

# The Pathogenesis of Focal Glomerulosclerosis - Nonimmunologic Mechanisms of Glomerular Injury in Renal Ablation Model -

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**Summary.** The incidence of focal segmental sclerosis was studied in the two experimental models using two rat strains, Wistar and Nagase analbuminemia rats (NAR). In the Wistar rats, one week after left 2/3 kidney infarction, the right kidney was removed in Group 1 and the right ureter was ligated in Group 2. Glomerular hypertrophy and morphological changes such as epithelial reabsorption droplets and focal segmental sclerosis were observed in Group 1, but these changes were less severe in the rats of Group 2. In another experiment, the incidence of glomerular injury was studied in NAR which were characterized by analbuminemia and severe hyperlipidemia. Ten NAR underwent 5/6 nephrectomy, and were divided into two groups. Four rats were treated with captopril (500 mg/L in drinking water) and 6 rats were untreated. After 4 weeks, untreated NAR exhibited severe hypertension and moderate proteinuria, but captopril-treated NAR showed normotension and mild proteinuria. Morphological studies revealed that glomerular hypertrophy, massive reabsorption droplets in epithelial cells and segmental sclerosis developed in untreated NAR. Nevertheless, no pathological lesions were detected in captopril-treated animals, in spite of the fact that the degree of hyperlipidemia did not differ significantly between the two groups. These data suggest that focal segmental sclerosis was always preceded by glomerular hypertrophy, and hyperlipidemia did not play a crucial role in developing focal segmental sclerosis in renal ablation model.

## INTRODUCTION

The natural course of renal insufficiency usually pro-

gresses to end-stage renal failure, regardless of the original cause of the renal disease. This fact suggests that a reduction of nephron mass causes eventual damage to the remaining nephrons. A model of partial nephrectomy, in which one kidney and a portion of the other were removed, has been used to study the consequences of reduced nephron mass. In 1932, Chanutin and co-workers<sup>1)</sup> first used this model to study chronic renal insufficiency in detail. They found that removing three fourths of the renal mass in rats resulted in hypertension, proteinuria and uremia. Morphologically, they observed extensive glomerulosclerosis, arteriolar sclerosis, tubular atrophy and interstitial fibrosis in the remnant kidneys of these rats. Koletsky and Goodsitt<sup>2)</sup> also studied various organs of the rats between 7 to 10 months after the reduction of three fourths of the renal mass, and found glomerular injury, which was focal at first and eventually diffuse. These findings were confirmed by Shimamura and Morrison<sup>3)</sup> who studied the pathological sequences of glomerular injury for 10 to 50 weeks following renal ablation of approximately 85%. When examined after three months, the glomerular hypertrophy was accompanied by ultrastructural changes, including vacuolization of epithelial cells and "fusion" of foot processes. By 6 months, the expansion of the mesangium was noted along with the focal areas where the endothelial and epithelial cells were denuded from the glomerular basement membrane. Thus they found that the progressive mesangial expansion and the collapse of capillary lumina eventually resulted in the appearance of focal and segmental glomerular sclerosis in initially normal

remnant glomeruli. Physiologically, Bricker et al.<sup>4)</sup> had reported that the increase in the glomerular filtration rate (GFR) was observed in the remaining kidney as a result of functional adaptation. The hemodynamic mechanisms underlying this physiological response were studied in the Munich-Wistar rats utilizing a micropuncture technique. Hostetter et al.<sup>5)</sup> reported that the marked increase in single nephron GFR (SNGFR) was observed in the remaining kidney following extensive renal ablation. The elevation of SNGFR was brought about by a significant rise in the mean net ultrafiltration pressure ( $P_{UF}$ ), which resulted from a rise in the mean transcapillary hydraulic pressure difference ( $\Delta P$ ) and a reduction in the average transcapillary oncotic pressure difference ( $\Delta\pi$ ). These changes were the consequences of extensive dilatation of the afferent and efferent arterioles that results in marked increases in mean glomerular hydrostatic pressure ( $P_{GC}$ ) and initial glomerular plasma flow rate ( $Q_A$ ). From these observations, it has been suggested that these hemodynamic changes in the remnant glomeruli following the reduction in nephron mass may cause progressive glomerular injury. Although extensive research was carried out to clarify the underlying mechanism that leads to the progression of glomerular damage, the relevant mechanism is still obscure.

In this paper, we will present the data from experiments that were intended to explain the mechanism of how the glomerulosclerosis occurs and discuss a couple of factors recently proposed as having important roles in pathogenesis of focal segmental sclerosis in the renal ablation model.

## MATERIALS AND METHODS

### Experiment 1

Studies were performed in three groups of male Wistar rats (purchased from Saitama Laboratory Animal) weighing 150 to 180 g.

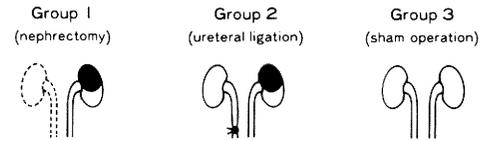
Group 1: Four rats underwent infarction of approximately two thirds of the left kidney by ligation of two or three extrarenal branches of the main renal artery. One week later, the right kidney underwent nephrectomy.

Group 2: As with group 1, four rats underwent left kidney infarction. One week later, the right ureter was ligated.

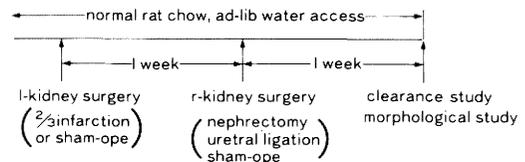
Group 3: Three rats served as a control group and underwent laparotomy and sham left renal ablation by the manipulation of renal pedicles but without

1. Animal: Wistar male rat (150-160g)

2. Rat surgery:



3. Experimental Schedule:



4. Clearance study:

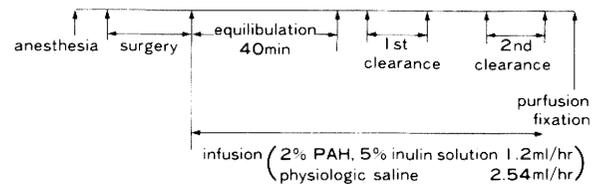


Fig. 1. Experimental design of Experiment 1.

destruction of renal tissue. One week later, right renal ablations were carried out with the same procedure. Rats of all groups were allowed free access to water and a standard rat chow (CE2, CLEA JAPAN INC.) before and after surgery. Functional and morphological studies were performed one week after the last surgery. The experimental design is illustrated in Figure 1.

### Experiment 2

Experiments were performed on female NAR weighing 200 to 250 g. All rats were fed *ad libitum* with a standard rat chow.

Group 1: Three rats underwent sham renal ablation and served as a sham operation group.

Group 2: Six rats underwent right nephrectomy followed by infarction of approximately two thirds of the left kidney as with Group 1 rats of Experiment 1. Group 1 (3 rats) and group 2 (6 rats) were allowed free access to water.

Group 3: Four rats underwent renal ablation as with Group 2. They were treated with angiotensin I converting enzyme inhibitor, captopril (Sankyo Co. Lmit. Tokyo), in a dose of 500 mg/L in drinking water. Administration of captopril was started the day after nephrectomy.

Four weeks after the renal surgery, functional and

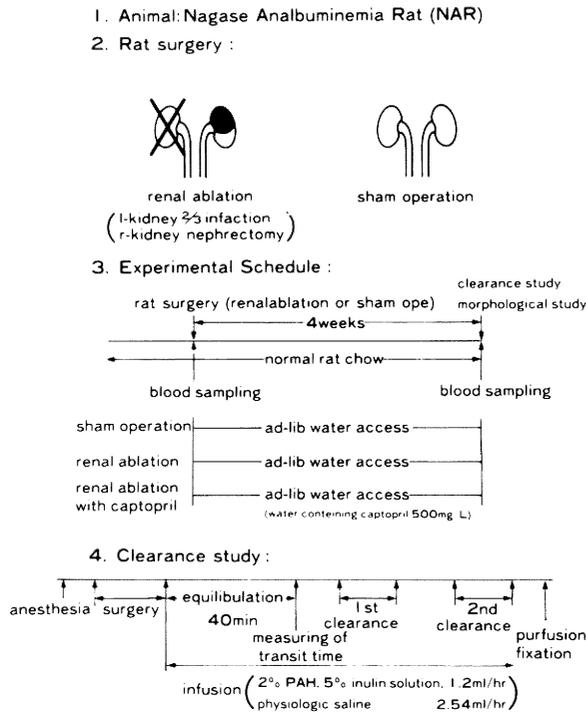


Fig. 2. Experimental design of Experiment 2.

morphological studies were performed. Along with renal surgery and clearance study, one milliliter of blood was collected from each animal by right jugular vein puncture. The experimental protocol is illustrated in Figure 2.

#### Clearance study

In both Experiment 1 and 2, a clearance study was performed. The rat was intraperitoneally anesthetized with pentobarbital, 20–40 mg/kg, and placed on an operating table. After tracheostomy, two polyethylene catheters (PE50) were inserted in the left jugular vein for the infusion lines. The right femoral artery was cannulated with a PE 50 catheter which was connected to the pressure transducer (PSA-101, Star Medical) and blood pressure was recorded. The left ureter was cannulated with a PE 10 catheter. Forty minutes prior to the clearance period, the solution containing 5% (v/v) inulin and 2% (v/v) paraaminohippuric acid (PAH) was given as a bolus of 0.5 ml, followed by a constant infusion at a rate of 1.2 ml/hr. Physiologic saline was infused from another line at a rate of 2.45 ml/hr. The clearance study was performed at two consecutive periods of 15 minutes, and the urine was collected from the left kidney during the clearance periods. Arterial blood was obtained at the

beginning and at the end of the clearance period. Hematocrit was measured in arterial blood samples. The concentration of inulin in the urine and plasma samples was measured by the Anthrone's colorimetric assay, and GFR was calculated according to the standard formulas.

Morphologic studies: As soon as the second clearance study was finished, the kidney was fixed by perfusion at the measured blood pressure with 0.1 M cacodylate buffer (pH 7.4) containing 1.5% (v/v) glutaraldehyde. The renal tissue was postfixed in 10% (v/v) buffered formaldehyde solution, and processed for light microscopy. The sections were stained with hematoxylin-eosin (HE), periodic acid Schiff (PAS), and Masson's trichrome stains.

The incidence of glomeruli showing glomerular sclerosis was determined by examining all glomeruli in each tissue section using light microscopy. The glomerular sclerosis was defined as the global or segmental areas where capillary lumens had collapsed, and these areas were often, but not always, associated with adhesions to Bowman's capsule. The number of glomeruli with the massive reabsorption droplets in the epithelial cells were counted. The incidence of the glomeruli which contained these lesions was given as the percentage of total number of glomeruli.

The glomerular size was estimated by point counting method using ocular lens equipped with 126-point mesh. The number of points in the area of individual glomerulus were counted at a magnification of  $\times 200$ . Thirty to forty glomeruli which swelled roundly and contained well-preserved glomerular components were counted randomly, and were listed in order according to the size. Ten larger glomeruli were picked up from them, and the glomerular sizes were expressed as mean  $\pm$  SD. The glomerular size of superficial glomeruli and juxtamedullary glomeruli were estimated separately in each section.

For electron microscopy, the tissue was rinsed in buffer, postfixed in 2% osmium tetroxide, dehydrated and embedded in epon 812. The thick sections (1  $\mu$ ) were stained with 0.5% toluidine blue in aqueous borax. The thin sections were stained with uranyl acetate and lead acetate, and examined by Hitachi H-7000 electron microscope at 75 kV.

#### Laboratory data

In experiment 2, serum level of blood urea nitrogen (BUN), total cholesterol, total protein and HDL-cholesterol were measured. The methods used for measuring each item are listed in Table 1.

**Table 1.** Methods of laboratory measurements and tissue processing

<b>Laboratory measurement</b>	
Inulin; Anthrone method	
PAH; 1-( $\beta$ -dethylaminoethyl)- $\alpha$ -naphtholamine	
Plasma Protein concentration; Buret method.	
Urine Protein concentration; Commassie Brilliant Blue binding assay	
BUN; OPA-amid method,	
T. Chol; COE • COD • POD method	
HDL-Chol; Enzymatic assay (dextran sulfate-Mg)	
<b>Tissue processing</b>	
LM; Fixation: 10% formaldehyde	
Staining:	
HE, PAS and Masson trichrome stain.	
EM; Fixation: 1.5% GA in 0.1 M cacodylate buffer.	
Post-fixation: 2% Osium tetroxide or reduced osmium.	
Staining: Uranyl acetate and Lead acetate	

*Statistics*

All data were given as mean  $\pm$  SD. Differences between groups were examined using paired Student's t-test.

**RESULTS****Experiment 1**

*Laboratory data:* Body weight, mean arterial pressure, hematocrit, urinary protein excretion, urine volume and results of inulin clearance are given in Table 2. Body weight and hematocrit did not differ among the three groups. Group 1 (nephrectomy) and Group 2 (ureteral ligation) developed hypertension, proteinuria and polyuria one week after reduction in functional nephron mass. Mean arterial pressure and urinary protein excretion were significantly greater in Group 1 than in Group 3 (sham operation) ( $P < 0.01$ ). In Group 2, although these parameters were greater

**Table 2.** Laboratory data of Experiment 1

	Body weight (g)	m-Blood pressure (mmHg)	Ht (%)	U. prot. (mg/day)	U.vol. (ml/day)	C <sub>IN</sub> (ml/min)	C <sub>PAH</sub> (ml/min)
Group 1 (n=4) (nephrectomy)	171.3 $\pm$ 14.9	163.0 $\pm$ 16.9*	52.3 $\pm$ 3.6	26.4 $\pm$ 16.8*	32.1 $\pm$ 8.0*	0.32 $\pm$ 0.11*	1.18 $\pm$ 0.46
Group 2 (n=4) (u. ligation)	173.8 $\pm$ 7.5	149.1 $\pm$ 38.0	51.4 $\pm$ 1.5	11.6 $\pm$ 6.8	27.9 $\pm$ 7.6*	0.29 $\pm$ 0.03*	1.27 $\pm$ 0.33
Group 3 (n=3) (sham-ope)	176.0 $\pm$ 5.3	121.0 $\pm$ 4.6	53.3 $\pm$ 0.6	4.8 $\pm$ 4.3	5.1 $\pm$ 1.8	0.63 $\pm$ 0.05	2.37 $\pm$ 0.78

The values are mean  $\pm$  SD. Abbreviations are: m-Blood pressure; mean arterial pressure, Ht; arterial hematocrit, U. prot.; urine protein excretion, U. Vol.; urine volume, C<sub>IN</sub>; inulin clearance rate, C<sub>PAH</sub>; paraaminohyprate clearance rate.

\*  $P < 0.01$  Group 1 vs Group 3. +  $P < 0.01$  Group 2 vs Group 3.

**Table 3.** Kidney weight and histological data in Experiment 1

	Kidney weight (g)	Glomerular size (points)		massive epithelial deposit(%)	FGS like lesion (%)
		superficial	juxtamedullary		
Group 1 (n=4) (nephrectomy)	1.49 $\pm$ 0.31*	29.06 $\pm$ 4.67*	31.69 $\pm$ 5.10*	42.0 $\pm$ 25.8*	5.4 $\pm$ 5.7
Group 2 (n=4) (u. ligation)	0.76 $\pm$ 0.11	23.47 $\pm$ 2.51	28.38 $\pm$ 1.91+	23.4 $\pm$ 13.4+	0.65 $\pm$ 1.3+
Group 3 (n=3) (sham-ope)	0.69 $\pm$ 0.03	18.40 $\pm$ 1.09	21.29 $\pm$ 1.02	0	0

The values are mean  $\pm$  SD. Glomerular sizes were estimated by point counting method and given number of points.

\*  $P < 0.01$ , Group 1 vs Group 2. +  $P < 0.01$ , Group 2 vs Group 3.

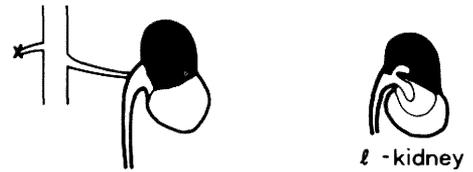
※  $P < 0.01$ , Group 1 vs Group 3.

than those of Group 3, there was no statistical difference. Urine volume of Group 1 and 2 increased approximately threefold that of Group 3.

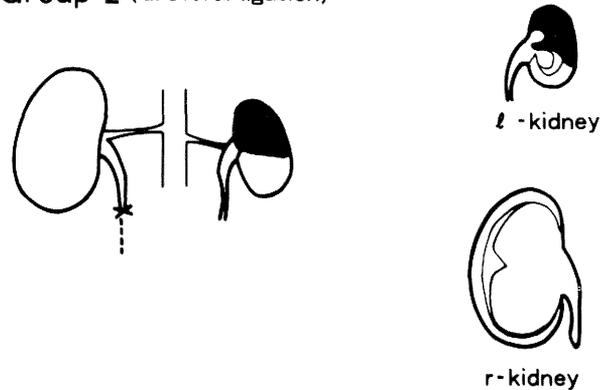
In all rats of Group 1 and 2, left kidney GFR, as measured by inulin clearance, decreased significantly to below the average value of  $0.63 \pm 0.05$  ml/min (mean  $\pm$  SD) seen in Group 3.

**Morphological Data:** In Group 2, the ureteral ligated right kidney enlarged due to hydronephrosis. Gross appearance of the kidneys is illustrated in Figure 3. The remaining left kidney of Group 1, enlarged more than that of Group 2. The left kidney weight is shown in Table 3. Increased left kidney weight was found in Group 1 when compared to Group 2 and 3. In Group 1, light microscopic findings of the remnant kidney showed slight increase of mesangial matrix and protein reabsorption droplets of glomerular epithelial cells. Occasionally the bleb-like formation in podocytes were noted (Fig. 4). Some glomeruli demonstrated segmental capillary collapse, with or without adhesions of the tuft to Bowman's capsules (Fig. 5). Focal interstitial lesions including round cell infiltration and fibrosis were present. Some tubules were dilated and contained hyaline casts. At the ultrastructural level, the glomerular visceral epithelial cells showed marked degenerative changes. Such alterations include focal fusions of foot processes, multiple osmiophilic bodies and coarse vacuoles of the cytoplasm and formation of cytoplasmic blebs (Fig. 6). In Group 2, these changes were less promi-

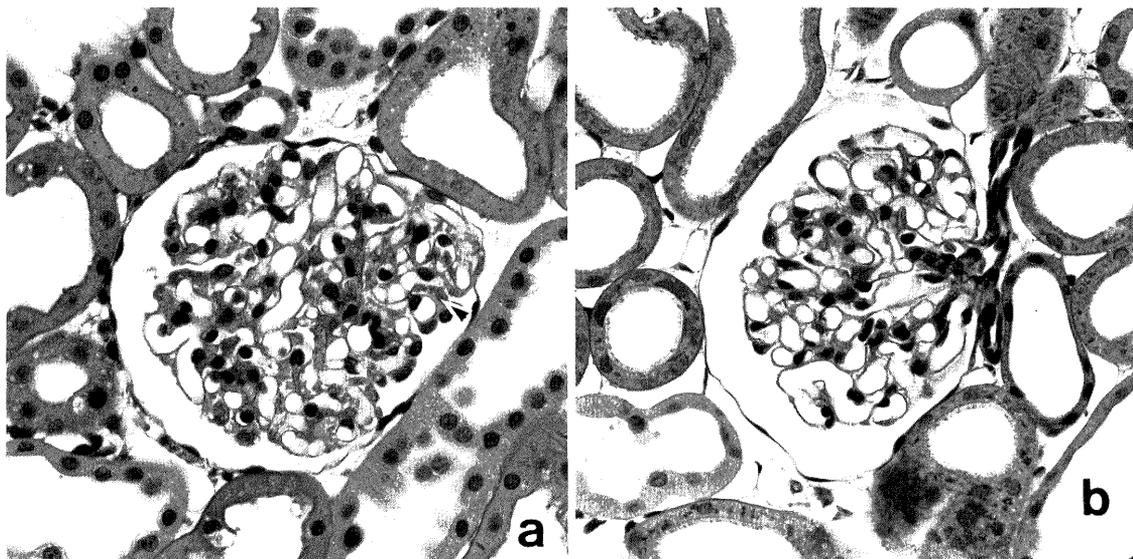
**Group 1 (nephrectomy)**



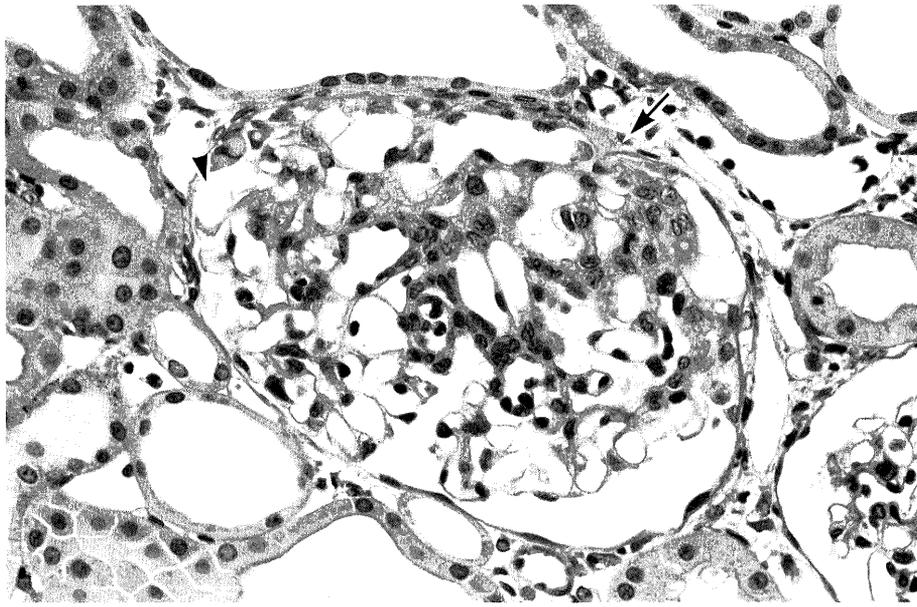
**Group 2 (ureteral ligation)**



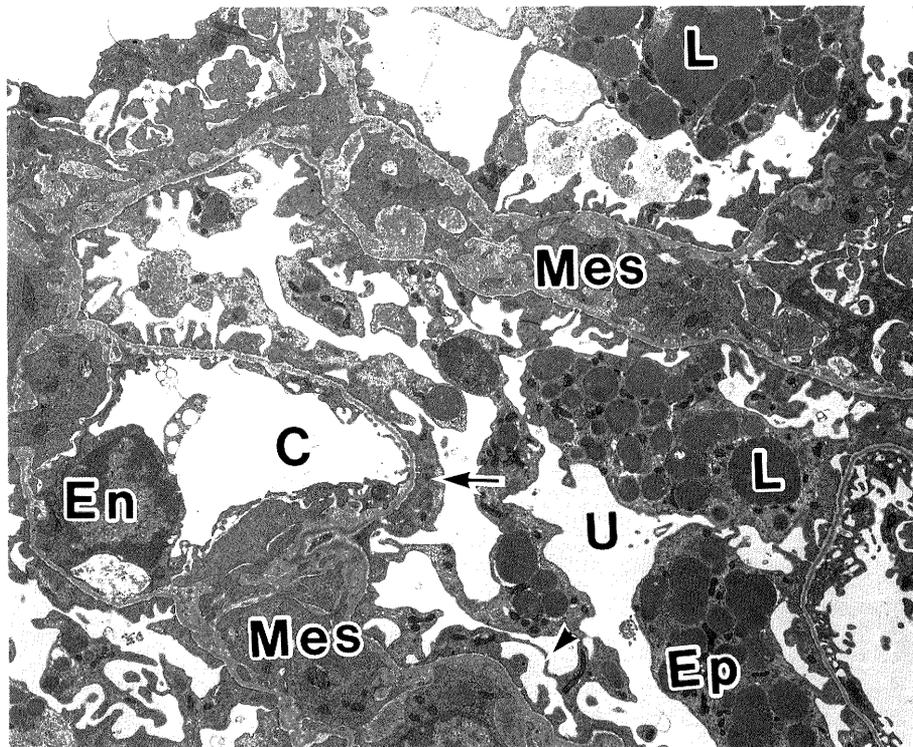
**Fig. 3.** Gross appearance of the kidneys in Experiment 1. The shaded areas indicate fibrotic tissue after renal infarction. The right kidney in Group 2 shows hydronephrosis with thinning renal tissue. The lower part of the left kidney is larger in Group 1 than in Group 2.



**Fig. 4.** Light micrograph of representative glomeruli from Experiment 1. **a;** Glomerulua from Group 1. Glomerular size is slightly enlarged. Epithelial reabsorption droplets (arrowhead) and mesangial matrix expansion are noted (PAS  $\times$  200). **b;** Glomerulus from Group 2. Glomerular size is smaller than that of Group 1 (PAS  $\times$  200).



**Fig. 5.** Glomerulus with segmental sclerosis from Group 1 animal. Pathological changes are: adhesion to Bowman capsule (arrow), degenerative change of epithelial cells (arrowhead) and mesangial matrix expansion (PAS×400).



**Fig. 6.** Transmission electron micrograph of representative glomerulus from Group 1 animal. There are mesangial matrix expansion and degenerative changes in epithelial cells. The epithelial cells show prominent lysosomes (L), focal fusion of foot processes (arrow) and attenuation of cytoplasm (arrowhead) (×2400). Abbreviations are: C; capillary lumen, U; urinary space, Mes; mesangium, Ep; epithelial cell, En; endothelial cell.

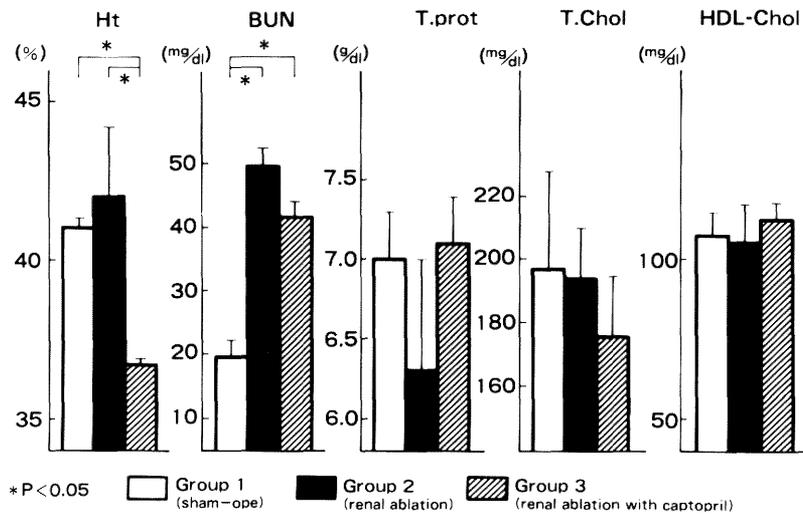


Fig. 7. Laboratory findings in Experiment 2. Abbreviations are: Ht; arterial hematocrit, BUN; blood urea nitrogen, T. prot; total protein, T. Chol; total cholesterol, HDL-Chol; HDL cholesterol. \* P < 0.05.

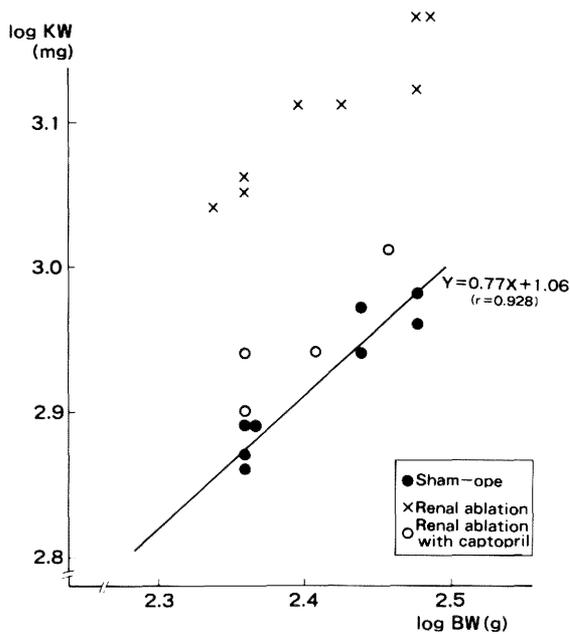


Fig. 8. Relationship between kidney weight and body weight in Experiment 2. The close correlation between log kidney weight and log body weight is observed in sham-operated animals. The data of an additional one sham-operated rat and two renal-ablated NAR rats are included in this figure.

ment, and there was no pathological abnormalities in the kidney of Group 3.

The size of glomerulus that was semi-quantitatively estimated by a point-counting method, the incidence of segmental sclerosis and incidence of massive reabsorption droplets in the epithelial cells are given in Table 3. The glomerular size of either superficial and juxtamedullary glomeruli was significantly larger in Group 1 animals compared to Group 3. The size of superficial glomeruli was slightly larger in Group 2, but there was no statistical significance compared to Group 3. Though the size of juxtamedullary glomeruli of Group 2 was larger than that of Group 3, it was smaller than that of Group 1 (P < 0.01, Group 2 vs Group 3). Although there was no difference in the renal function of the remnant kidney between Group 1 and 2, the incidence of glomeruli which contained either massive epithelial droplets and segmental sclerosis was higher in Group 1 than Group 2.

### Experiment 2

*Laboratory data:* The data of blood examination are shown in Figure 7. Hematocrit in captopril-treated animals (Group 3) was decreased compared to Group 1 and 2. BUN was increased in renal ablation rats treated with or without captopril. Though total protein concentration was slightly decreased in renal ablation rats that were not treated with captopril (Group 2), there was no statistical significance. Total cholesterol level and HDL cholesterol level were similar in these three groups.

Body weight, mean arterial pressure, urinary protein excretion, urine volume and inulin clearance rate are given in Table 4. Body weight did not differ among the three groups. Mean arterial pressure was significantly elevated in the renal ablation rats which were not receiving captopril, but it remained normotensive in captopril-treated animals. Urinary protein excretion was about sevenfold higher in the renal ablation animals which were not given captopril than in the sham-operated animals. In captopril-treated animals, urinary protein excretion was twofold higher than that of sham-operated animals. Urine volume was approximately twofold greater in untreated renal ablation rats than that seen in sham-

operated and treated animals. GFR measured by inulin clearance was reduced in renal ablated Group 2 and 3, though it was slightly higher in captopril treated Group 3 rats than untreated Group 2.

Morphologic data: Kidney weight, glomerular size and incidence of glomeruli containing massive epithelial droplets and presenting segmental sclerosis are given in Table 5. Left kidney weight was higher by approximately 50% in Group 2 compared to sham-operated animals ( $1.20 \pm 0.10$  g vs.  $0.79 \pm 0.10$  g), but remained nearly the same as that of rats in the control group (Group 3) ( $0.88 \pm 0.09$  g). Figure 8 shows the relationship between kidney weight and body weight. When log kidney weight was plotted against log body

**Table 4.** Laboratory data of Experiment 2

	B. W. (g)	m-B.P. (mmHg)	U. prot. (mg/day)	U. vol. (ml/day)	C <sub>IN</sub> (ml/min)
sham-ope (n=3)	253.3±40.4	122.0±12	5.6±2.5	19.2±4.3	0.75±0.11
renal ablation (n=6)	242.5±22.2	168.0±9*	37.3±7.8*	37.4±15.8*	0.31±0.09*
renal ablation with captopril (n=4)	252.5±28.7	120.0±3 <sup>+</sup>	10.9±1.6 <sup>+</sup>	23.0±4.3	0.59±0.08 <sup>+</sup>

\* P<0.01 sham-ope vs renal ablation

+ P<0.01 renal ablation vs renal ablation with captopril

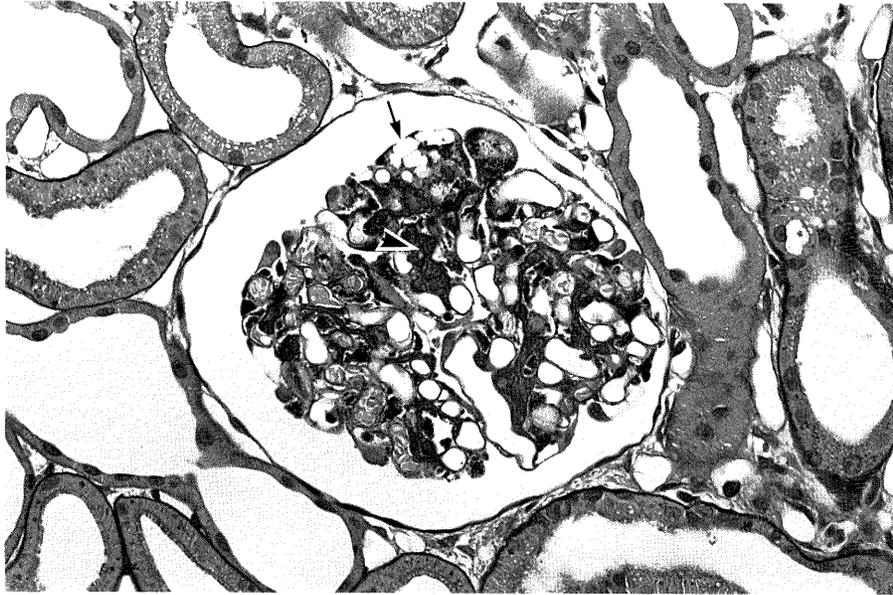
The values are mean ± SD. Abbreviations are: B. W., body weight; m-B.P., mean arterial pressure; U. prot, urine protein excretion; U. vol; urine volume; C<sub>IN</sub>, inulin clearance rate.



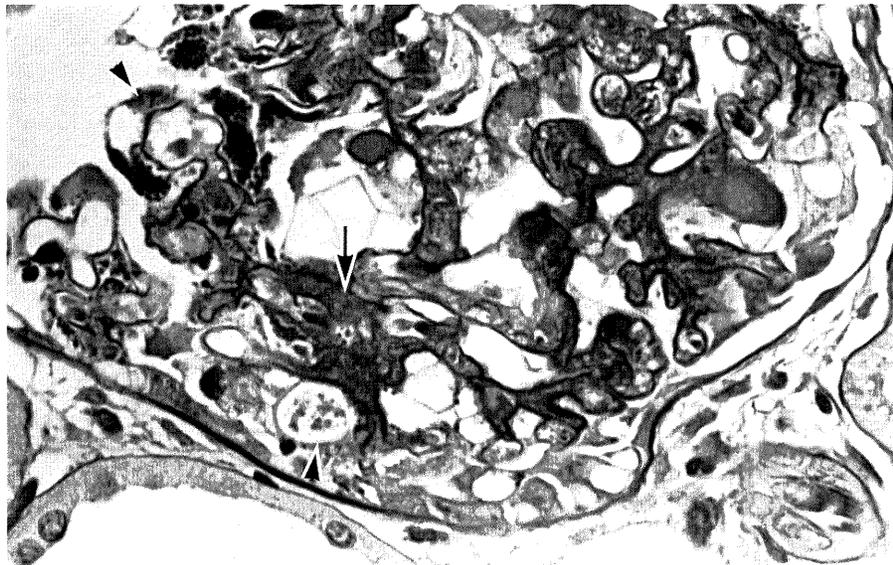
**Fig. 9** Light micrograph of the glomerulus from sham-operated Group 1 animal. No pathological lesion is observed. Note well-preserved glomerular component (Masson×200).

weight, the close correlation between kidney weight and body weight was observed in Group 1 sham-operated animals (closed circles). The regression equation for this Group was  $Y=0.77x+1.058$ ,  $r=0.928$ . The plots were shifted upward from this line in Group 2 (cross marks), and remained near this line in

Group 3 (open circles). Light microscopic findings of representative glomerulus from Group 1 are shown in Figure 9. There were no pathological lesions and the glomerular components were well preserved in this group. In Group 2, the glomerular size was slightly enlarged. The pathological lesions, such as marked



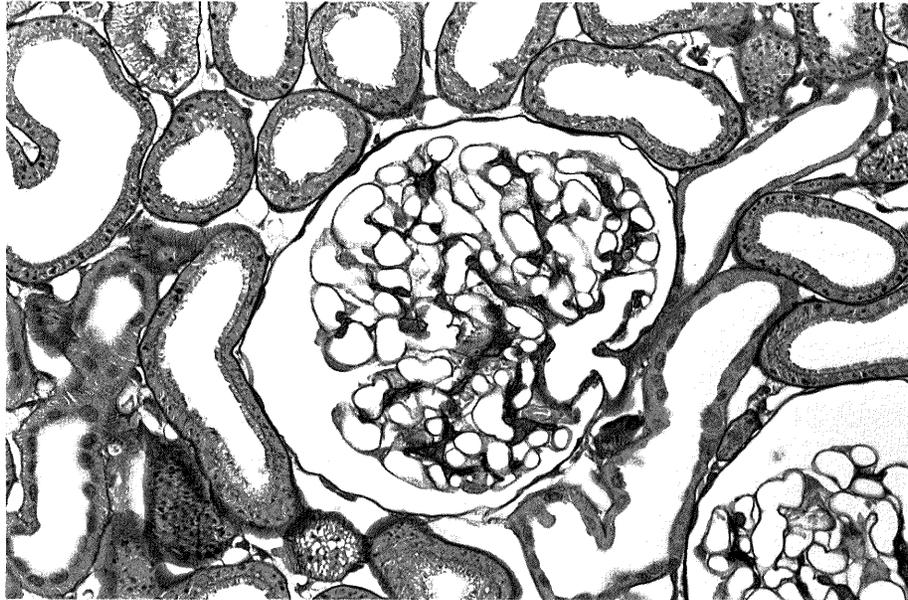
**Fig. 10** Light micrograph of the glomerulus from renal-ablated Group 2 rat. Glomerular size increased. Note an expansion of mesangium (arrowhead) and degenerative epithelial cells. These changes are massive reabsorption droplets and vacuolization in cytoplasm (arrow) (Masson  $\times 200$ ).



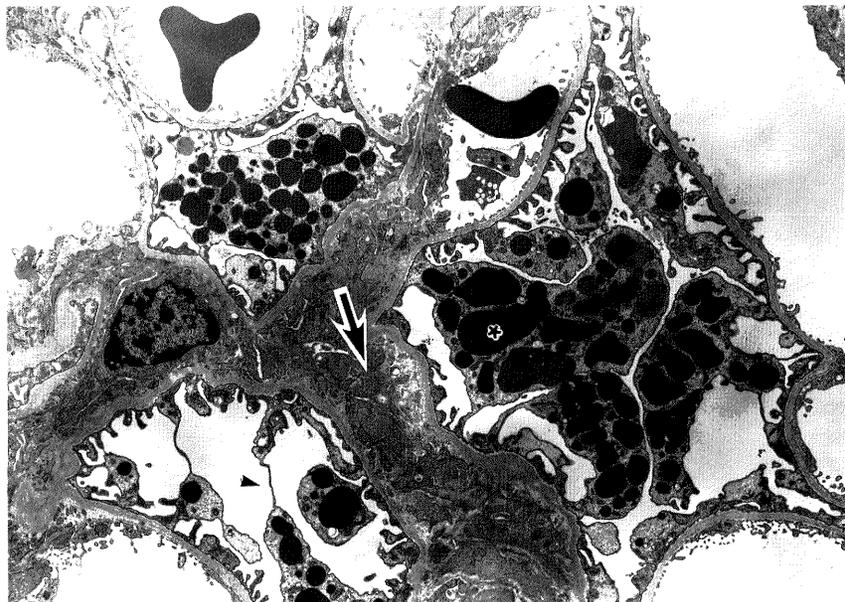
**Fig. 11** Light micrograph of the glomerulus with segmental sclerosis. The glomerular size is markedly increased. The glomerular segment shows collapse of capillaries and prominent vacuolization of epithelial cell (arrowhead). Mesangial matrix expansion is prominent in this area (arrow) (Masson  $\times 400$ ).

reabsorption droplets in epithelial cells, bleb formation of epithelial cells, and mesangial matrix expansion, were prominent in this group (Fig. 10). Some glomeruli revealed segmental sclerosis of glomerular tufts associated with capsular adhesion (Fig. 11). The

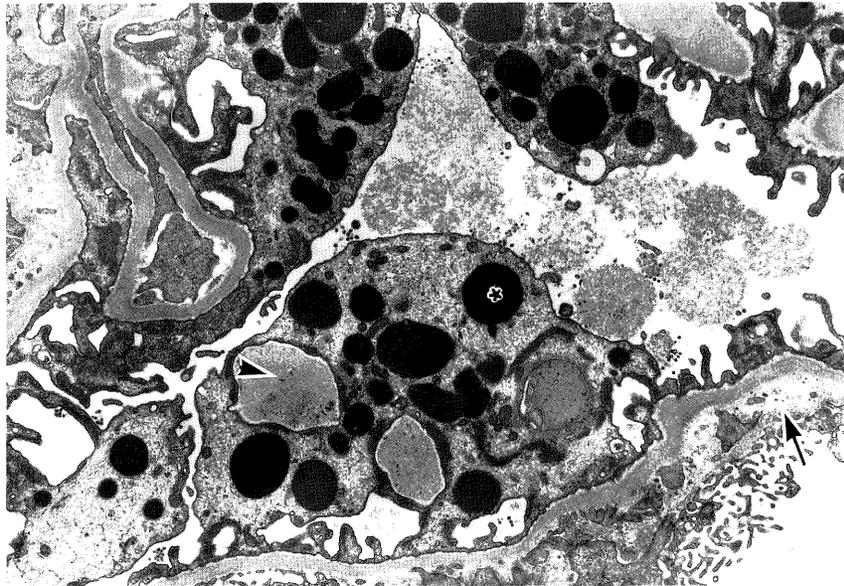
glomerular capillaries occluded with thrombi were seen in a few glomeruli. In the captopril-treated animals, these changes were significantly lessened and segmental sclerosis was detected only in one rat. Instead, small droplets in epithelial cells were occa-



**Fig. 12.** Light micrograph of the glomerulus from captopril-treated animal. The glomerular size is smaller than that of Group 2. Note a marked diminishment of epithelial changes.



**Fig. 13.** Electron micrograph of the glomerulus from Group 2 animal. There is expansion of mesangial matrix (arrow). The epithelial cells show prominent lysosomes (\*) and attenuation of cytoplasm (arrowhead) ( $\times 3000$ ).



**Fig. 14.** Electron micrograph of the glomerulus from Group 2 animal. The lysosomes in the epithelial cell show osmiophilic bodies (\*) and residual bodies (arrowhead). The widening of subendothelial space is noted (arrow) ( $\times 6000$ ).

**Table 5.** The kidney weight and histological data in Experiment 2

	Kidney weight (g)	Glomerular size (points)		massive droplet (%)	FGS-like lesion (%)
		superficial	juxtamedullary		
Group 1 (sham ope)	0.79 $\pm$ 0.10	18.1 $\pm$ 2.7	24.0 $\pm$ 1.5	0	0
Group 2 (renal ablation)	1.20 $\pm$ 0.10*	28.2 $\pm$ 1.9*	32.8 $\pm$ 1.6*	28.3 $\pm$ 21.8*	3.4 $\pm$ 3.6
Group 3 (renal ablation with captopril)	0.88 $\pm$ 0.09 <sup>+</sup>	23.8 $\pm$ 4.7 <sup>+</sup>	28.0 $\pm$ 2.2 <sup>+</sup>	0	0.15 $\pm$ 0.3

\*  $P > 0.05$ , \*  $P > 0.01$  sham ope vs renal ablation

‡  $P > 0.05$ , +  $P > 0.01$  renal ablation vs renal ablation with captopril

The values are mean  $\pm$  SD.

sionally seen in the glomeruli in this group (Fig. 12). At the ultrastructural level, the pathological findings were similar in the untreated renal ablation animals compared to those of Group 1 in Experiment 1. These pathological changes were: focal fusions of epithelial foot processes, multiple osmiophilic bodies and coarse vacuoles of the cytoplasm, formation of cytoplasmic blebs, mesangial matrix expansion and widening of subendothelial space (Fig. 13, 14). The glomerular size, in both superficial and juxtamedullary glomeruli, measured by a point-counting method was significantly increased in Group 2 and was slight-

ly increased in Group 3 when compared to Group 1. The glomeruli containing massive epithelial reabsorption droplets were detected only in Group 2, and were not detected in Group 1 and 3, even though a few droplets were seen in some glomeruli in Group 3. The incidence of glomeruli presenting segmental sclerosis was higher in Group 2 than the other groups. In Group 1, there was no glomeruli presenting segmental sclerosis, and in Group 3, only one animal revealed a few segmental sclerotic lesions (Table 5).

## DISCUSSION

A number of experiments, using animal models of chronic renal failure, have attempted to clarify the mechanisms that may lead to progressive renal injury. In the rat subtotal nephrectomy model, for example, increased glomerular pressures,<sup>5-7)</sup> altered renal prostaglandins,<sup>8-10)</sup> and coagulation abnormalities<sup>11,12)</sup> have been shown to contribute to glomerulosclerosis.<sup>13)</sup>

Relatively high systemic blood pressure was thought to be an important factor in the pathogenesis of glomerulosclerosis.<sup>6,14)</sup> Brandis et al.<sup>15)</sup> recently described a relative resistance to glomerulosclerosis in the spontaneously hypertensive Milan strain. They suggested that systemic hypertension *per se* does not have to increase intraglomerular pressure or induce progressive glomerulosclerosis. It is likely that the afferent vasoconstriction may be crucial in protecting glomeruli from systemic blood pressure.<sup>16,17)</sup> The elevation of intracapillary pressure which may occur after renal ablation<sup>5)</sup> or after high protein intake<sup>18)</sup> or in experimental diabetes mellitus,<sup>19)</sup> could be the most important factor in the pathogenesis of glomerulosclerosis.<sup>20)</sup> Therefore, the alteration in vascular resistance is important in the pathogenesis of glomerulosclerosis in a renal ablation model. This idea has been confirmed by the observations that the various manipulations listed in Figure 15 could modify the incidence of glomerulosclerosis in a renal ablation model.<sup>6,8,16,17,21-23)</sup> Additional support for this concept is reported by Bidani et al.<sup>24)</sup> who demonstrated the loss of autoregulation which appeared

**Fig. 15.** Various manipulations for lessened glomerular injury in renal ablation model.

1. Diet
  - protein restriction
  - phosphate restriction
  - caloric restriction
2. Antihypertensive agent
  - triple therapy (hydrochlorothiazide, reserpine, hydralazine)
  - angiotensin 1 converting enzyme inhibitors
  - calcium entry blockers
3. Anticoagulant
  - heparin
  - warfarin
4. Others
  - inhibitors of cyclooxygenase (ASA)
  - inhibitors of thromboxane synthetase (OKY-1581)
  - Lipid lowering agent
  - pituitary ablation
  - thyroidectomy

before glomerulosclerosis in the rats with renal ablation. However, these speculations are based on the findings that the severity of increased intraglomerular pressure is in close correlation with the degree of glomerulosclerosis during experimental manipulations.

On the contrary, the lack of correlation between glomerular hemodynamics and glomerulosclerosis has been observed in the recent study. Yoshida et al.<sup>25)</sup> carried out serial micropuncture measurements on the same glomeruli of the subtotal renal ablation rats. The values of SNGFR and  $P_{GC}$  were compared with a histological score which showed the degree of glomerular damage in the same glomeruli that were punctured. Since the glomerular damage did not correlate with the highest value of SNGFR or  $P_{GC}$ , the authors concluded the altered hemodynamics may act as a trigger of other injurious processes, rather than causing direct damage.

Anderson et al.<sup>16)</sup> suggested that the glomerular hypertrophy is important in pathogenesis of glomerulosclerosis. They observed that glomerular volume was increased in the model of renal ablation and was associated with severe glomerular injury. Recently the importance of hypertrophy for the development of glomerulosclerosis has been proposed by Grond et al.<sup>26)</sup> They compared the incidence of glomerular damage after uninephrectomy in the Wistar rats and in the PVG/c rats that had 20% more glomeruli than did Wistar rats. The Wistar rats, after renal ablation, developed glomerular hypertrophy and progressive glomerular injury, but the PVG/c rats did not manifest these changes. The glomerular volume of PVG/c rats after unilateral nephrectomy was smaller than even that of sham-operated Wistar rats. From this background, we focused on the glomerular hypertrophy which could have some role in pathogenesis of glomerulosclerosis in a renal ablation model. It has been known that the increased contralateral kidney growth begins immediately following the unilateral ureteral ligation.<sup>27)</sup> The mitotic activity in the kidney of contralateral ureteral ligation peaks at 48 h after ligation. The pattern of growth up to two weeks resembles that following unilateral nephrectomy, but one principal difference is that the increases in kidney weight during the first 2 or 3 weeks after unilateral nephrectomy are about half as great in the kidney of contralateral ureteral ligation.<sup>28,29)</sup> We focused on the different growth characteristics of these two models, and decided to carry out Experiment 1 to define whether growth response may contribute to the development of segmental glomerulosclerosis.

Although glomerular hemodynamics were not esti-

mated in our study, GFR in the remnant kidney was similar in Group 1 (nephrectomy) and Group 2 (ureteral ligation). Nevertheless, the incidence of glomeruli with segmental sclerosis was higher in Group 1 compared to Group 2. These data suggest that the compensatory growth response could have some roles in the development of segmental glomerulosclerosis in a renal ablation model.

A similar study was reported by Yoshida et al.<sup>30)</sup> They combined partial nephrectomy on the right side with ureteral diversion (UD), nephrectomy or sham operation on the left kidney. Both the UD and nephrectomy groups showed similar degrees of glomerular hypertension, hyperfiltration and hyperperfusion. The glomerular hypertrophy was observed in the nephrectomy group but was not seen in the UD group. The glomerular volumes of UD group were identical with those of the sham operation group. Despite the presence of alteration in glomerular hemodynamics, the UD group had fewer sclerotic glomeruli than the nephrectomy group. The authors concluded that glomerular hypertrophy must precede the development of segmental sclerosis and that the glomerular hyperfunction alone has little effect on the induction of hypertrophy and glomerulosclerosis. Our data of Experiment 1 seem to support this concept, but the question is what is the factor most responsible for induction of glomerular hypertrophy and how the glomerular hypertrophy or hypertrophic processes cause glomerular sclerosis. One possible mechanism of injurious effects of hypertrophy was proposed by Fries et al.<sup>31)</sup> Recently they produced adriamycin nephrosis in Munich-Wistar rats. Four weeks later, they performed 4/5 nephrectomy in some of them. They found that the combination of increased  $P_{GC}$  and hypertrophy was associated with the most severe injury. They evoked Laplace's Law, which states that wall tension is equal to the product of radius and pressure. Thus, for a given increase in  $P_{GC}$ , the tension will be greater in capillary loops that are dilated as part of hypertrophy. The exact mechanisms of injury secondary to wall tension are likely to be mechanical, but further study is required. Another mechanism which may be involved in the pathogenesis of glomerular injury as a consequence of glomerular hypertrophy<sup>32)</sup> is proposed from a couple of works.<sup>31,33)</sup> The studies on the turnover of glomerular cells in a renal ablation model demonstrated that, in contrast to endothelial and mesangial cells, visceral epithelial cells do not undergo cell division in response to such stimuli. The lack of proliferation leads to attenuation of epithelial cells within the greatly enlarged glomeruli. The increased surface

area of peripheral capillary walls must be covered by the same number of hypertrophied epithelial cells. Thus, the lack of podocyte proliferation during this process may contribute to the distortion of foot processes, the detachment of the epithelial cells from underlying basement membrane. Therefore epithelial cell damage may be an important factor in the development of focal segmental sclerosis.<sup>34)</sup>

Another factor which may have an important role in pathogenesis of glomerulosclerosis was proposed by Moorhead et al.<sup>35,36)</sup> They offer the hypothesis that lipids may be directly toxic to endothelial cells, may stimulate mesangial cell proliferation and are capable of producing glomerular injury. A couple of instances of supportive evidence were reported.<sup>37)</sup> Kasiske et al.<sup>22,38)</sup> studied the effect of lipid-lowering agents on the development of glomerulosclerosis. Using either clofibric acid or mevinolin they succeeded in reducing the number of sclerotic glomeruli in Zucker rats,<sup>38)</sup> which are characterized by elevated serum cholesterol and triglycerides, and in Sprague-Dawley (SD) rats with 5/6 nephrectomy.<sup>22)</sup> In the latter experiment, the rats were treated with clofibric acid during the ten weeks following 5/6 nephrectomy. This resulted in a decrease in serum cholesterol level, in proteinuria and in the incidence of glomerulosclerosis. Physiological data revealed no differences in SNGFR,  $P_{GC}$ ,  $Q_A$  or  $K_f$  between the treated and untreated animals. Though these lipid-lowering agents have many actions, the mechanism involved in the protection of glomerulosclerosis is not clear. Therefore, we focused on the abnormal lipid metabolism to determine whether it has an important role in the pathogenesis of glomerulosclerosis.

In 1977, Nagase et al.<sup>39)</sup> found an analbuminemic rat among hypercholesterolemic Sprague-Dawley rats. They established a rat strain having analbumi-

**Fig. 16** General features of Nagase analbuminemia rat (NAR).

Inheritance	: autosomal recessive
Clinical Feature	: essentially healthy no edema
Histological Feature	: essentially normal
Hematological Finding	: slight anemia
Serum Protein	: total proteins.....nearly normal albumin.....less than 1 mg/dl globulins.....increased
Serum Lipid	: total cholesterol...increased phospholipids.....increased
Reason for Lack of Albumin	: mutation of mRNA processing of albumin

nemia (Nagase Analbuminemia Rat: NAR) through breeding experiments. Analbuminemia of this strain was inherited as an autosomal recessive trait. Total serum protein in NARs was similar to that of control SD rats, with an increased globulin fraction.<sup>40)</sup> These rats of the NAR strain develop not only analbuminemia but also hyperlipidemia, which greatly increases their serum cholesterol and triglyceride concentrations. General features of NAR are summarized in Figure 16. Renal hemodynamics of these rats were reported by Sanfelice et al.<sup>41)</sup> They studied extracellular volume and glomerular hemodynamics in male rats of this particular NAR strain. They found that the blood volume, mean arterial pressure, extracellular volume and plasma renin activity in NARs were not different from values observed in SD rats. They also showed that plasma oncotic pressure and glomerular hydraulic transcapillary gradient in NARs were lower than SD rats, but that Kf was higher in NARs than in SD rats. Therefore, SNGFR in NAR remained normal or slightly increased.

We employed this particular rat strain as hyperlipidemic rats, and carried out Experiment 2 in order to make clear whether hyperlipidemia worsened glomerular injury in the renal ablation model. Animals in experiment 2 exhibited severe hyperlipidemia. Usually the serum cholesterol levels of Wistar and SD rats are somewhere around 50 mg/dl to 70 mg/dl. In NAR, the serum cholesterol level is somewhere around 180 to 220 mg/dl, and the serum triglyceride level is more than 500 mg/dl. Our study showed that early treatment with captopril in 5/6 nephrectomized Nagase analbuminemia rats resulted in normotension, less proteinuria, less hypertrophy of the glomeruli and lowering incidence of glomerular injury, although the degree of hyperlipidemia did not differ between captopril-treated and untreated animals. These data suggest that hyperlipidemia *per se* did not have a crucial role in the development of focal segmental sclerosis, at least in this model.

Similar observation was made by Fujihara et al.<sup>42)</sup> who studied the effect of enalapril on the development of glomerulosclerosis in uninephrectomized analbumineima rats. They found that the enalapril lowered  $P_{GC}$  and limited the glomerulosclerosis without altering serum cholesterol level. They suggested that the marked glomerular hypertension, rather than high cholesterol level, is the key to the development of severe glomerulosclerosis in this model. However, it is noteworthy that the NAR did not reveal any glomerulosclerosis and atherosclerosis until 18 months after birth (unpublished observation) when we studied NAR in another experiment. Zatz et al.<sup>43)</sup>

also reported the incidence of glomerulosclerosis in aged analbuminemia rats. Despite persistent hypercholesterolemia, analbuminemia rats failed to develop glomerulosclerosis. Therefore, it is possible that these rats have some protective factors against lipid toxicity on glomerular injury. In our experiment, the HDL-cholesterol level as well as total cholesterol level is markedly elevated in NAR serum, and may be involved in the protective effect on progression to glomerulosclerosis. Although hyperlipidemia or some component of hyperlipidemic serum are thought to cause endothelial injury and atherosclerosis,<sup>44)</sup> the role of abnormal lipid metabolism on the pathogenesis of glomerulosclerosis is not yet clarified.

It is known that there is a sharp decrease in the percentage of sclerotic glomeruli, accompanied by a lesser degree of proteinuria, when converting enzyme inhibitor (CEI) was used to lower systemic blood pressure in the renal ablation model.<sup>16,17)</sup> Micropuncture studies demonstrated that, in addition to decrease in systemic blood pressure, there was a decrease in  $P_{GC}$  but not in either SNGFR or  $Q_A$ . In our study, GFR of the remnant kidney is slightly higher in captopril-treated animals than in the untreated. The possible explanation for this increase in GFR is the activation of the renin angiotensin system after renal ablation, because low plasma oncotic pressure as a consequence of massive proteinuria occurs. Unfortunately, plasma renin activity was not measured in our experiment.

The reduction of protein excretion, observed in the report of Meyer et al.,<sup>17)</sup> was also observed in the captopril-treated animals in our study. However, Beukers et al.<sup>8)</sup> reported the lack of this effect of captopril on protein excretion in uninephrectomized aging Wistar rats. The discrepancy between their study and our study could be due to the differences in the models (the remnant kidney model versus the milder model of unilateral nephrectomy) or in the strain of rats (Wistar strain versus analbuminemia rats).

The captopril-treated animals in our experiment developed anemia, although glomerular hypertrophy and glomerular injury were lessened. One possible explanation for this anemia is that the inhibition of extrarenal erythropoietin production which is stimulated by renin is blocked by relatively high doses of captopril.<sup>45,46)</sup> Recently, Garcia et al.<sup>47)</sup> have shown that inducing anemia by an iron-deficient diet in rats with renal ablation resulted in lowered blood pressure, decreased  $P_{GC}$ , increased  $Q_A$  and lessened proteinuria. Therefore, it is possible that anemia may contribute to the diminishing of glomerular injury in

this model.

Another interesting observation of our experiment is that the glomerular hypertrophy was markedly diminished after captopril treatment, although GFR of the remaining kidney was slightly increased when compared with untreated renal ablation animals. Similar observation was made by Dworkin et al.<sup>48)</sup> who studied the effect of calcium channel blocker in DOCA salt hypertension rat. When nifedipin was given to the rat with DOCA salt hypertension, in spite of no reduction in  $P_{GC}$ , systolic blood pressure was lowered, proteinuria was reduced and glomerular sclerosis was prevented. From these observations, it has been suggested that glomerular hypertension did not have a crucial role in glomerular hypertrophy and glomerulosclerosis.

Recently, the factors involved in glomerular hypertrophy and growth response have been refocused<sup>49,50)</sup> from this background, although it has been studied for many years. The factors which control glomerular hypertrophy are not entirely understood. One possible mediator of glomerular hypertrophy is Insulin-like growth factor 1 (IGF-1).<sup>51)</sup> Doi et al.<sup>52)</sup> have examined transgenic mice of for growth hormone, growth hormone-releasing factor or IGF-1, and reported that IGF-1 surely mediated glomerular hypertrophy, but that additional factors which related to growth hormone were needed for the development of glomerulosclerosis.

In summary, the results of the present studies suggest that glomerular hypertrophy always precedes glomerulosclerosis, and may play a role in the pathogenesis of glomerulosclerosis in a renal ablation model. In contrast, hyperlipidemia did not play a crucial role in the pathogenesis of glomerulosclerosis in our renal ablation model.

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