Virchows Archiv A Pathol Anat (1991) 419:281–289

Virchows Archiv A
Pathological Anatomy and Histopathology

Atubular glomeruli, renal function and hypertrophic response in rats with chronic lithium nephropathy

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Received January 17, 1991 / Accepted April 24, 1991

Summary. Experimental lithium nephropathy was induced by administering lithium orally to newborn rats for 8 weeks; thereafter the rats were randomized into four groups which were studied after 8 weeks of further treatment. One group was left untreated, one group was given a high (40%) protein diet, one group was unilaterally nephrectomized, and one group was unilaterally nephrectomized and received high protein diet after nephrectomy. Comparable control groups (not lithium-treated) were also studied. Stereological methods were used to estimate the total volume of different parts of the nephron, interstitial fibrosis, and the distribution of the volume of individual glomeruli. The structural integrity between the glomerulus and the proximal tubule was investigated on serial sections. No sclerotic glomeruli were present. The most extensive degree of hypertrophy with almost a doubling of the total volume of proximal and distal tubule cells was seen in the groups that were both nephrectomized and fed a high protein diet. In both controls and lithium-treated animals, high protein and nephrectomy induced enlargement of the glomerular tufts to volumes from 4 to 5 times the normal size. A pronounced heterogeneity of the glomerular population was found in the lithium-treated groups with 36–54% atubular glomeruli with small volumes, and 34–48% enlarged glomeruli connected to qualitatively normal proximal tubules. Only glomeruli connected to proximal tubules had a potential for hypertrophy. In multiple regression analysis the percentage of glomeruli connected to normal proximal tubules was correlated with the reciprocal of plasma creatinine, but the volume of fibrosis also contributed to the decreased renal function.

Key words: Atubular glomeruli – Glomerular volume – High protein diet – Kidney – Lithium

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Introduction

In previous studies we have shown that lithium has a more harmful effect on kidneys in newborn rats than in adult rats (Christensen and Ottosen 1983, 1986). In adult rats, the minor renal changes caused by lithium may be compared with those observed in humans treated with therapeutic doses of lithium, where a decrease in kidney function is only seen rarely (Schou 1990). Stereological investigation of the kidney after lithium treatment of newborn rats has shown that many atubular glomeruli (i.e. apparently normal glomeruli but without connection to a proximal tubule) were present, and that the volumes of these glomeruli were decreased (Marcussen et al. 1989). A few glomeruli, all connected to normal proximal tubules, were hypertrophic. Ultrastructurally, the small, atubular glomeruli showed only minute changes while the hypertrophic glomeruli had a qualitatively normal structure (Marcussen et al. 1990).

Hypertrophy and hyperfiltration of glomeruli after renal injury such as ablation is supposed to lead to segmental and later to global glomerulosclerosis (Brenner 1985). A high protein diet, given to normal or uninephrectomized animals, has in some studies been shown to lead to glomerulosclerosis (Bertani et al. 1989; Hostetter et al. 1986; Kenner et al. 1985). Uninephrectomy alone generally leads to an increase in the structural components and in the size of the contralateral kidney (Bradley et al. 1974; Seyer-Hansen et al. 1985). While the mechanism underlying the hypertrophy of the glomeruli in general has been studied in various conditions, relatively little effort has been made to investigate the mechanisms involved in the hypertrophy of individual glomeruli when the kidney is exposed to various injuries. We therefore found it of importance to study the glomerular and tubulo-interstitial changes in a model where the kidneys were subjected to an additional challenge superimposed to the injury caused by lithium itself. The aims of the study were: (1) to investigate the possible relationship

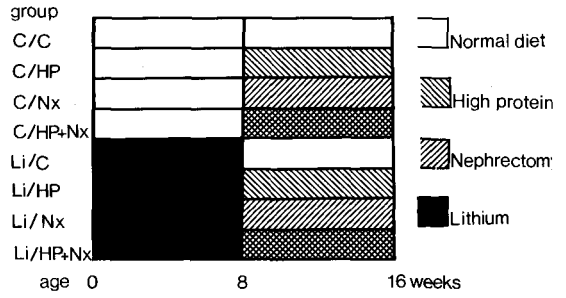

Fig. 1. The experimental protocol. Four groups of newborn rats were left untreated from time of birth and four groups of newborn rats were treated with lithium. After 8 weeks the animals were randomized into eight groups as shown

between marked structural changes and renal function; and (2) to study the renal response to injury, especially the capacity of glomeruli to hypertrophy, in further detail.

Materials and methods

Seventy newborn female Wistar rats were used in the study. They were kept with their mothers for the first 3 weeks, i.e. the suckling period. During the first 8 weeks, the litters and mothers were given either a control diet with 19% protein content (Altromin no. 1320; Altromin International, Lage, FRG) or identical pellets to which were added 50 mmol lithium chloride/kg. At the age of 8 weeks control (C) and lithium-treated (Li) rats were randomized according to their mean plasma urea concentration into one of the following treatment regimens (Fig. 1): control diet (group C/C and Li/C), high protein diet (40%) (group C/HP and Li/HP), nephrectomy (C/Nx and Li/Nx), and high protein and nephrectomy (C/HP + Nx and Li/HP + Nx). At the age of 14 weeks the systolic blood pressure was recorded by the non-invasive tail cuff method, using a blood pressure recorder (Ugo Basile, Italy). Further non-invasive investigations were performed at the age of 15 weeks, and these results have been published elsewhere (Christensen et al., submitted for publication).

At the age of 16 weeks, the animals were anaesthetized with halothane 1–3% in nitrous oxide/oxygen. The abdomen was opened and the vascular pedicle of the right kidney was ligated. Blood was drawn from the aorta for analysis and the left kidney was perfused in situ with 1% glutaraldehyde in Tyrode's buffer (total osmolality 350 mosmol/kg) through the abdominal aorta at a pressure of 180 mmHg for 2.5 min (Maunsbach 1966). After immersion in the same fixative for at least 24 h the kidney was weighed and cut into a series of slices of alternating thickness (2 mm and 1 mm) by use of a set of fixed razor blades with random position with respect to the kidney (Gundersen et al. 1988a). All 2-mm-thick slices were dehydrated and embedded in paraffin. They were cut at 3 μm and stained with periodic acid-Schiff (PAS) and Sirius red. From every second rat, one-third of the 2 mm slices were sampled systematically and cut into 40 serial sections at a nominal thickness of 6 μm by the same technician. These sections were stained with Sirius red.

The cortex was defined as the zone containing proximal tubules, i.e. including the outer stripe of the outer medulla. Regarding all stereological estimates, a glomerulus was defined as the glomerular tuft. The glomeruli were regarded as globally isotropically oriented particles. The 3-μm-thick, Sirius-red-stained sections were studied at a magnification of ×31, and the volume fraction of the cortex, V_v *(cortex/kidney)*, was estimated using point counting (Gundersen et al. 1988a; Weibel 1979). The volume fractions of proximal tubule cells, V_v *(PTC/cortex)*, proximal tubule lumina, V_v *(PTL/cortex)*, distal tubule cells, V_v *(DTC/cortex)*, fibrotic interstitium, V_v *(fib/cortex)*, total interstitium, V_v *(int/cortex)*, and glomerular tufts, V_v *(glom/cortex)*, were estimated on the same sections by point counting at a magnification of ×320 (Marcussen 1990). From the cortical volume fraction in the whole kidney and the respective volume fractions in the cortex the total volumes of fibrosis, glomerular tufts, and proximal and distal tubule cells were calculated assuming that the specific gravity of kidney is 1 g/cm^3 (Seyer-Hansen et al. 1985).

Assuming global isotropy of proximal tubules the length density of the proximal tubules within the cortex, L_v *(PT/cortex)* is $2Q_A$ *(PT/cortex)*, where Q_A *(PT/cortex)* is the ratio between the number of proximal tubular profiles and the area of the cortex in the unbiased counting frame (Gundersen et al. 1988a). L_v *(PT/cortex)* was estimated at a magnification of ×320. The average cross sectional area perpendicular to the axial direction of a tubular structure is $\bar{a} = V/L$, which is estimated by V_v/L_v. Assuming a circular cross-section, the average diameter of tubules can be calculated as $\bar{d} = 2\sqrt{(\bar{a}/\pi)}$, and the average height, $\bar{h}$, of tubular cells is then calculated as $(\bar{d}_e - \bar{d}_l)/2$ where suffixes e and l indicated external and luminal diameter, respectively.

In a subsample of animals the volumes of individual glomeruli were estimated, and the connection between the glomeruli and the proximal tubules investigated. The total glomerular profile including Bowman's capsule was followed from top to bottom in serial sections, so that the origin of the proximal tubule (urinary pole) could be located. In each glomerulus the proximal tubule was classified as normal, atrophic or absent. An average of 15 ± 3 (±SD) glomeruli in each sampled kidney was investigated. The glomeruli were sampled using the disector, which is a stereological sampling device ensuring that arbitrary-shaped particles can be sampled unbiasedly and uniformly using two planes through the specimen (Gundersen et al. 1988b; Sterio 1984). The individual volume of each disector-sampled glomerulus was estimated in the serial sections using the Cavalieri principle (Gundersen and Jensen 1987), and from these individual volumes were the mean glomerular volume calculated (Marcussen et al. 1989). It is necessary to know the mean section thickness of the serial sections for the estimation of the glomerular volume. An unbiased estimate of the mean section thickness of the serial sections was made using a stereological method that has been described in detail (Marcussen et al. 1989). The mean section thickness was 7.6 ± 0.4 μm (mean ± SEM, $n = 5$) and this value was used in the calculations.

Plasma creatinine was determined by an enzymatic colorimetric method, using an automated process (Hitachi 704/705).

Statistical comparisons between groups were tested using analysis of variance, which partitioned total variance in the data into partial variances attributable to lithium, high protein diet, nephrectomy and interactions between these components. Multiple regression analysis was used to fit a hierarchical model to explain the variation in renal function (reciprocal plasma creatinine) and blood pressure in terms of structural variables. For that purpose the rats were divided into two groups, which were investigated separately: lithium- and not lithium-treated. Statistical differences within groups were tested using the paired t-test. Statistical significance was accepted at the 5% level.

Results

The significant differences attributable to lithium, diet and nephrectomy are shown in Table 1. Interaction between lithium and nephrectomy was found regarding body weight. The body weight was found to be decreased more by the combination of nephrectomy and lithium than would be expected by simple addition of the effects of these two treatments. In the lithium-treated groups the mean body weight was 232 g ± 28 (SD), significantly less than that of 276 g ± 25 (SD) in the non-lithium-treat-

Table 1. Analysis of variance

Variable	Significance of effect attributable to				
	Lithium (A)	High protein diet (B)	Nephrectomy (C)	Interactions (A × C)	Interactions (B × C)
Kidney weight	↓$P<0.001$	↑$P<0.001$	↑$P<0.001$	NS	↑$P<0.005$
Body weight	↓$P<0.001$	↑$P<0.05$	NS	↓$P<0.05$	NS
Blood pressure	↑$P<0.001$	NS	NS	NS	NS
Plasma creatinine	↑$P<0.001$	↓$P<0.005$	↑$P<0.005$	NS	NS
V(Glom)/g body wt.	NS	NS	↑$P<0.001$	NS	NS
V(PTC)/g body wt.	↓$P<0.001$	↑$P<0.001$	↑$P<0.001$	NS	NS
V(DTC)/g body wt.	↓$P<0.001$	NS	↑$P<0.001$	NS	NS
V(Fib)	↑$P<0.001$	NS	↑$P<0.001$	NS	↑$P<0.05$
V(IS)	↑$P<0.001$	↑$P<0.05$	↑$P<0.001$	↑$P<0.05$	↑$P<0.05$
Height of PTC	↓$P<0.01$	↑$P<0.01$	↑$P<0.001$	NS	NS
$\bar{v}_N$(Glom), all glomeruli	NS	↑$P<0.001$	↑$P<0.001$	NS	↑$P<0.005$
$\bar{v}_N$(Glom), connected to normal PT	↑$P<0.001$	NS	↑$P<0.05$	NS	NS
% Glomeruli with normal PT	↓$P<0.001$	NS	NS	NS	NS
% Atubular glomeruli	↑$P<0.001$	NS	NS	NS	NS

All interactions of type (A × B) and (A × B × C) were non-significant
Arrows indicate that the values behind this *P*-value generally are elevated (↑) or decreased (↓)
V, Absolute volume; $\bar{v}_N$(Glom), mean glomerular volume; DTC, distal tubule cells; Glom, glomerular tuft; IS, interstitial tissue; Fib, interstitial fibrosis; PTC, proximal tubule cells; PT, proximal tubule; NS, not significant

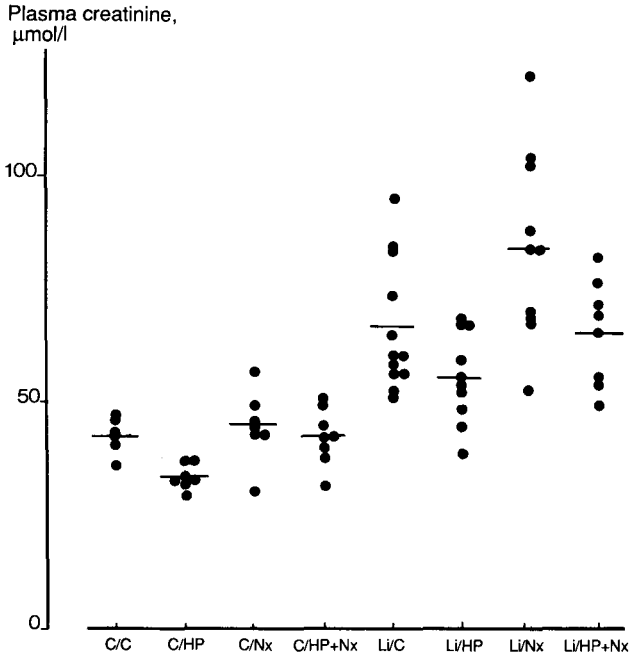

Fig. 2. Plasma creatinine in the eight groups of rats. The *horizontal bars* indicate group means

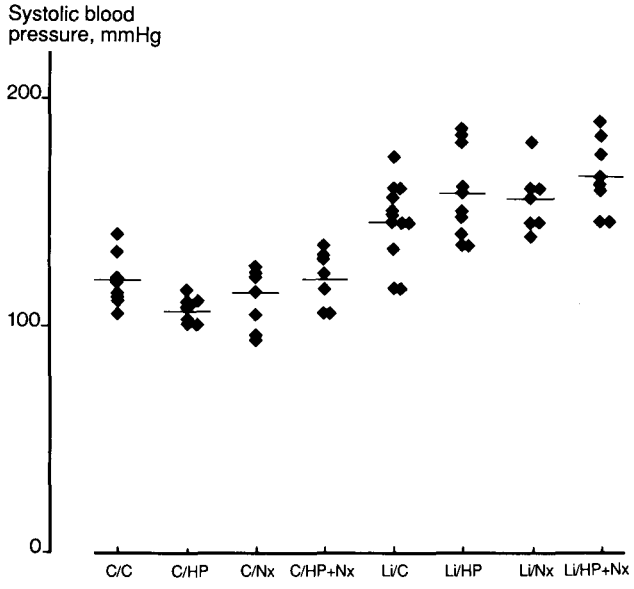

Fig. 3. Tail-cuff systolic blood pressure in the eight groups of rats. The *horizontal bars* indicate group means

ed groups. Lithium treatment was followed by a decrease in kidney weight, whereas high protein diet and nephrectomy led to an increase (Table 1). Positive interaction existed between diet and nephrectomy; the combination of these two led to an increase in kidney weight of 101% and 141% above respective controls in groups Li/HP + Nx and C/HP + Nx, respectively.

Lithium treatment was followed by an increase in plasma creatinine (Fig. 2). High protein feeding caused a remarkable decrease in plasma creatinine levels in all groups. Blood pressure was elevated in all lithium-treated groups (Fig. 3). In contrast, high protein and nephrectomy seemed to be of no importance for the elevation of blood pressure.

Macroscopically, all kidneys from the lithium-treated rats showed some degree of polycystic changes in the cortical areas. The kidneys from animals additionally exposed to uninephrectomy or to the combination of high protein and uninephrectomy were enlarged and the cysts more pronounced.

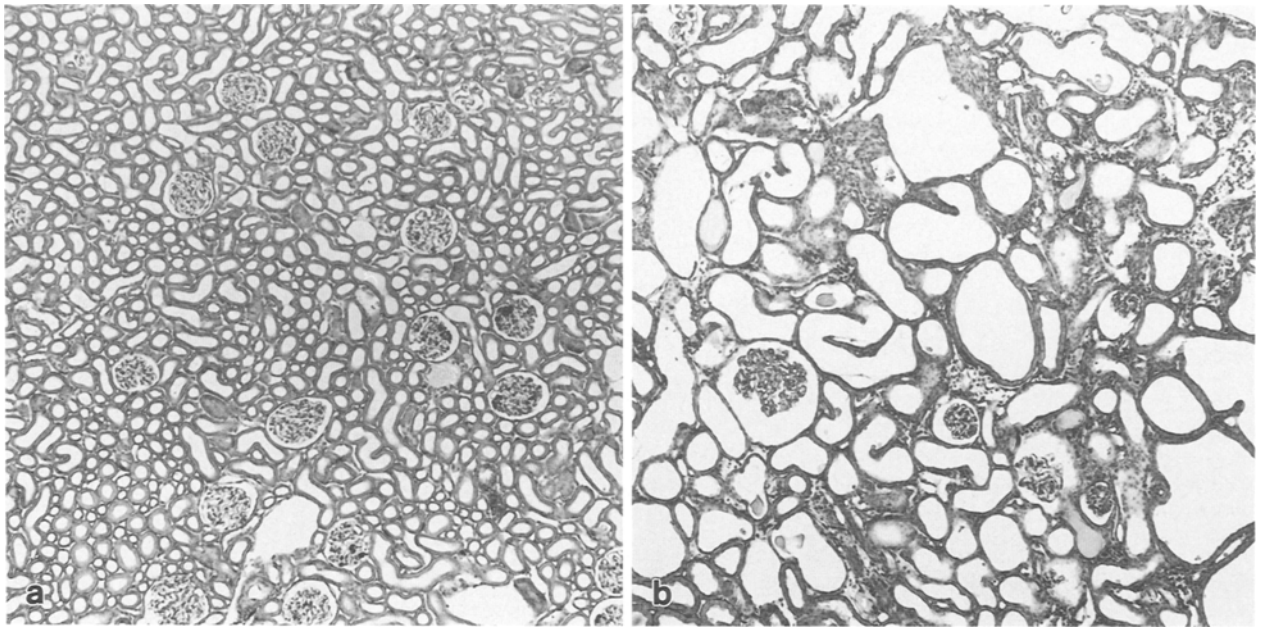

Fig. 4. a Cortex from a normal rat (group C/C) compared with **b** from a lithium-treated, nephrectomized rat fed high protein diet (group Li/HP+Nx). Dilatations of tubules and interstitial tissue increment is present. The glomeruli are intact with open capillaries and without signs of sclerosis. Periodic acid-Schiff (PAS), × 50

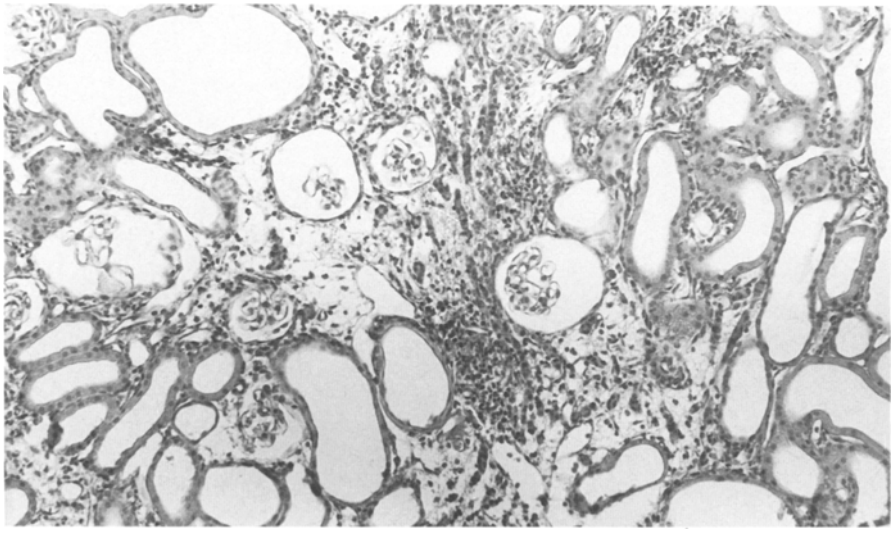

Fig. 5. Kidney from lithium-treated rat (group Li/HP+Nx) showing interstitial fibrosis, tubular atrophy and inflammatory cell infiltrates focally present. PAS, × 130

Microscopically, the kidneys in the high protein and nephrectomized groups showed dilatations of both distal and proximal tubules. In the C/HP+Nx, and especially in the Li/HP+Nx group, the tubular dilatations were pronounced (Fig. 4). In the lithium-treated groups cysts were present in the cortex. No fibrosis was found in the controls, whereas the lithium-treated kidneys showed interstitial fibrosis and chronic inflammatory cell infiltrates focally (Fig. 5). In the Li/HP+Nx group oedema of the interstitium was found in some kidneys. Subjectively, the glomeruli in the lithium-treated groups varied in size but they had open capillaries and showed no sclerosis (Fig. 4). In a few kidneys in the Li/HP+Nx group several large glomeruli had collapsed tufts with only few recognizable capillaries. Many tubules in the lithium-treated groups were atrophic and could not be seen to be of either distal or proximal origin. In the medulla slight fibrosis was present in stripes and some tubular dilatation was seen.

On stereological investigation no significant changes were found in the relative volume of cortex among the groups (data for relative structural values not shown). The absolute glomerular volume per gram body weight was increased. In all the lithium-treated groups the volume fractions and absolute volume of proximal tubule cells were decreased to roughly 75% of the respective

Table 2. Absolute structural values (±SD) in the eight groups

Group	(*n*)	Proximal tubule cells (mm^3/g body wt.)	Distal tubule cells (mm^3/g body wt.)	Fibrosis (mm^3)	Interstitial tissue (mm^3)	Height of proximal tubule cell (μm)
C/C	(8)	1.3 ±0.2	0.26±0.01	41±8	52±14	10.4±1.0
C/HP	(7)	1.5 ±0.4	0.34±0.10	42±18	50±18	11.8±1.9
C/Nx	(7)	2.0 ±0.3	0.47±0.16	62±23	78±24	12.6±1.7
C/HP+Nx	(8)	2.3 ±0.4	0.48±0.11	96±34	122±43	14.2±2.2
Li/C	(12)	0.86±0.17	0.20±0.05	69±24	98±27	10.9±1.8
Li/HP	(9)	1.1 ±0.2	0.24±0.08	70±34	99±38	10.5±1.0
Li/Nx	(10)	1.4 ±0.3	0.32±0.08	108±48	167±70	10.8±1.5
Li/HP+Nx	(7)	2.0 ±0.5	0.37±0.08	147±59	229±104	12.5±1.4

Table 3. Mean volume and percentage of glomeruli connected to normal, atrophic or no tubule

Group	(n)	$\bar{v}_N$ of glomeruli with normal proximal tubules 10^6 μm^3	$\bar{v}_N$ of glomeruli with atrophic tubules 10^6 μm^3	$\bar{v}_N$ of atubular glomeruli 10^6 μm^3	% Glomeruli with normal proximal tubules	% Glomeruli with atrophic tubules	% Atubular glomeruli
C/C	(4)	0.68±0.11	–	0.48±0.62	96.6±4.5	0	3.4±4.5
C/HP	(4)	0.76±0.12	–	0.33±0.45	93.6±5.1	0	6.4±5.1
C/Nx	(4)	0.83±0.11	–	0.72	97.8±3.9	0	2.2±3.9
C/HP+Nx	(4)	1.25±0.20	–	–	100	0	0
Li/C	(5)	1.04±0.21	0.41±0.31	0.24±0.08	48.1±19.0	15.8±10.9	36.1±13.5
Li/HP	(5)	1.57±0.69	0.45±0.21	0.29±0.36	44.3±26.3	13.8±10.4	42.0±18.6
Li/Nx	(5)	1.78±1.05	0.31±0.06	0.24±0.07	33.6±24.3	12.8±13.3	53.6±17.1
Li/HP+Nx	(4)	2.19±0.63	0.67±0.18	0.26±0.09	47.9±25.3	14.4±16.0	37.8±12.4

Values are mean±SD

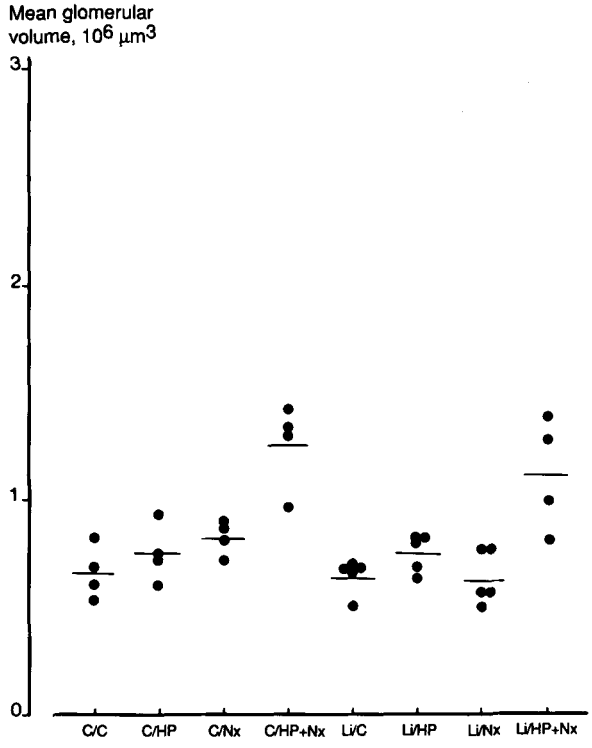

Fig. 6. Mean volume ($\bar{v}_N$) of all glomeruli in each group. The *horizontal bars* indicate group means

controls (Table 2). The volume of proximal tubule cells per gram body weight was increased in the groups given high protein or nephrectomized (Table 2). The same was found regarding the cell heights of proximal tubule cells in these groups, whereas the cell heights were decreased by lithium treatment. The lithium-treated rats were found to have decreased volume of distal tubule cells per gram body weight. The relative and absolute amount of fibrotic and interstitial tissue were increased in the lithium-treated animals (Table 2), and the absolute volume was also increased in the nephrectomized groups (Table 2). The effects of lithium and nephrectomy on the total volume of interstitial tissue were more than additive showing significant interaction (Table 1).

One-third to one-half of the glomeruli in the lithium-treated groups were found to be without connection to a proximal tubule (atubular) (Table 3). Only 34–48% were connected to a normal proximal tubule in these groups. In the controls nearly all glomeruli were found to be connected to a normal proximal tubule.

The volume of glomeruli was slightly increased by nephrectomy or high protein (Fig. 6), and positive interaction was found by the combination of high protein and nephrectomy (Table 1). In all lithium-treated groups the volume of glomeruli connected to normal proximal tubules was significantly larger than the volume of glomeruli connected to atrophic proximal tubules or the

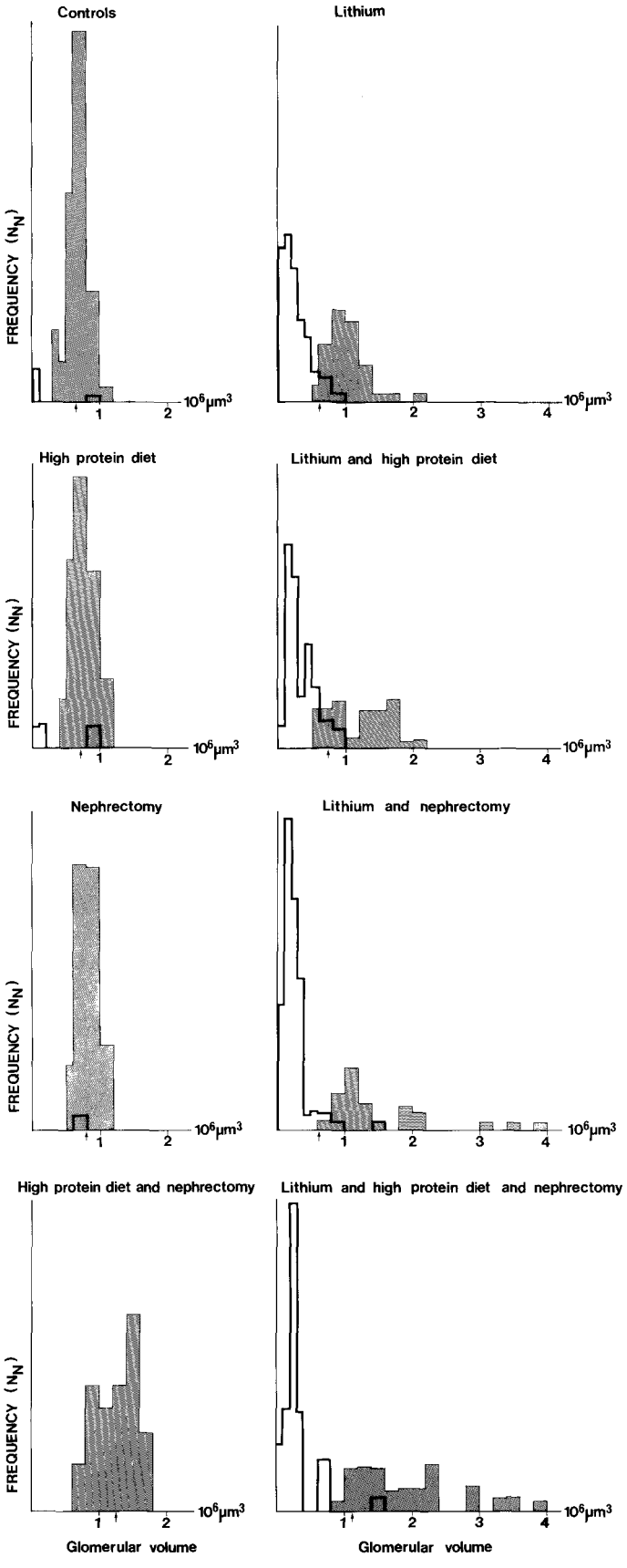

Fig. 7. The distribution of glomerular volumes. *Hatched histograms*, distributions of volumes of glomeruli connected to normal proximal tubules; *open histograms*, distributions of volumes of atubular glomeruli or glomeruli connected to atrophic tubules. *Arrow*, mean volume ($\bar{v}_N$) of all glomeruli in each group

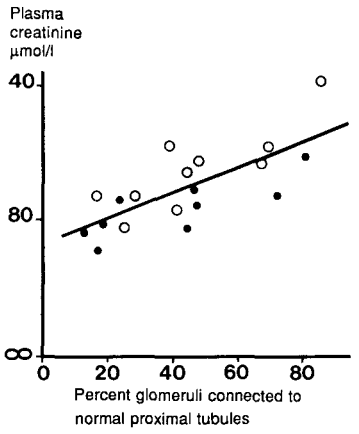

Fig. 8. Relationship between percentage of glomeruli connected to a normal proximal tubule and the reciprocal plasma creatinine. Regression line obtained in groups Li/C and Li/HP (*open circles*), and groups Li/Nx and Li/HP+Nx (*closed circles*) ($r=0.75$, $2P<0.001$)

volume of atubular glomeruli (Table 3). The mean volume of glomeruli connected to normal proximal tubules in the lithium-treated groups was 2–4 times larger than that of the glomeruli in the C/C group (Table 3). The volume of atubular glomeruli was only one-third of the mean volume in the C/C group.

The distribution of glomerular volumes showed that the variation in the lithium-treated groups was huge compared with the control groups (Fig. 7). Many small glomeruli were present and some gigantic ones with volumes up to 4×10^6 μm^3. As can be seen from the distributions only glomeruli connected to normal proximal tubules had hypertrophied. The distributions of the glomerular volumes in the C/HP and C/Nx groups were not different from that of the C/C group, except that the mean volumes were increased. In the C/HP+Nx group the variance and mean of the glomerular volumes were increased compared with the C/C group (Fig. 7).

Multiple regression analysis revealed that no structural variables could explain the variation in plasma creatinine or blood pressure in the controls (non-lithium-treated groups). In the lithium-treated groups no meaningful correlation was found between structural variables and blood pressure. Performing a multiple regression analysis stepwise showed that the reciprocal of plasma creatinine was correlated to both the percentage of glomeruli with connection to normal proximal tubules (Fig. 8) and inversely to the volume of fibrosis. The variation of the reciprocal plasma creatinine was determined by the percentage of glomeruli connected to normal proximal tubules and the volume of fibrosis (both variables were significant alone and in combination, irrespective of their sequence in the hierarchical analysis). The highest level of significance was found by the combination of the percentage of glomeruli connected to normal proximal tubules and the volume of fibrosis (multiple $r=0.83$, $P<0.0001$). Addition of other variables did not add further significant explanation of the decrease in renal function.

Discussion

Lithium-induced renal changes in rats have been described previously (Christensen et al. 1982; Marcussen et al. 1989; Ottosen et al. 1984). It is only possible to induce severe interstitial nephropathy with chronic renal failure and atubular glomeruli if lithium is given to the animals from the time of birth. Previous studies have shown that the first morphological and functional lithium-induced changes occur in the distal nephron (Dørup et al. 1988; Walker et al. 1986). It has been found that the fibrosis occurs primarily around the affected collecting ducts, and it is hypothesized that this initial fibrosis leads to secondary changes in the proximal tubules (Christensen et al. 1982). Atrophy of the proximal tubules resulting from interstitial fibrosis may thus be important in the lithium nephropathy and could explain the formation of atubular glomeruli. The concept that the formation of atubular glomeruli is a secondary condition is supported by the findings that large numbers of atubular glomeruli have also been found in patients with chronic pyelonephritis (Marcussen and Olsen 1990), in rats with chronic pyelonephritis (Heptinstall et al. 1963), in patients with renal artery stenosis (Marcussen, in press) and in adult rats with cisplatin-induced chronic nephropathy (Marcussen 1990). In the kidneys from patients with chronic pyelonephritis and from rats with cisplatin-induced chronic nephropathy, the formation of atubular glomeruli is probably due to direct destruction of tubules caused by the inflammatory or toxic process in the kidney, whereas the formation of atubular glomeruli in kidneys with renal artery stenosis is probably caused by ischaemia. However, the possibility cannot be excluded that in the rats fed lithium from birth the disconnection between tubules and glomeruli is an induced developmental defect.

Renal ablation is followed by hypertrophy of the remaining renal tissue including overall hypertrophy of the glomeruli (Lombet et al. 1989; Seyer-Hansen et al. 1980), which has also been demonstrated in the present study, where the size distribution of glomerular volume has also been studied. The capability to hypertrophy is also present in rats with chronic interstitial nephropathy, and despite the damage produced by lithium, all parts of the intact nephrons could hypertrophy. In contrast, in the lithium-treated animals the atubular glomeruli were not larger in the nephrectomized groups when compared with the groups with two kidneys. These findings indicate that glomerular hypertrophy is dependent on the presence of a functioning tubule. At the whole kidney level a circulating "renotropin" has been proposed to mediate the renal hypertrophy (Malt 1983; Yoshida et al. 1989). The present study shows that another factor also has to be considered and that feedback from the tubule to the glomerulus seems to mediate the glomerular hypertrophy. This feedback is obviously lacking in the atubular glomeruli and could be related to that which regulates the function of the nephron (Thurau and Schnermann 1965).

Loss of functioning nephrons in the rat leads to increased glomerular filtration rates in the residual nephrons. This hyperfiltration is the result of increased hydraulic pressure and/or flow rates within remaining glomerular capillaries (Hostetter 1984). The glomerular hypertension is sometimes associated with pathological changes, such as glomerulosclerosis which occurs in rats with unilateral nephrectomy or 4/6 nephrectomy within 4–8 months (Grond et al. 1982; Hostetter et al. 1986). In the present study, virtually no glomerulosclerosis was present. This could, however, be explained by the relative short observation time used in the study (16 weeks). The small volume of the atubular glomeruli and their non-functioning state makes it possible that some of the atubular glomeruli over a long observation time will disappear or become sclerotic. No direct support for that suggestion has been provided and in a study of atubular glomeruli in cisplatin-induced nephropathy the number of glomeruli was not changed 12 weeks after the start of cisplatin (Marcussen 1990). In the present study the number of atubular glomeruli was not modified by high protein diet or uninephrectomy and no sclerotic atubular glomeruli was found. However, in an electron microscopic study of lithium-induced nephropathy (Marcussen et al. 1990), the atubular glomeruli were found to have increased volume fraction of mesangium, decreased diameter of the capillaries and an increased basement membrane thickness, whereas the hypertrophic glomeruli had values within the normal range.

Evidence from studies in rats supports the notion that unrestricted protein ingestion or a high protein diet accelerates the progression of renal failure (Hostetter et al. 1986; Kenner et al. 1985; Provoost et al. 1989; Savin et al. 1989). However, in humans and especially in experimental animals other than rats, the effect of dietary protein in chronic renal failure has been conflicting (Polzin et al. 1988; Rosman et al. 1989; Walker et al. 1989; Walser 1988). Tapp et al. (1989) demonstrated that food restriction prevented the development of end-stage renal pathology (defined as a relative interstitial volume of more than 40%) in chronic renal failure in rats regardless of the protein intake. Bertani et al. (1989) exposed rats to diets with different protein content, and found a significant correlation between the degree of tubulointerstitial damage and the percent of glomeruli with focal segmental sclerosis. They suggested that tubulointerstitial damage may contribute to the development of glomerular sclerosis. However, no adverse reactions to the high protein were seen in the present study. Conversely, the high protein groups had lower plasma creatinine level at the end of the study than their respective control groups. These functional changes are in accordance with the hypertrophy of proximal tubules and glomeruli seen in the high protein groups. Previous studies have revealed enlarged kidneys but shown conflicting results regarding glomerular sizes (Bouby et al. 1988; Kenner et al. 1985). In the study by Bouby et al. (1988), the authors found that it was the juxtamedullary glomeruli which showed increased cross-sectional surface areas, whereas Kenner et al. (1985) found no increase in the glomerular diameters when rats were given 37% protein diet for up to 6 months.

The present results may contribute to our understanding of the pathogenesis of chronic renal failure. A remarkable compensatory hypertrophy was observed

in glomeruli connected to normal proximal tubules. Using stereological methods this hypertrophy has been shown to be due mainly to increase in the number of glomerular capillaries and not to increase in the length of individual capillaries (Marcussen, in preparation). It is noticeable that high protein diet and especially nephrectomy were able to induce a significantly larger hypertrophy of the intact glomerular population than lithium nephropathy alone. The glomeruli connected to normal proximal tubules are most likely connected to the hypertrophic, dilated tubules with normal appearing epithelium. It has not, however, been shown whether tubules without connection to glomeruli exists in different chronic interstitial nephropathies. In this particular model, glomerulosclerosis was of no importance in the development of renal failure. On the contrary, tubular destruction leading to formation of many non-functioning glomeruli played a major role. The "intact nephron hypothesis" (Bricker 1969) states that in chronic renal diseases the nephron population consists of intact nephrons, which contribute to the function of the kidney, and of destroyed nephrons, which are of no use. The present findings provide some support for that hypothesis, especially by the multiple regression analysis showing that the declining percentage of glomeruli connected to normal proximal tubules provided a major explanation for the decrease in renal function. However, the finding that the volume of interstitial fibrosis also contributed to the decrease in kidney function may be explained by an impaired interplay between tubules and interstitium. In this respect it is of interest that Møller and Skriver (1985) found an increase in the distance between tubules and capillaries caused by an increment in the interstitium.

In conclusion, the experimental model investigated in this study shows that the chronically diseased kidney has a remarkable capacity to hypertrophy when challenged by an extra load. The hypertrophy solely involves the residual intact glomeruli and probable also exclusively the tubules connected to these glomeruli. The decrease in kidney function observed in chronic interstitial renal diseases may be explained by the formation of atubular glomeruli in response to tubular injury, but also the degree of interstitial fibrosis has some importance.

Acknowledgements. Professor Steen Olsen and H.J.G. Gundersen are thanked for valuable discussions regarding the manuscript. The skilful technical assistance from Birthe J. Jensen and Annette Funder is gratefully acknowledged. The study was supported by grants from the Danish Research Council, Fonden til Lægevidenskabens Fremme, Viholm fonden og Nyreforeningens Forskningsfond.

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