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Renal Hypertrophy in Experimental Diabetes

A Morphometric Study

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Summary. Renal hypertrophy in rats with streptozotocin diabetes or after unilateral nephrectomy was studied by sterological techniques. – After 4 days of diabetes total glomerular volume had increased by 30%, and after 47 days by 43%. Glomerular growth was more pronounced than whole kidney growth during the first 4 days, but subsequently whole kidney growth exceeded glomerular growth. In control rats glomerular volume was 4.9% of total kidney volume; after 4 days of diabetes it was 5.4% and after 47 days 4.1%. – Proximal tubule length increased from 366 m/kidney in control rats to 447 m/kidney after 47 days of diabetes; tubular luminal diameter increased from 26.8 μm to 31.4 μm in the same rats. Tubular length and luminal diameter were, however, not increased after 4 days of diabetes. – In unilaterally nephrectomised rats there was no early rapid glomerular growth. Glomerular fractional volume was 4.9% in controls, 4.4% at four days, and 4.2% at 24 days after nephrectomy. – The results indicate a disturbed glomerulo-tubular balance in the early phases of diabetic renal hypertrophy.

Key words: Diabetes, glomerulus, hypertrophy, kidney, morphometry, nephrectomy, proximal tubule, rats, stereology.

Kidney size and function are increased in diabetes mellitus of recent onset [1]. Morphometric studies of renal biopsies from diabetics have revealed an increase in glomerular size [2]. In streptozotocin diabetic rats the kidney weight increases within the first few days of diabetes [3]. In the present study this kidney growth has been characterised by morphometric analysis of the various anatomical structures at different intervals after the onset of diabetes. For comparison a less detailed study has been made of kidneys from unilaterally nephrectomised rats.

Material and Methods

Female Wistar rats aged five to eight months and weighing 240–270 g at the start of the experiments were used. They were fed commercial rat pellets (Fakse Dampmølle) without restriction. Diabetes was induced by IP injection of streptozotocin (55 mg/kg). Only animals with a moderate degree of diabetes were used, the criteria for inclusion being a blood glucose exceeding 300 mg/ml at death, and a loss in body weight of less than 35 g during the experimental period. The diabetic rats were studied 4, 24, and 47 days after streptozotocin injection.

Two groups of non-diabetic rats were studied 4 and 24 days after removal of the right kidney.

At the end of the experimental period rats were anaesthetised with pentobarbitone (40 mg/kg). The right kidney was removed after ligation of the renal pedicle. A gauge 18 cannula was inserted into the aorta just above the bifurcation; the left renal vein was cut and during digital compression of the aorta proximal to the renal artery the left kidney was perfused in situ with glutaraldehyde. The perfusion procedure was essentially as described by Maunsbach [4]. Perfusion was maintained at a pressure of 140 mm Hg for 3 min. Kidneys that failed to blanch immediately after the start of the perfusion were discarded.

The perfusion medium consisted of 1 g/100 ml glutaraldehyde in modified Tyrode's buffer, containing 22.5 g/l dextran T 40, which was prepared freshly a few hours before the experiment. The osmolality of the medium was checked each time and varied from 330 to 357 mOsm/l.

After perfusion the kidney was removed, trimmed free of fat and capsule, and post-fixed in the perfusion medium for 90 min. It was then stored in the modified Tyrode's buffer without glutaraldehyde for seven days before further processing.

The kidney was cut into a series of slices of alternating thickness (2 and 0.8 mm) by use of a set of fixed razor blades with random position with respect to the kidney. All thick slices with a right-handed cut surface were dehydrated, embedded in paraffin and one PAS-stained section from each cut surface was used. From the thin slices a number of small blocks was taken systematically [5] dehydrated and embedded in Vestopal. One thin (400 nm)

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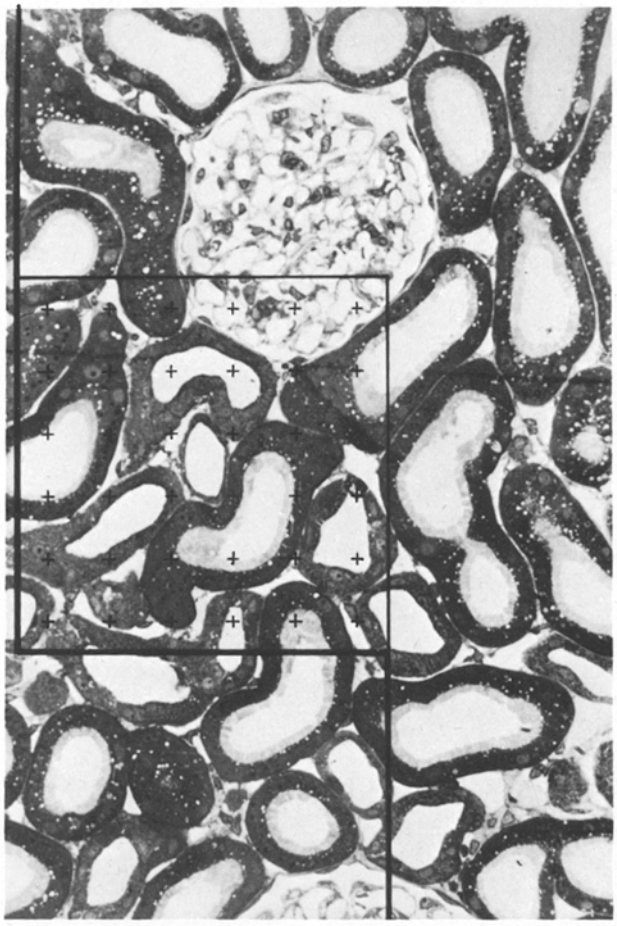

Fig. 1. Plastic embedded section of renal cortex with rectangular counting frame superposed. Irrespective of the presence of lumina, all proximal tubular profiles were counted according to the rule: All profiles entirely or partially within the rectangle are counted provided they are not intersected by any full-drawn edge or extended exclusion line [7]

PAS-stained section from each of five blocks selected and oriented at random was used in each animal for the analysis of proximal tubular dimensions (Fig. 1).

By use of standard stereological techniques [6] the volume fractions of glomeruli and cortex were estimated from the paraffin sections at a magnification of ×308. A glomerulus was defined as the minimal convex figure enclosing the glomerular tuft. Cortex was defined as that part of the kidney where proximal tubules were present. In each animal an average of 160 fields of vision were analysed corresponding to a total area of 43.3 mm^2. The total area of the five cortical plastic sections averaged 0.41 mm^2 per animal. On these sections the cortical volume fractions of proximal tubular cells and lumina were estimated at a magnification of ×400 (the brush border was included in tubular cells).

In addition, the external proximal tubular profiles were counted, employing the unbiassed counting role [7]. An average of 112 profiles per animal was counted.

The volume fraction, $V_V(X/R)$, of a structure X in a reference volume R is estimated by $\frac{\Sigma P(x)}{\Sigma P(r)}$, where $\Sigma P(x)$ is the total number of points hitting X on sections and $\Sigma P(r)$ is the total number of points hitting the reference volume.

In a separate investigation the specific gravity of kidneys from normal and diabetic rats was measured using a pychometer after fixation in situ, as described above. Nine normal kidneys weighing between 808 and 1147 mg had specific gravities between 1.001 and 1.045, and eleven kidneys from diabetic rats (diabetes duration 4 days to 6 months) weighed between 983 and 1600 mg, with specific gravities between 1.015 and 1.035. This minimal variation allowed all volumes of the kidney structures to be calculated on the basis of the weight of the fixed kidney, assuming a density of 1 g/ml, as follows.

The total glomerular volume in the kidney is:

V_V(glomeruli/kidney) × kidney weight.

The total tubular volume is:

V_V(tubules/cortex) × V_V(cortex/kidney) × kidney weight.

The total length per unit volume, L_V, of a tubular structure is estimated by $2 \times N_A$, where N_A is the number of tubular profiles per unit cortical area on the thin plastic sections. The total length of tubules in one kidney is therefore:

$2 \times N_A$(tubules/cortex) × V_V(cortex/kidney) × kidney weight.

The average cross sectional area perpendicular to the longitudinal direction of a tubular structure is $\bar{A} = \frac{V}{L}$, which is estimated by $\frac{V_V}{L_V}$.

Assuming a circular cross section, the average diameter of tubules can be calculated as $\bar{d} = 2\sqrt{\frac{\bar{A}}{\pi}}$ and the average height, $\bar{h}$, of tubular cells is then calculated as $^1/_2 \times (d_e - d_l)$ where suffixes e and l indicate external and luminal diameter, respectively.

Student's t-test was used for statistical analysis.

Results

The results are shown in Tables 1 and 2. All the kidneys weights were obtained after the fixation procedure was completed. In all animals with two kidneys the non-perfused kidney was weighed separately. Perfusion fixation increased renal weight by 21–24%, but this increase was the same in all groups. As calculations in the unilaterally nephrectomised rats could be made only from fixed renal weight, this variable was used in all groups.

No systematic investigation was made of the possible changes in control rats with increasing age. This was because the ages of the animals in each experimental group varied by more than the 47 days, which was the longest experimental period.

During 47 days of diabetes kidney weight increased 73%. The relation between cortex and medulla remained the same, the cortex constituting about 80% of the volume of kidney.

The total volume of proximal tubular cells increased by 75% during the 47 days. Tubular growth was manifested as an increase in cell height

Table 1. Kidney weight and glomerular volume at various intervals after induction of diabetes or after unilateral nephrectomy. D-4, D-24, and D-47 are rats with a diabetes duration of 4, 24, and 47 days. Nx-4 and Nx-24 are rats 4 and 24 days after unilateral nephrectomy. Results are mean values ± SEM. NS = not significant

	n	Body weight (g)	Kidney weight (mg)	Cortical fraction (V_V)	Glomerular fraction (V_V)	Glomeru- lar volume (mm^3/kidney)
Control	12	254 ± 3	863 ± 24	0.82 ± 0.01	0.049 ± 0.001	42.6 ± 1.4
D-4	12	242 ± 4	1044 ± 25	0.79 ± 0.01	0.054 ± 0.001	56.0 ± 1.7
D-24	7	245 ± 5	1322 ± 42	0.80 ± 0.01	0.045 ± 0.001	59.0 ± 1.8
D-47	8	224 ± 5	1490 ± 46	0.77 ± 0.01	0.041 ± 0.001	61.1 ± 3.2
Nx-4	7	244 ± 4	1143 ± 35	0.82 ± 0.01	0.044 ± 0.001	50.0 ± 1.5
Nx-24	7	256 ± 4	1296 ± 34	0.80 ± 0.01	0.042 ± 0.001	53.9 ± 1.9
Significance of differences, 2p:						
Control	v. D-4		< 0.001	< 0.05	< 0.005	< 0.001
Control	v. Nx-4		< 0.001	NS	< 0.005	< 0.005
D-4	v. Nx-4		< 0.05	NS	NS	< 0.05
D-24	v. Nx-24		NS	NS	NS	NS

NS = $2p > 0.05$

Table 2. Dimensions of the proximal renal tubules after various periods of diabetes. Results are mean values ± SEM. NS = not significant

Diabetes duration (days)	n	Tubule length (m/kidney)	Tubule cell volume (mm^3/kidney)	Epithelial cell height (μm)	Luminal diameter (μm)
0	9	366 ± 6	239 ± 10	6.5 ± 0.3	26.8 ± 0.7
4	10	371 ± 23	306 ± 11	7.7 ± 0.2	27.3 ± 0.6
24	7	400 ± 15	413 ± 17	8.2 ± 0.2	31.9 ± 0.8
47	8	447 ± 25	421 ± 16	7.8 ± 0.2	31.4 ± 0.9
Significance of differences, 2p:					
0 v. 4		NS	< 0.001	< 0.005	NS
0 v. 24		< 0.005	< 0.001	< 0.001	< 0.001

NS = $2p > 0.05$

(19%), tubule length (22%), and tubular luminal diameter (17%). There was not, however, a uniform growth of the tubules in all directions throughout the experiments. Tubule cell height had grown almost to its final size after 4 days of diabetes, whereas the luminal diameter and the tubular length were unchanged at this time.

The total volume of glomeruli increased by 43% during the 47 days. The greater part of this growth took place during the first few days.

In Table 1 renal compensatory hypertrophy after unilateral nephrectomy is compared to diabetic hypertrophy. The increase in total kidney weight was the same in the two groups, but glomerular volume was smaller after compensatory growth. This difference was manifested most clearly by the glomerular fraction of the kidney, which after four days of diabetes had risen from 4.9% to 5.4%, but four days after nephrectomy had decreased to 4.4%.

Discussion

We have previously reported on the kidney growth in streptozotocin diabetes [1]; the growth rate found in the present experiments was similar to that previously found. This renal growth can be prevented by insulin treatment [1] and is thus not a direct effect of streptozotoxin, but due to the metabolic derangement of diabetes. We have not included insulin treated rats in the present study, so theoretically some of the early morphological changes (e. g. tubule cell hypertrophy) could be due to streptozotocin toxicity. However, we think it improbable, as all the changes persist or become more pronounced throughout a seven week period.

Increased glomerular size has been described in human [2, 8] and experimental diabetes [9, 10] by authors using different techniques. We have not measured average glomerular volume, but total vol-

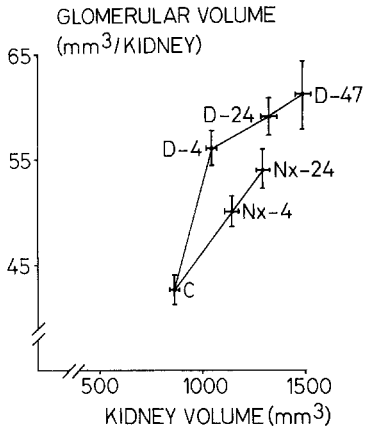

Fig. 2. Relationship between glomerular volume and kidney volume in diabetic rats (D) and in uninephrectomised rats (Nx). The figures give the duration of the experimental condition in days. Bars represent SEM, C, control rats

ume, and our results are therefore not directly comparable. As it is most unlikely that new nephrons are formed in adult rats however [11], the increase in total glomerular volume probably reflects a similar increase in mean glomerular volume. Measurements of tubular size in diabetes have not previously been published.

The principal finding of this part of the study is the nonuniformity of the renal growth. Initially glomeruli grow very quickly, faster than the rest of the kidney, but after a few days of diabetes glomerular growth rate slows down. After a few weeks the glomeruli comprise a smaller proportion of the kidney than in control rats. Tubules on the other hand grow more slowly, but more persistently.

The relationships between morphological changes and alterations in renal function are unclear. Glomerular filtration rate has been found elevated in streptozotocin diabetic rats [12]. The increased glomerular size, which is accompanied by an increased filtration surface area [13], may contribute to this, but not if filtration equilibrium occurs in the glomerular capillaries, as has been described in Munich-Wistar rats by Brenner et al. [14]. Tubular growth, by which diameter increased by 20% after 24 days, but length only by 10%, might result in a decreased resistance to flow in the tubule, and that would also increase glomerular filtration rate.

There have been controversies in the literature concerning glomerular size in compensatory renal hypertrophy. Our findings support those of Olivetti et al. [15]. They found (using a different technique) a 70% increase in mean glomerular volume 35 days after unilateral nephrectomy. We have found a somewhat smaller, but still definite, increase in the total glomerular volume.

Table 1 confirms our previous findings that the kidney growth is of the same order of magnitude in diabetic and compensatory renal hypertrophy [16]. However, the mode of growth seems different in the two situations. The very marked increase in glomerular volume which is seen in diabetes is absent in compensatory growth.

In Figure 2 glomerular volume has been plotted against total kidney volume in the various experimental groups and the different growth patterns of the kidney in nephrectomised and diabetic rats are illustrated. It is known that functional glomerulotubular balance is preserved in compensatory renal hypertrophy [17]. The straight line, which connects the control group with the nephrectomised groups, can be taken as a graphical expression of some kind of anatomical glomerulo-tubular balance in these rats (since the total kidney volume is closely related to the total volume of proximal tubules). When depicted in this way there seems to be a gross glomerulotubular disequilibrium in the early stages of streptozotocin diabetes, but a gradual approach towards equilibrium with time. Studies are needed to show whether this is reflected in glomerular and tubular function in the hypertrophying diabetic kidney.

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