clear-cut hypertension is one of the more striking findings in this study. A numerical tendency to higher pressure was noted, but it was not statistically significant. Furthermore, the numerical increase was generally small and inconsistent. By contrast, rats with partial ablation regularly demonstrate systolic pressures of 30 or more mm Hg greater than controls [1–3]. However, arterial hypertension can be induced in the C57BL/6 mouse. Hypertensive models associated with direct perturbation of the renin-angiotensin-aldosterone system have produced two-kidney and one-kidney clip renovascular disease, mineralocorticoid excess, and insertion of excess copies of angiotensinogen transgenes [26–28]. The reason for the absence of hypertension after partial renal ablation is unknown.

In summary, the remnant kidney produced by nephrectomy and partial infarction in the C57BL/6 mouse yields compensatory growth but almost none of the characteristic pathophysiology of the model seen in various rat strains. Perhaps other strains of mouse will prove more susceptible to this model.

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