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Ultrastructural quantitation of atubular and hypertrophic glomeruli in rats with lithium-induced chronic nephropathy

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Summary. The very heterogeneous population of glomeruli in rats with lithium-induced chronic nephropathy which includes small glomeruli without connection to a proximal tubule (atubular glomeruli) and large hypertropic glomeruli with connection to a normal proximal tubule, was studied at the ultrastructural level, using stereological methods. After 8 weeks of lithium treatment followed by 8 weeks without lithium the hypertrophic glomeruli showed no changes in their relative ultrastructural composition, including normal mesangium, basement membrane-like material and peripheral basement membrane. The absolute quantities of each component were, however, increased due to the increased volume of the glomeruli. The atubular glomeruli had increased volume fractions of mesangium, peripheral basement membrane, basement membrane-like material and epithelium, whereas the absolute quantities were decreased due to the decreased volume. The thickness of the basement membrane was within normal limits in the group of hypertrophic glomeruli but increased by 31% above controls in the group of atubular glomeruli. Both groups of glomeruli in lithium-treated animals showed normal mean foot process width, but with a slightly abnormal distribution. The atubular glomeruli showed a disproportionate large decrease in peripheral filtration surface and capillary length, compared with the reduction in glomerular volume, whereas the hypertrophic glomeruli showed changes in proportion with the increased volume.

Key words: Atubular glomeruli – Chronic nephropathy – Electron microscopy – Lithium – Stereology

Introduction

In an earlier study of lithium-treated rats with chronic renal failure a marked heterogeneity of the glomerular population was demonstrated (Marcussen et al. 1989). Some of the glomeruli were hypertrophic, but most were found to be without connection to a normal proximal tubule, and to have small volumes. Some of the latter were connected to severely atrophic tubules, but the majority were without connection to a proximal tubule at all (atubular). Despite the compensatory hypertrophy and probable hyperfiltration of some glomeruli, the rats were uraemic and some became even more uraemic after lithium was withdrawn.

It has been suggested (Brenner 1985; Hostetter 1984) that hyperfiltration in the remaining intact glomeruli plays a major role in the deterioration of renal function in chronic renal diseases. This compensatory hyperfiltration is considered to be detrimental to glomerular structure and function leading to proliferation of mesangial cells and ultimately to segmental and global sclerosis. Other authors (Bohle et al. 1981; Ottosen et al. 1984) have stressed the importance of interstitial and tubular changes in the pathogenesis of chronic renal failure.

The purpose of the present study was to throw some light on the consequences of hyperfunction/hypertrophy in a well-defined glomerular population. Moreover, the quantitative ultrastructure of non-functioning, atubular glomeruli was studied in order to gain some insight into the interplay between glomerular function and structure. A large number of glomerular structures were quantified in each of the two glomerular populations: atubular glomeruli and hypertrophic glomeruli with connections to normal proximal tubules.

Materials and methods

New-born male Wistar rats were used. Lithium was administered orally for 8 weeks, followed by 8 weeks without lithium. During the suckling period of 3 weeks the animals received lithium through their mothers' milk, and throughout this period the mothers were fed a diet containing 40 mmol lithium/kg dry weight (Christensen and Ottosen 1983). The animals received a similar lithium-containing diet after weaning. The rats were anaesthetized with pentobarbital and the left kidney was fixed by perfusion with 1% glutaraldehyde and modified Thyrode's buffer (Maunsbach 1966).

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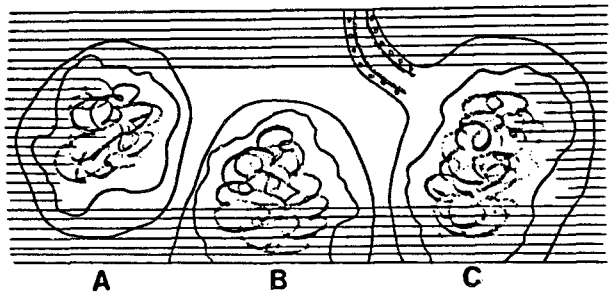

Fig. 1. Glomeruli were sampled using the disector. Only those glomeruli that were seen connected to a normal proximal tubule (glomerulus *C*) or that were completely in the serial sections and were found to be atubular (glomerulus *A*) were sampled for ultrastructural quantitation. Glomerulus *B* was not included because it could not be assigned to one of these categories

The animals used in the present study were all included in the material presented in previous papers (Christensen and Ottosen 1983; Marcussen et al. 1989; Ottosen et al. 1984). For the ultrastructural investigation five control rats (group C/C) and seven lithium-treated rats (group Li/C) were used.

Sampling of glomeruli. Using a simple device with parallel razor blades [see Fig. 6 in Baddeley et al. (1986)] the perfusion fixed kidney was cut into thin parallel slices. From these slices two 1.5 × 1.5 mm blocks were randomly sampled from the cortical area. These blocks were post-fixed in 1% osmium tetroxide for 1 h, washed in malic acid, dehydrated in alcohol, washed in acetone and embedded in Epon. From each of the blocks a minimum of 25 serial sections were made at a thickness of 4 μm. The sections were stained with toluidine blue.

The disector is a stereological sampling device which ensures that particles are sampled unbiasedly and uniformly using two parallel sections simultaneously (Sterio 1984). The probability that a particle is hit by one of the planes (the reference plane) but not the other plane (the look-up plane) is the same for all particles irrespective of their size, i.e. small particles are sampled with the same probability as large particles. Thus, using the disector in consecutive parallel sections glomeruli are sampled with identical probabilities. Using the first 6–7 sections in each series a number of glomeruli were sampled (Fig. 1). These sampled glomeruli fell into two categories. One category contained glomeruli that had their whole Bowman's capsule represented in these sections. These small glomeruli were followed in the serial sections and found to be connected to an atrophic proximal tubule or no tubule at all. From this category the atubular glomeruli were included for further study (glomerulus "A" in Fig. 1). Another category was glomeruli which were only partly represented in the sections (glomeruli "B" and"C" in Fig. 1). Of these glomeruli only those seen to be connected to a normal proximal tubule were studied (glomerulus "C" in Fig. 1). The serial sections were numbered consecutively and one section through the glomerular tuft was sampled for electron microscopy using random numbers.

The sampled 4-μm-thick sections were re-embedded in Epon and thin (50 nm) sections containing the sampled glomeruli were stained with uranyl acetate and lead citrate. Each glomerular cross-section was photographed in a Philips electron microscope at a magnification of × 3300. A systematic random sample, comprising an average of 12 micrographs/cross-section, was photographed at a final magnification of × 16500. In the control group a total of 11 glomeruli (all connected to normal proximal tubules) were investigated, whereas the numbers were 11 in the group of hyper-

Fig. 2. Electron micrograph of an atubular glomerulus at low magnification with grid superposed. The minimal convex figure enclosing the epithelial side of the basement membrane, called the string polygon, is shown on the micrograph with a *solid line*. The length of a test line is 8.6 μm. × 2200

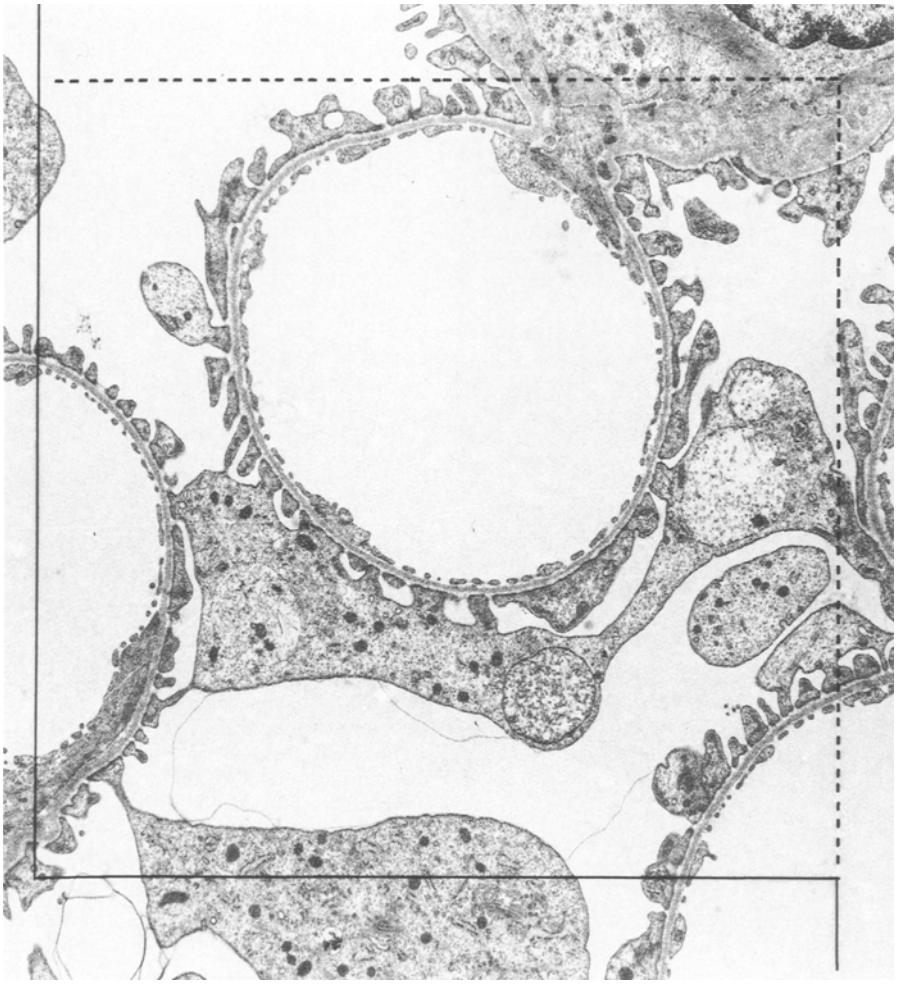

Fig. 3. Electron micrograph of a hypertrophic glomerulus at high magnification with an unbiased counting frame superposed. All foot process profiles that have any part inside the central test area are measured provided they are not intersected by any of the full drawn lines. The ruler shown below the test-area was used for measuring the linear apparent foot process width. The side of the square frame is 9.7 μm. ×11000

trophic glomeruli and 12 in the group of atubular glomeruli. The means to be estimated were within animals, that is separate summation of the estimated values in each animal was carried out over the sampled glomeruli.

Stereological investigation. Most of the stereological techniques employed in this study are described in the paper by Østerby and Gundersen (1980) and the recent reviews by Gundersen et al. (1988a, b). On the low-magnification (LM) photographs the minimal convex figure enclosing the epithelial side of the basement membrane (BM) was drawn (Fig. 2). On the LM photographs the densities of surface and length were expressed in relation to this reference space. The nacked tuft volume (the combined volume of mesangial regions, endothelium, peripheral BM, and capillary lumen) was used as the reference volume for the volume fractions on the LM photographs and in the quantitative analysis of the high magnification (HM) photographs. In addition to the naked tuft volume, the volume of the mesangium was reference space for the basement membrane-like material (BMLM) of the mesangium. The peripheral BM (PBM) was defined as the BM in the capillary wall, and the transition between the PBM and BMLM of mesangial regions was determined on the basis of widening of the distance and disappearance of the parallellism between the endothelial and epithelial cells as described by Østerby (1973). The following variables were estimated on the LM photographs: capillary luminal space (Cap), mesangium (Mes), endothelial cells (En) and epithelial cells (Ep), whereas the PBM and BMLM were estimated on the HM photographs.

Both on the LM and the HM photographs, the fractional volumes were estimated by point counting (Fig. 2) (Weibel 1979). For example, the volume fraction of mesangium was estimated by counting the points of a randomly placed grid hitting the mesangium and the tuft, respectively:

$$V_V(\text{Mes/Tu}) = \sum P(\text{Mes}) / (4 \times \sum P(\text{Tu})),$$

where $\sum P(\text{x})$ is the total number of points hitting compartment x, and 4 is the ratio of two point-sets in the test system used for mesangium and tuft, respectively. In the calculation of the relative quantities, separate summation in numerator and denominator was carried out over the 1–3 glomeruli investigated in each category of glomeruli in each kidney.

The surface density of the PBM was estimated by:

$$S_V(\text{PBM/glomerulus}) = 2 \times \sum I(\text{PBM}) / [l(p) \times \sum P(\text{polygon})],$$

where $\sum I$ is the total number of intersections between the surface trace of the PBM and test lines, $l(p)$ is the real test line length per test point, and $\sum P(\text{polygon})$ is the total number of test points hitting the string polygon. The length density of glomerular capillaries was estimated by:

$$L_V(\text{Cap/glomerulus}) = 2 \times \sum Q(\text{lumina}) / [a(p) \times \sum P(\text{polygon})],$$

where $\sum Q(\text{lumina})$ is the total number of luminal profiles and $a(p)$ is the real area associated with a test point. The length and

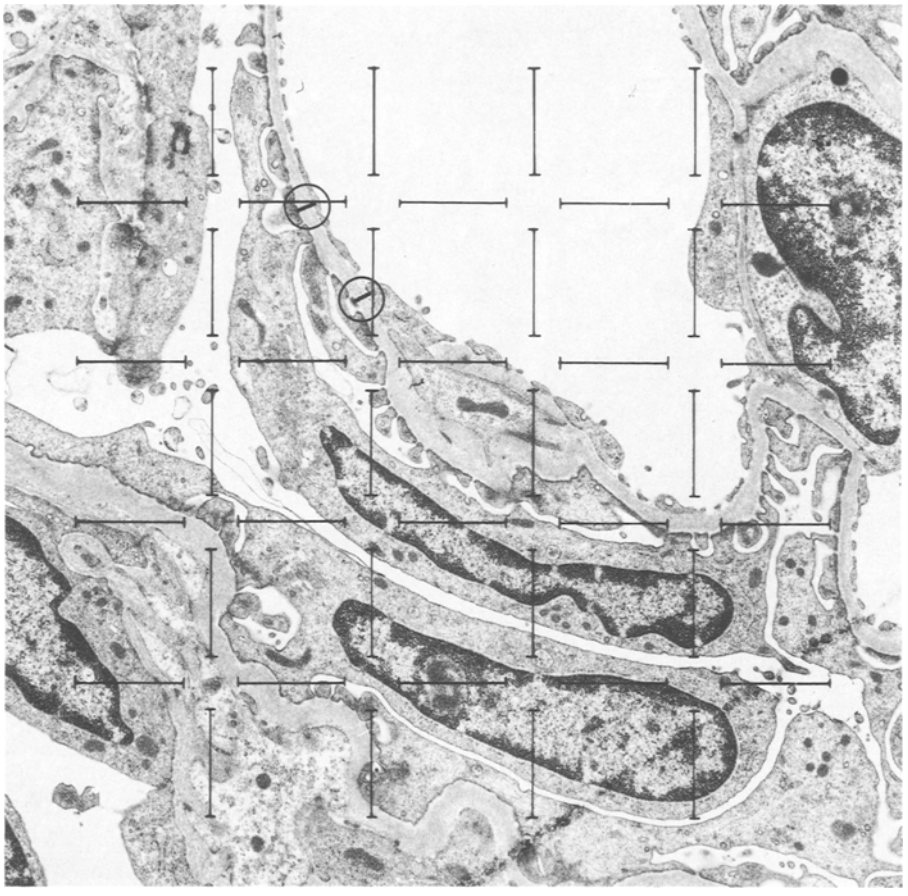

Fig. 4. Electron micrograph of an atubular glomerulus at high magnification. A grid with lines is placed over the micrograph, and at each place where a line intersects the endothelial side of the peripheral basement membrane the orthogonal intercepts (*circles*) are classified with a ruler. The length of a test line is 1.4 μm. ×9400

surface densities were estimated on the LM photographs. The average capillary cross-sectional area perpendicular to the capillary lumen was estimated by

$$\bar{A} = V_V(\text{Cap/glomerulus})/L_V(\text{Cap/glomerulus}).$$

The width of individual glomerular epithelial foot processes (FP) was estimated using a method that is based on geometric probability theory pertaining to a specific geometric model. The true width of the FP is defined as the linear, orthogonal separation between two neighbouring filtration slits, that in to say, the distance between two parallel lines in space (Gundersen et al. 1980). On the micrographs, the apparent width of a FP is defined as the linear width at the level of the slit diaphragm including the width of one slit in each FP width. Under the assumption of random orientation, it is possible mathematically to transform (unfold) any observed distribution of apparent width into the underlying distribution of true width and thus to eliminate all variation due to sectioning angle (Jensen et al. 1979). Moreover, some simple relationships exists between certain parameters of the apparent and true width distributions (Jensen et al. 1979). It is therefore possible to calculate the mean as well as other parameters, e.g. SD of true width directly from parameters of apparent width. The mean used in this study was the harmonic mean ($\bar{t}_h$).

To avoid bias due to the edge-effect at the margins of the micrograph, the unbiased counting frame (Gundersen 1977) was placed on each HM micrograph independently of glomerular structures (Fig. 3). The apparent width of all FPs within the frame was measured or classified using a ruler which has equidistant classes on a log reciprocal scale (Fig. 3). An average of 390 FP widths was measured per glomerular cross section. FP profiles abutting on the mesangial regions and on the PBM were measured separately. A particular FP was considered to belong to, for example, a mesangial region if its directionally defined limit (the clockwise end), was over that region.

Glomerular basement membrane (GBM) thickness was estimated with the orthogonal intercept method (Jensen et al. 1979). Only the thickness of the PBM was estimated. A grid with solid lines (Fig. 4) was placed on each HM micrograph and at each point where a line intersects the endothelial aspect of the PBM the length of the intercept was measured from the point perpendicularly to the epithelial border. For this measurement a ruler which incorporated ten classes on a log reciprocal scale was used. An average of 70 intercepts was classified per glomerular cross-section. Using the same unfolding principle as for the estimation of FP widths the harmonic mean thickness ($\bar{t}_h$) was calculated.

The mean volume of glomeruli with or without connection to a proximal tubulus in each animal had been estimated by light microscopy in a previous study (Marcussen et al. 1989). These values were used in this study disregarding the bias which might be introduced due to the volumes being estimated in paraffin-embedded tissues. It was decided to use the mean glomerular volumes for all the glomeruli in a given category, mainly due to the relatively small number of hypertrophic glomeruli investigated in some of the animals in the lithium group in the previous study. The mean glomerular volumes were 0.97×10^6 μm^3, 1.81×10^6 μm^3, and 0.29×10^6 μm^3 in the controls, hypertrophic glomeruli and atubular glomeruli, respectively. Using the mean glomerular volume of each category the variances of the estimates in the present study were minimized.

Since the glomerular volume was decreased by a factor 2–3 in the atubular glomeruli and increased by a similar factor in the hypertrophic glomeruli it was only of minor interest to look for differences in the absolute structural quantities. Moreover, the real proportions between the elements composing the glomerulus are only directly measurable irrespective of changes in total volume

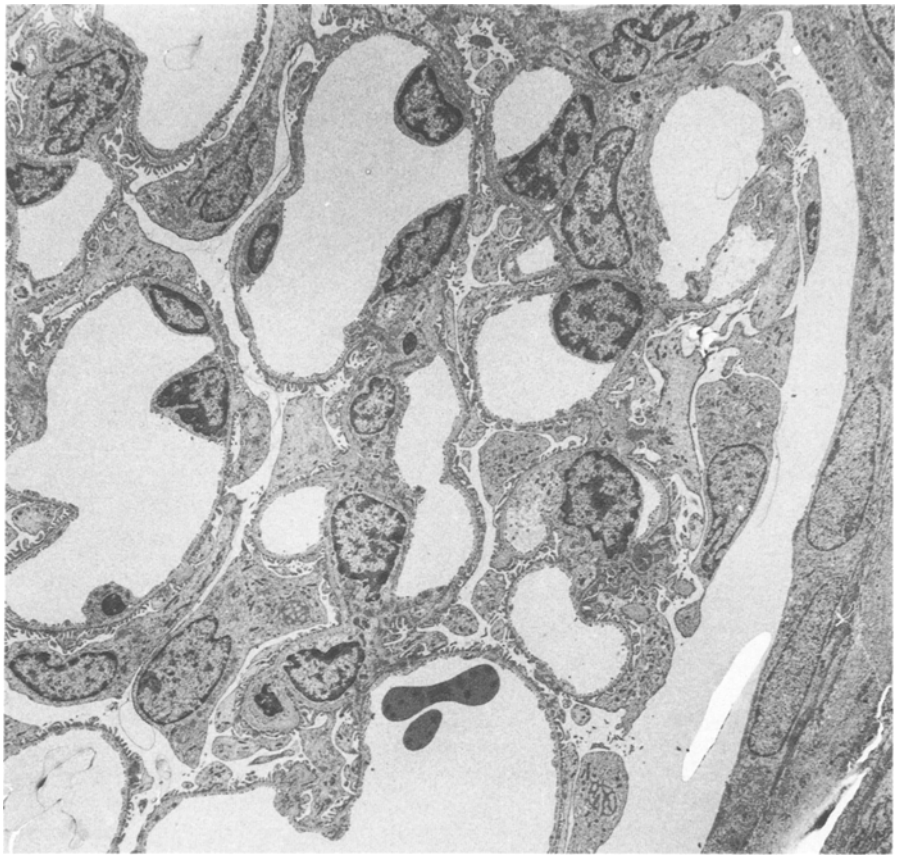

Fig. 5. Electron micrograph of an atubular glomerulus. The nuclei of the endothelial cells have an abnormally peripheral location in the capillary loops. ×2700

in the case of the dimensionless volume fractions (of formal dimension $\mu m^3/\mu m^3$). All other relative structural quantities change when the glomerular volume changes, as has been fully explained in the paper by Østerby and Gundersen (1980). However, only when the absolute quantities corresponding to these structural quantities are known is it possible to construct estimators that are dimensionless. Such estimators have earlier been known as "shape factors", but the name "coefficients of isomorphic change" is more appropriate and will be used in this paper. The coefficients of isomorphic change are estimators which are independent of the absolute reference volume. For example, as described in the above-mentioned paper, $S^{3/2}/V = 6 \times \sqrt{\pi} = 10.6$ for an assembly of spheres irrespective of their size distribution. By comparing such coefficients from different categories of glomeruli one can test for isomorphous change of the structural quantities, that is, are the changes in the quantities of the magnitude that could be expected from the change in the reference volume.

Statistics. Estimates of the parameters are provided in terms of means ± CV (coefficient of variation = SD/mean, where SD is the ordinary standard deviation between animals). Differences between mean values were tested with the Student's unpaired *t*-test. A *P* value is as usual only an indicator of significant changes. In the present paper the variation between absolute values was only a part of the true variation, due to the use of a constant mean glomerular volume within each category of glomeruli, whereby the variation in glomerular volumes was eliminated. To "compensate" for this, only a 2*P* value less than 0.01 was considered statistically significant.

Results

The functional obtained values are part of data published in previous papers (Christensen and Ottosen 1983; Ottosen et al. 1984; Marcussen et al. 1989). The plasma urea levels were 6.2 mmol/l ± 0.9 (mean ± SD) (values for three animals) in the C/C group and 14.0 mmol/l ± 5.7 in the Li/C group after 8 weeks. At 16 weeks the plasma urea levels were 6.4 mmol/l ± 0.7 (mean ± SD) and 18.5 mmol/l ± 6.3 in the C/C group and Li/C group, respectively. All animals in the Li/C group showed unchanged or increased plasma urea level from week 8 to week 16. The mean body weight in the two groups after 16 weeks was 326 g ± 16 (mean ± SD) in the C/C group and 262 g ± 30 in the Li/C group.

The variation in glomerular size in the Li/C group was remarkable. The small glomeruli were either connected to atrophic proximal tubules or without connection to a proximal tubule at all. No qualitative difference was seen at electron microscopy between these two populations of small glomeruli, and only atubular glomeruli were included in the quantitative study. The small glomeruli were denser with smaller capillary lumina and much less urinary space. In some areas the BM was wrinkled and the PBM seemed to be thickned. Fusion of FPs was only seen in a few places. No detachment of endothelial or epithelial cells from the BM was observed, but in several glomeruli it was noted that the nucleus of endothelial cells had a more peripheral location in the capillary loops (Fig. 5), and was not placed over the mesangium as it is normally.

The large, hypertrophic glomeruli were all connected to a normal proximal tubule. They appeared very much like the glomeruli from the control group. No sclerotic

Table 1. The dimensionless glomerular volume fractions

	V_V (Tu/Pol)	V_V (Cap/Tu)	V_V (Mes/Tu)	V_V (PBM/Tu)	V_V (BMLM/ Tu)	V_V (En/Tu)	V_V (Ep/Tu)	V_V (BMLM/ (Mes)
Control								
Mean	0.60	0.67	0.17	0.048	0.034	0.087	0.23	0.20
CV	0.075	0.054	0.094	0.30	0.14	0.16	0.22	0.18
Hypertrophic glomeruli								
Mean	0.61	0.62	0.19	0.052	0.041	0.11	0.29	0.21
CV	0.10	0.16	0.22	0.31	0.52	0.36	0.22	0.39
Atubular glomeruli								
Mean	0.65	0.55	0.26	0.080	0.053	0.13	0.33	0.21
CV	0.070	0.16	0.15	0.21	0.17	0.22	0.17	0.17
$2P$: Control versus hypertrophic glomeruli	NS	NS	NS	NS	NS	NS	NS	NS
$2P$: Control versus atubular glomeruli	NS	NS	0.001	<0.01	0.001	NS	<0.01	NS

NS $= 2P > 0.01$

The volume fractions (V_V) are with respect to the tuft (Tu) as reference space regarding the capillary luminal space (Cap), total mesangial region (Mes), peripheral basement membrane (PBM), endothelial cells (En) and epithelial cells (Ep). For the tuft (Tu), the string polygon (Pol) is used as reference space. Basement membrane-like material (BMLM) has Tu as well as Mes as reference space. The means and coefficients of variation (CV) between animals are presented

Table 2. Absolute volumes per glomerulus: for each subgroup the means and coefficients of variation between animals are given

	V(Tu) 10^6 μm^3	V(Mes) 10^6 μm^3	V(PBM) 10^6 μm^3	V(BMLM) 10^6 μm^3	V(En) 10^6 μm^3	V(Ep) 10^6 μm^3
Control						
Mean	0.580	0.100	0.028	0.020	0.050	0.129
CV	0.075	0.12	0.28	0.21	0.11	0.17
Hypertrophic glomeruli						
Mean	1.110	0.213	0.059	0.045	0.125	0.317
CV	0.102	0.23	0.38	0.54	0.41	0.24
Atubular glomeruli						
Mean	0.188	0.048	0.015	0.010	0.024	0.062
CV	0.069	0.11	0.24	0.17	0.21	0.14
$2P$: Control versus hypertrophic glomeruli	<0.001	0.001	<0.01	<0.01	NS	<0.001
$2P$: Control versus atubular glomeruli	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001

NS $= 2P > 0.01$

areas nor an increase in mesangial matrix was seen. The cells were unremarkable.

Stereological data showed that the volume fractions of the tuft components (mesangium, PBM, BMLM and endothelium) and epithelium did not show any difference between the control group and the glomeruli in the lithium group which were connected to a normal proximal tubule (Table 1). The group of atubular glomeruli showed larger volume fractions of mesangium, PBM, BMLM and epithelium compared to the controls. However, no differences was found between the experimental groups regarding the volume fractions of endothelium as well as BMLM related to the mesangium [V_V(BMLM/Mes)] (Table 1).

The absolute volumes of the various glomerular compartments showed significant changes in nearly all the

Table 3. Relative and absolute quantities per glomerulus of glomerular structures

	S_V(PBM) μm^{-1}	S_V(Mes) μm^{-1}	L_V(Cap) μm^{-2}	S(PBM) 10^6 μm^2	S(Mes) 10^6 μm^2	L(Cap) 10^3 μm^2	A(Cap) μm^2	T_h(BMT) nm	T_h(FP) nm
Control									
Mean	0.19	0.048	0.010	0.186	0.047	9.7	40	124	260
CV	0.059	0.11	0.12	0.059	0.11	0.12	0.17	0.064	0.044
Hypertrophic glomeruli									
Mean	0.16	0.049	0.009	0.292	0.089	16.7	45	142	269
CV	0.31	0.51	0.29	0.31	0.51	0.29	0.43	0.19	0.079
Atubular glomeruli									
Mean	0.21	0.071	0.016	0.062	0.021	4.6	24	163	251
CV	0.25	0.14	0.19	0.25	0.14	0.19	0.31	0.16	0.092
$2P$: Control versus hypertrophic glomeruli	NS	NS	NS	NS	NS	0.01	NS	NS	NS
$2P$: Control versus atubular glomeruli	NS	0.001	<0.01	<0.001	<0.001	<0.001	<0.01	<0.01	NS

NS = $2P > 0.01$

S_V and S, surface density and total surface area, respectively; L_V and L, length density and total length, respectively; A(Cap), average capillary cross-sectional area; T_h(BMT), the harmonic mean basement membrane thickness; T_h(FP), the harmonic mean width of foot processes. For each group the means and coefficients of variation (CV) between animals are presented

structures investigated in the lithium-treated animals (Table 2). Generally, an increase was seen in the structures in the hypertrophic glomeruli and a decrease in the atubular glomeruli compared to controls.

The surface densities and the length density of the capillaries only showed differences between the control glomeruli and the atubular glomeruli (Table 3). The surface density of the mesangium towards the urinary space and the length density of the capillaries were increased by 48% and 60%, respectively, in the atubular glomeruli compared to the control glomeruli. As expected, the absolute surface of PBM and the absolute surface of mesangium towards the urinary space were increased, although not statistically significant, in the hypertrophic glomeruli by 57% and 89%, respectively, compared to the control glomeruli, and decreased by 67% and 55%, respectively, in the atubular glomeruli. The total length of the glomerular capillaries was increased by 72% and decreased by 53% in the hypertrophic and atubular glomeruli, respectively. The average cross-sectional capillary area showed a relatively large interindividual variation in the three groups, but was significantly decreased in the atubular glomeruli compared to controls.

As shown in Table 3, a 31% thickening of the glomerular capillary basement membrane was seen in the atubular glomeruli. No significant thickening was found in the hypertrophic glomeruli. The harmonic mean width of the FPs was not changed between the groups. The harmonic mean width of FPs over the mesangial areas was 2%, 14% and 9% larger than the mean width of foot processes over the peripheral basement membrane in the controls, hypertrophic and atubular glomeruli, respectively. The distributions of FP width are shown in Fig. 6. As expected (Gundersen et al. 1980), the curve from the control group was bimodal with peaks at about 500 and 900 nm. The curves from the atubular and hypertrophic glomeruli were slightly abnormal with loss of the bimodality due to loss of FPs around 500 nm. The curves showed peaks at about 900 nm.

Comparison of the coefficients of isomorphic change showed that they were within normal limits in the hypertrophic glomeruli (Table 4). The coefficients $S^{3/2}$(PBM)/

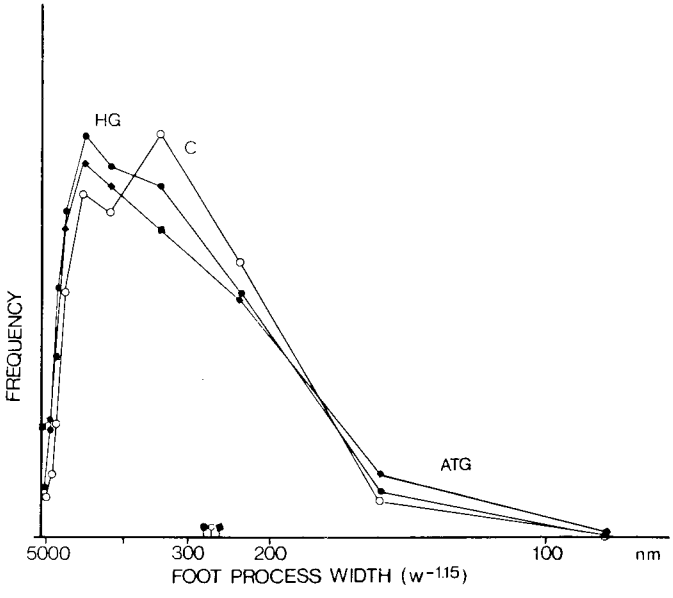

Fig. 6. The distribution of true foot process width in the controls (C), in atubular glomeruli in lithium-treated rats (ATG), and in hypertrophic glomeruli in lithium-treated rats (HG). The group mean values are indicated on the reciprocal abscissa which is equidistant on a scala of width$^{-1.15}$ in order to facilitate the unusual separation of the two peaks in the distribution of normal foot process width

Table 4. Coefficients of isomorphic change: for each subgroup the means and coefficients of variation (CV) between animals are presented

	$\frac{S^{3/2}(PBM)}{V(Tu)}$	$\frac{S^{3/2}(Mes)}{V(Tu)}$	$\frac{L^3(Cap)}{V(Tu)}$	$\frac{A^{3/2}(Cap)}{V(Tu)}$
			10^6	10^{-3}
Control				
Mean	139.9	17.5	1.65	0.445
CV	0.077	0.20	0.44	0.22
Hypertrophic glomeruli				
Mean	146.8	25.5	3.33	0.288
CV	0.43	0.66	0.52	0.74
Atubular glomeruli				
Mean	83.8	15.9	0.55	0.639
CV	0.34	0.18	0.54	0.44
$2P$: Control versus hypertrophic glomeruli	NS	NS	NS	NS
$2P$: Control versus atubular glomeruli	<0.01	NS	<0.01	NS

NS = $2P > 0.01$

V(Tu) and L^3(Cap)/V(Tu) were decreased by 40% and 67%, respectively, in the atubular glomeruli below that expected from the already decreased tuft volume. No significant changes were found concerning $S^{3/2}$(Mes)/V(Tu) and $A^{3/2}$(Cap)/V(Tu).

Discussion

The findings in lithium-induced uraemia of a loss of connections to proximal tubules in some glomeruli and a large variation of glomerular volume due to hypertrophy of others (Marcussen et al. 1989) offers a unique possibility for investigating a very heterogeneous population of glomeruli in chronic renal disease. Hypertrophy of glomeruli has most often been studied in partially nephrectomized rats (Shea et al. 1978). The recent finding of atubular glomeruli in rats with chronic renal failure due to lithium treatment (Marcussen et al. 1989) provided a good explanation for the reduction in renal function. The hypertrophic glomeruli seemed to attempt to compensate for the reduction in renal function without success, and an investigation of such hypertrophic glomeruli is relevant with regard to the hyperfiltration hypothesis (Brenner 1985; Hostetter 1984). According to this hypothesis, the remaining glomeruli in chronic renal diseases try to compensate the loss of kidney function by hyperfiltering, but this hyperfiltration is detrimental to the structure of the glomerulus and leads to segmental sclerosis and ultimately global sclerosis. The implications of the results for the pathogenesis of chronic renal disease are important and will be discussed in the light of prevailing and new theories.

The atubular glomeruli showed the most pronounced changes. These glomeruli showed an increase in the volume fractions of mesangium, BM material and epithelial cells, but no increase in the fraction of BMLM in the mesangium. The average cross-sectional area of the capillary lumen was decreased in the atubular glomeruli, and it seems possible that this decrease in the reference volume is the major reason for the above-mentioned increased relative volumes. Volume fractions are generally difficult to interpret and the explanation must therefore be taken with care. Expectedly, the absolute volumes showed a decrease of the different structures compared to normal. The thickness of the capillary BM was increased. This increase could be due to a decreased degradation or could be explained by an increased synthesis of new BM material, like the BM thickening seen in diabetes mellitus et al. (Hirose et al. 1982; Brekke et al. 1985), where, however, the reason for the thickened capillary BM is probably quite different.

The hypertrophic glomeruli that were connected to normal proximal tubules showed no changes in the volume fractions compared to normals, whereas the absolute values showed the expected increase. The capillaries had normal cross-sectional areas and showed no increase in BM thickness. It was, however, found that the investigated structures in this group had a larger variation between animals than in the controls.

It is remarkable how well the width of the FPs was regulated. The interindividual variation was very low in all three groups, indicating a special biological importance of the distance between neighbouring filtration slits in according with earlier studies of this structure (Gundersen et al. 1980; Seefeldt et al. 1981). The hypertrophic and atubular glomeruli (mostly the last one) showed an abnormal distribution of FP width with fewer FPs of medium width; despite this, however, the mean FP width was normal in all three groups. The reason for the abnormal distribution of FP width is unknown. It might be due to proteinuria, which, however, normally leads to a larger mean (Seefeldt et al. 1981). Lithium might have an specific effect, as well as other components of the uraemia.

The hypertrophic glomeruli showed isomorphic changes, that is, the changes of all the investigated structures are in balance with the change in tuft volume. In a study of streptozotocin diabetic rats, Østerby and Gundersen (1980) also found isomorphic changes in glomerular structural composition after 47 days of diabetes. The atubular glomeruli, on the other hand, showed changes of the peripheral filtration surface and of the length of the capillaries that were decreased more than could be explained by the decrease in tuft volume. This might be considered understandable since the atubular glomeruli have no use of their filtration surface and their capillaries have no other role than functioning as a shunt to the interstitial capillary network.

There are good reasons to believe that the hypertrophic glomeruli were hyperfiltering (Marcussen et al. 1989), and despite that we did not find any significant sclerosis in these glomeruli. This is in contrast to previous studies in which it has been shown that hyperfiltering glomeruli develop segmental sclerosis (Brenner 1985; Hostetter 1984). There are several possible explanations for this discrepancy. First, there are differences regarding the animal models used. In previous studies renal ablation with the removal of approximately 85% of the normal kidney tissue was used to induce hyperfiltration. Secondly, in renal ablation studies, animals given a high protein diet develop glomerulosclerosis earlier and the sclerosis is more severe than in animals given low protein diet (Hostetter et al. 1986). As the lithium-treated rats were in a state of malnutrition with a significantly lower body weight than the controls, they most likely had a low protein intake. However, we are presently doing a study on lithium-treated rats with high protein intake, and it appears that also these animals do not develop glomerulosclerosis after 16 weeks (Marcussen et al., study in progress). Thirdly, in the present study we used newborn rats, and their developing renal tissue could react different than renal tissue in adult rats. Fourthly, one cannot exclude the possibility that a longer period of study would have led to changes in the hypertrophic glomeruli. However the lithium model of chronic nephropathy, at least morphologically, resembles the human chronic non-glomerular renal diseases more than the renal ablation does.

Other studies have also failed to demonstrate a correlation between hyperfiltration and glomerulosclerosis. In a study by Yoshida et al. (1988), using micropuncture and histological sections, the authors found that the degree of early glomerular hyperfiltration or hypertension did not correlate with the extent of subsequent structural damage. In a study on congenitally obstructed kidneys, Steinhardt et al. (1988) found that focal segmental glomerulosclerosis occurred almost exclusively in areas of the kidney also involved with acute and chronic inflammation and fibrosis.

In many studies (Bohle et al. 1977; Mackensen et al. 1981; Møller and Skriver 1985), including some in lithium-treated rats (Ottosen et al. 1984), correlations have been found between the reduction in renal function and the degree of fibrosis as well as the reduced amount of proximal tubules. Two main hypotheses have been put forward concerning these relationships. One hypothesis favours the role of the interstitial changes in supposing that an increase in the interstitial connective tissue may reduce the glomerular filtration rate (GFR) by means of constriction of the post-glomerular capillaries (Bohle et al. 1977). The other hypothesis proposes that a reduced reabsorption of tubular fluid by pathologically damaged proximal tubular cells may reduce the GFR by way of the negative feed-back mechanism triggered by an increased load of sodium chloride to the macular densa region of the distale tubules (Bohle and Thurau 1974; Møller and Skriver 1985).

The observations in this study naturally lead to the proposal of a third hypothesis. We suggest that in many non-glomerular chronic renal diseases a progressive primary destruction of the tubules takes place, and that this destruction ultimately leads to total or partial atrophy of the tubules with their substance being replaced by fibrosis. The glomeruli are not primarily involved in this process but the tubular atrophy gives rise to the formation of atubular non-filtering glomeruli. This hypothesis gains support from studies of chronic renal diseases with severe atrophy of the proximal tubules (Møller et al. 1984; Christensen et al. 1982) and also from the micropuncture study by Kramp et al. (1974) demonstrating the absence of flow in heavily atrophied tubules. We have also demonstrated, in human chronic pyelonephritis many atubular glomeruli, predominantly in severely fibrotic areas (Marcussen and Olsen 1990). It has to be noted that a further decrease in renal function in several rats occurred from week 8 to week 16 in the present study despite the removal of the nephrotoxic agent (lithium). This indicates that the process is self-perpetuating, leading to continuous tubular destruction with the formation of more atubular glomeruli. The reason for this continuous destruction of the remaining functioning tubules is speculative.

In conclusion, this ultrastructural morphometric study demonstrates that the glomerular population in lithium-induced chronic interstitial nephropathy is extremely heterogeneous. The glomeruli with the most pronounced changes in ultrastructural composition are those that are not connected to proximal tubules, whereas the hypertrophic glomeruli only show changes proportional to their larger size. The results indicate that the destruction of glomeruli is mediated by tubular changes with a loss of proximal tubular substance and not by the accompanying hypertrophy and hyperfiltration in glomeruli connected to normal proximal tubules.

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