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Glomerular sclerosis in wistar rats: analysis of its variable occurrence after unilateral nephrectomy

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Summary. Individual differences in susceptibility to the development of focal and segmental glomerular hyalinosis and sclerosis (FSGHS) were studied in rats after unilateral nephrectomy. A total of 20 male Wistar rats underwent unilateral nephrectomy at 3 months of age. Glomerular number in the removed kidney ranged from 17 000 to 32 700 with a mean value of 25 997 ($n=19$). At death 30 weeks later, the incidence of glomeruli with FSGHS in the remaining kidney varied from 0 to 20% with a mean of 4.4%. However, the percentage of glomeruli with FSGHS and the degree of proteinuria did not correlate either with glomerular number per kidney (left and right kidneys were shown to be equivalent for glomerular number) or with glomerular volume on killing. The occurrence of FSGHS correlated significantly with mean protein excretion ($P<0.01$) and serum cholesterol levels ($P<0.01$). In conclusion, in the rat a relatively low number of nephrons in the kidneys does not increase susceptibility to the development of FSGHS.

Keywords: focal sclerosis, renal ablation, glomerular number

The course of many different glomerular and tubulo-interstitial diseases, diabetes mellitus and conditions with a reduction in functional renal mass such as unilateral renal agenesis, unilateral nephrectomy, and renal transplantation, may be complicated by the development of focal and segmental glomerular hyalinosis and sclerosis (FSGHS), a condition associated with proteinuria and progressive loss of renal function (Brenner 1983). Interestingly however, some patients will develop FSGHS in these conditions whereas others will not. The reasons for these individual differences in susceptibility may be manifold and comprise among others the extent of renal functional reserve capacity and different environmental and genetic factors.

In many rat strains proteinuria and glomerular lesions, akin to FSGHS in man, develop spontaneously during ageing (Couser & Stilmant 1975; Elema & Arends 1975). Experimental manipulations, such as reduction of renal mass (Hostetter *et al.* 1981) or administration of puromycin aminonucleoside (Glaser *et al.* 1977), considerably accelerate the occurrence of nephrotic syndrome and FSGHS. As in the human disease, the occurrence of FSGHS in the rat varies widely. In our laboratory from 0 to 7% of the glomeruli of 1 year old male Wistar rats showed FSGHS; 1 year after unilateral neph-

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rectomy the percentage of diseased glomeruli in the remaining kidney ranged from 9 to 44% (Grond *et al.* 1986). In addition, a considerable interstrain variability in susceptibility to FSGHS has been reported in rats (Feld *et al.* 1981; Grond *et al.* 1986).

The present study focuses on the individual susceptibility to FSGHS of Wistar rats after unilateral nephrectomy and, in particular, on the possible pathogenetic role of the number of nephrons.

Materials and methods

Male Wistar rats underwent unilateral nephrectomy at 3 months of age and the following parameters were studied: (1) number of glomeruli in the removed kidney; (2) urine protein and blood chemistry during 30 weeks following surgery; (3) renal morphology and (4) glomerular volumes on killing.

Animals. A total of 20 male inbred Wistar rats were used. They were fed a normal rat chow with a sodium content of 0.44% and a digestible protein content of 22% (Hope Farms Inc., Woerden, The Netherlands) with free access to tap water throughout the course of the experiment. Right sided nephrectomy was performed through a flank incision under ether anaesthesia. The animals were studied during a 30 weeks period. Body weights were recorded every other week.

Glomerular number. The glomerular number of the kidneys obtained at unilateral nephrectomy was determined in the following manner. After weighing and removal of the capsule, the kidneys were digested in 10 ml of a 6 M hydrochloric acid solution during $1\frac{1}{2}$ hours at 37°C (Damadian *et al.* 1965). The material was rinsed in distilled water and stored at 4°C for 24 h. Subsequently, 15 g of glucose was added and the vial was gently stirred for 60 min to obtain a homogeneous suspension of glomeruli, tubular fragments and vascular structures. By this procedure, the glomeruli remain histologically intact when checked by light microscopy. One millilitre of the suspension was pipetted into a counting chamber as designed by Bonvalet *et al.* (1972) and the glomerular number was counted by light microscopy. Two aliquots were counted and a difference between both results of less than 10% was considered acceptable. Otherwise, the material was stirred again to improve homogeneity of the suspension and the counting procedure was repeated.

Pilot experiments in eight male Wistar rats using this digestion method indicated no significant differences between left and right kidneys for glomerular numbers ($y = 1.08x - 2.11$, $r = 0.94$, $P < 0.001$) or organ weights of individual rats.

Urine protein and blood chemistry. Urine was collected 2 weeks before unilateral nephrectomy and subsequently every other week by housing the rats in metabolic cages for 24 h with access to water only. Blood was obtained by orbital plexus puncture under ether anaesthesia after the first urine collection and on killing. The total protein content of urine and serum was determined by the biuret method.

Serum levels of cholesterol and triglycerides were determined enzymatically (Boehringer Mannheim Diagnostics kits, Germany, nr 237574 and 70191).

Renal morphology. At autopsy, the kidneys were removed, weighed and processed for light microscopy, immunofluorescence and lipid staining. From each kidney three cross sectional slices were cut at the level of the vascular pedicle. The two outer slices were fixed by immersion in 8% buffered formalin and embedded in glycol methacrylate. From each tissue block, two consecutive 2 μm sections were cut. One set was stained with periodic acid Schiff (PAS), the other by the silver methenamine method. At least 100 glomeruli per kidney were studied.

The central tissue slice was snap frozen in precooled freon at −90°C. Two μm cryostat sections were cut. For immunofluorescence,

the indirect method was used with rabbit antisera to rat immunoglobulin M, C_3, and fibrinogen as the first layer and fluorescein isothiocyanate-labelled goat anti-rabbit as the second. For the detection of lipids, frozen sections were stained by the Oil red O method.

Glomerular volumes. For morphometric analysis silver methenamine-stained sections were used. The sections were screened, moving through the cortex from surface to medulla and vice versa, at a magnification of × 376 using a light microscope equipped with a side tube attachment and a drawing prism. The image of a given glomerulus present in the picture was traced with a cursor along the Bowman capsule over the surface of a graphic tablet (Computex, GT 50/10, USA) connected to a Digital PDP 11/10 computer (Digital Equipment Int., Galway, Ireland). At least 50 glomerular profiles per kidney were traced. Mean glomerular diameters were calculated according to Van Damme & Koudstaal (1976). Glomerular volumes were estimated from mean glomerular radius using the formula $4/3\ \pi r^3$.

Statistical analysis. Results are presented as mean ± 1 standard error of the mean. Linear regression analysis was performed using the method of least squares. Statistical evaluation was carried out using the two-sided Student's *t*-test with the level of significance being defined as $P<0.05$.

Results

During the experimental period one rat died and was excluded from the study. The other remained in good health and showed a normal weight gain (Table 1).

Glomerular numbers

The numbers of glomeruli in the right kidneys removed at unilateral nephrectomy are presented in Fig. 1. A broad variation from 17 100 to 32 700 glomeruli per kidney was found with a mean glomerular number of 25 997. The actual kidney weight and relative kidney weight (in relation to body weight) at unilateral nephrectomy and on killing are given in Table 1. Body weight and single kidney weight increased in proportion after unilateral nephrectomy without alteration of the ratio of kidney to body weight. When linear regression analysis was applied to the right kidney weight versus glomerular

Table 1. Body weight, kidney weight, kidney weight in relation to body weight and serum levels of cholesterol, triglycerides, and total protein in 3 months old male Wistar rats ($n=19$) at unilateral nephrectomy and 30 weeks later

	Unilateral nephrectomy	30 weeks after unilateral nephrectomy	
Body weight (g)	208±4	401±13	$P<0.001$
Kidney weight (g)	1.08±0.03	1.97±0.07	$P<0.001$
$\frac{\text{Body weight}}{\text{Kidney weight}}$ (%)	0.49±0.02	0.49±0.01	P=NS
Cholesterol (mmol/l)	1.61±0.07	2.24±0.12	$P<0.01$
Triglycerides (mmol/l)	1.53±0.05	1.81±0.15	P=NS
Total protein (g/l)	74.2±2.9	69.3±1.0	P=NS

Results are expressed as mean ± 1 SE.

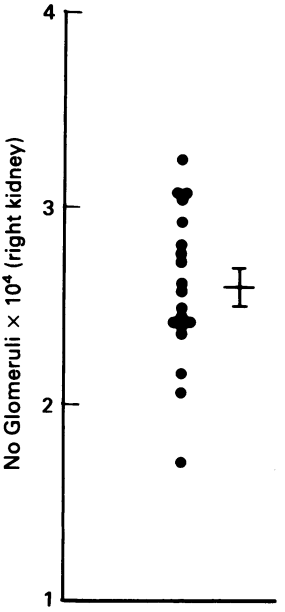

Fig. 1. Number of glomeruli in 19 right kidneys removed at unilateral nephrectomy. Bars indicate mean ± 1 SE.

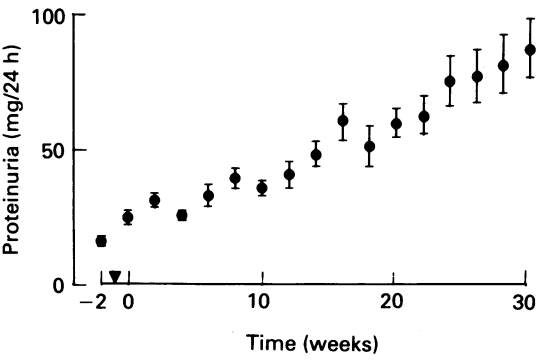

Fig. 2. Urinary protein excretion before and during 30 weeks following unilateral nephrectomy. Each point indicates mean proteinuria of 19 rats ± 1 SE.

number, no significant relationship was found. Likewise, no relationship was observed between glomerular number in the right kidney and the relative increase of the left kidney weight (ratio of left to right kidney weight) 30 weeks after unilateral nephrectomy.

Urine protein and blood chemistry

Urinary protein excretion increased steadily from a mean of 17 ± 1 mg per 24 h before unilateral nephrectomy to 89 ± 11 mg per 24 h at the end of the experimental period ($P < 0.001$, Fig. 2).

Mean protein excretion (the total of the results of all 24 h protein excretion measurements divided by the number of collected specimens) did not correlate either with glomerular number in the removed right kidney at unilateral nephrectomy or with the ratio of body weight to left kidney weight on killing or with the ratio of glomerular number to right kidney weight.

Serum levels of cholesterol, triglycerides, and total protein are given in Table 1. A significant rise of serum cholesterol level was found 30 weeks after unilateral nephrectomy.

Renal morphology

Characteristic FSGHS lesions, consisting of segmental mesangial and subendothelial deposition of hyalinized PAS-positive material with an increase of mesangial matrix substance and capillary wall wrinkling with collapse and synechiae to the Bowman capsule, were observed in 15 of the 19 rats (Fig. 3*a*). Diseased glomeruli were seen equally in the juxta-medullary or outer portion of the cortex. Protein reabsorption droplets were found in glomerular and tubular epithelial cells. Protein casts were present in some tubules. The percentage of diseased glomeruli ranged from 0.9% to 20% (Fig. 4). Mean percentage for the whole group was 4.4%. By immunofluorescence and Oil red O staining, diseased glomeruli showed segmental depositions of IgM, C_3, fibrinogen, and lipid material with large deposits of proteins and lipids in fully developed FSGHs lesions (Fig. 3*b*). No significant relationship could be demonstrated between the incidence of FSGHS in the left kidney and glomerular

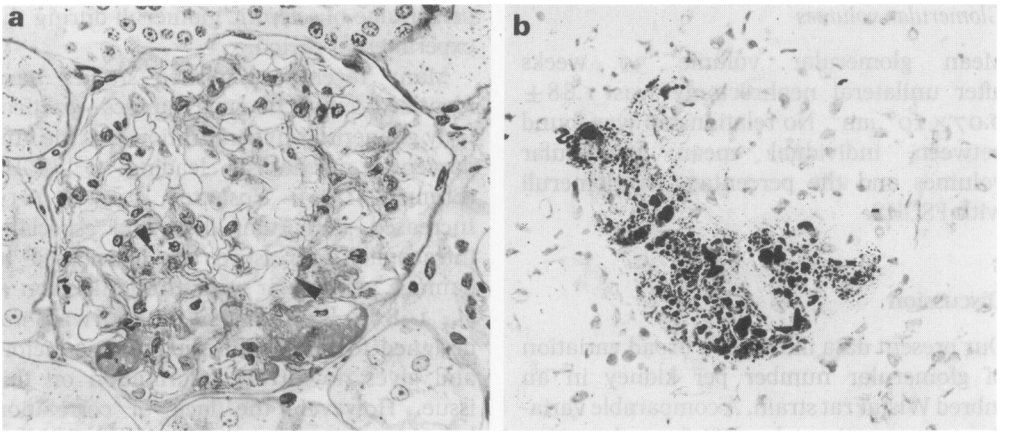

Fig. 3. *a.* Representative glomerulus with segmental hyalinosis and sclerosis. Protein reabsorption droplets are present in visceral epithelial cells (arrowheads). PAS, ×250. *b.* FSGHS glomerulus showing massive lipid accumulation in the affected glomerular area. Oil red O and hematoxylin, ×250.

number of the right kidney removed at unilateral nephrectomy (Fig. 5). Regression analysis revealed a weak correlation between body weights at death and the percentage of FSGHS ($r=0.53$, $P<0.05$); no relationship was found between the relative increase of body and kidney weight and the percentage of glomeruli with FSGHS.

FSGHS strongly correlated with mean protein excretion after unilateral nephrectomy ($r=0.85$, $P<0.01$) and serum cholesterol levels on killing ($r=0.71$, $P<0.01$). No correlation with serum levels of triglycerides could be demonstrated.

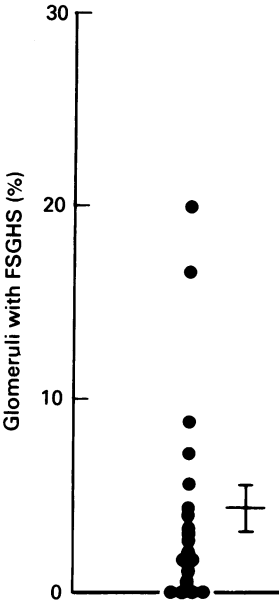

Fig. 4. Percentage of glomeruli with FSGHS 30 weeks after unilateral nephrectomy. Bars indicate mean ± 1 SE.

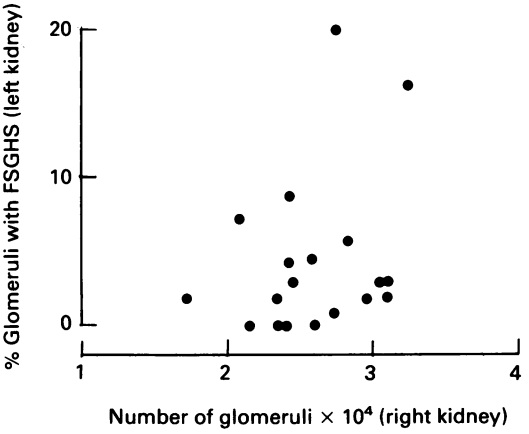

Fig. 5. The number of glomeruli of the right kidneys, removed at unilateral nephrectomy. plotted against the percentage of glomeruli with FSGHS in the left kidneys on killing after 30 weeks. The scatter of points is quite wide and no significant relationship can be seen.

Glomerular volumes

Mean glomerular volume 30 weeks after unilateral nephrectomy was $1.88 \pm 0.07 \times 10^6$ μm^3. No relationship was found between individual mean glomerular volumes and the percentage of glomeruli with FSGHS.

Discussion

Our present data illustrate a broad variation of glomerular number per kidney in an inbred Wistar rat strain. A comparable variation of approximately 25% from the mean glomerular count has been reported in other animal studies using neonatal and adult Sprague–Dawley rats (Larsson *et al.* 1980), mice (Bonvalet *et al.* 1977), and guinea-pigs (Chevalier 1982). Glomerular number of the removed kidneys at unilateral nephrectomy was assumed to be equivalent to that in the remaining contralateral kidney since a highly significant relationship between left and right kidneys for glomerular number was found and no formation of new nephrons has been reported to occur after unilateral neprectomy in neonatal or adult rats (Larsson *et al.* 1980). The weight of the remaining kidney, however, nearly doubled 30 weeks after unilateral neprectomy due to compensatory renal growth. Similarly, it has been reported that, in guinea-pigs, glomerular number did not correlate with the contralateral weight after unilateral nephrectomy and compensatory renal growth (Chevalier 1982).

The composition and amount of food intake have been related to the occurrence of FSGHS, and dietary restriction has been shown to lower the incidence of the lesions (Tucker & Mason 1976). Although a weak correlation was observed between body weights at death and presence of FSGHS, individual differences in food intake can be rejected as a major factor contributing to the variable incidence of FSGHS in the current study since no relationship was observed between relative increase of body weight and percentage of sclerotic glomeruli during the experimental period.

Many recently published studies have identified renal haemodynamics—particularly glomerular hyperfiltration—as the fundamental mechanism leading to FSGHS (Brenner 1983; Hostetter *et al.* 1981). Increased glomerular flow and especially intracapillary pressure are considered to damage glomerular structure (Anderson *et al.* 1985). The current study was not designed to explore haemodynamic factors and gives no further information on this issue. However, the lack of correlation between glomerular number and individual susceptibility to FSGHS after nephrectomy was unexpected since one would expect a higher workload per glomerulus in kidneys with low nephron numbers to lead to enhanced development of FSGHS. Glomerular size is likely to be determined by the interplay of intraglomerular pressures and glomerular tissue compliance. The latter is unlikely to vary significantly in the inbred rat strain used in this study, and differences in glomerular volume are probably the result of differences in intraglomerular pressure. However, no relationship was found between individual mean glomerular volumes and the percentage of glomeruli with FSGHS. Adaptive increase of glomerular filtration rate may therefore be attained in different ways in different animals. We suggest that rats relatively resistant to FSGHS after unilateral nephrectomy, may achieve an increase of glomerular filtration rate by an increase of ultrafiltration coefficient or glomerular capillary plasma flow rather than by a rise of intracapillary hydraulic pressure. The lack of correlation in individual rats between mean glomerular volume and percentage of affected glomeruli, suggest that these adaptive mechanisms are not mutually exclusive, but that the development of FSGHS and proteinuria depend in part on elevated intracapillary pressure.

In the rat, FSGHS can be produced in the absence of glomerular hyperfiltration by repeated injections of puromycin aminonuc-

leoside (Glasser *et al.* 1977; Grond *et al.* 1984). This experimental model of focal sclerosis illustrates the involvement of other, probably non-haemodynamic factors in the initiation and progression of FSGHS. In chronic puromycin aminonucleoside nephrosis, FSGHS glomeruli show massive accumulations of protein and lipid (Velosa *et al.* 1977; Grond *et al.* 1984). In the present study depositions of similar macromolecular substances were found within the lesions. The relationship between serum cholesterol levels and incidence of the lipid-laden FSGHS lesions suggest that the glomerular lipid is derived at least in part from serum lipoproteins. The pathogenetic significance of glomerular lipid deposits is unclear but is being investigated further.

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