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Dimensional changes of proximal tubules and cortical capillaries in chronic obstructive renal disease

A light microscopic morphometric analysis

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Summary. The study was carried out to determine the proximal tubular length, surface area and length of peritubular capillaries and the nephron numbers in kidneys with chronic nephropathy and varying increase in the cortical interstitial volume. Kidneys of pigs with varying chronic obstructive nephropathy were used for the experiments. Two subgroups of ureterobstructed kidneys were defined arbitrarily according to the volume of cortical interstitium. One subgroup (I) comprised kidneys with a volume fraction of cortical interstitium less than 30% (mean 17.2%; mean of controls 9.7%). The other subgroup (II) consisted of kidneys with severe chronic nephropathy and with a volume fraction of interstitium more than 30% (mean 44.5%). Proximal tubular length and length and surface area of peritubular capillaries were assessed by conventional morphometric techniques on 1 μm thick sections of plastic embedded material. Nephron numbers were determined by a stereological method for counting glomeruli.

The results demonstrated that proximal tubular length and capillary dimensions were significantly reduced in subgroup II, whereas no significant changes were observed in subgroup I. The mean number of glomeruli was not significantly different from control values in any of the subgroups.

The results are in line with observations from previous quantitative analyses of proximal tubular cross-sections indicating that proximal tubular dimensions become reduced mainly at advanced stages of chronic nephropathy. The results also indicate that shortening of individual tubules rather than loss of entire nephrons is responsible for the observed reduction in total length of proximal tubules. Finally, the present observations suggest that reduced dimensions of the cortical capillary network may have pathogenetic significance for ongoing proximal tubular atrophy in chronic renal disease.

Key words: Proximal tubular length – Cortical capillaries – Nephron numbers – Chronic renal disease

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Introduction

Ultrastructural analyses of proximal tubules in human (Møller and Skriver 1984) and experimental (Møller et al. 1986) chronic obstructive nephropathy have demonstrated that luminal and peritubular diameters and cell organelles of the tubules become reduced when the cortical interstitium increases. These studies also suggest that the increase in cortical interstitium may influence the structure of the proximal tubules by altering their spatial relationships with the peritubular capillaries. Only a few previous studies have been concerned with the interrelationships between cortical interstitial volume, proximal tubular length and dimensions of the peritubular capillaries. Bohle et al. (1981) in a study of renal biopsies from patients with various types of chronic renal disease demonstrated that an increase in cortical interstitial volume is associated with significant reductions in the number of peritubular capillaries and in capillary area per area unit. Ottosen et al. (1984) found that proximal tubular length is significantly reduced in rat kidneys with a severe cortical interstitial fibrosis following chronic lithium administration. However, it is not known whether reductions in length of proximal tubules and peritubular capillaries occur at an early stage of chronic renal disease with slight increase in cortical interstitium or mainly in

advanced chronic nephropathy with severe cortical fibrosis.

The present study was carried out to determine the length of the proximal tubules and the length and surface area of cortical capillaries in chronic ureter obstructed kidneys having either a slight or severe degree of cortical interstitial fibrosis. A further aim was to assess the number of glomeruli and thereby estimate the number of nephrons. Such determination may provide additional information on the mechanisms underlying proximal tubular changes in chronic nephropathy.

Materials and methods

The study is based on the same kidneys used in a previous ultrastructural analysis of proximal tubules and cortical interstitium in chronic obstructive nephropathy. Details concerning the Materials and methods have been given elsewhere (Møller et al. 1986).

Female pigs of Danish Landrace breed, weighing from 36–60 kg, were used for the experiments. In 15 animals a slight to moderate degree of unilateral, chronic obstructive nephropathy was induced by partial ureteral obstruction for 10 weeks. Four animals of this series were excluded because of disease of the animals or inadequate perfusion fixation of the kidneys. Five additional animals were subjected to unilateral, total ureteral obstruction for 7 days, which according to preceding pilot experiments was a sufficiently long period of time for the development of even severe degrees of chronic hydronephrotic atrophy of the kidneys. Two animals were excluded due to infection of the kidneys. Thus, a total of 11 partially and 3 totally obstructed kidneys from 14 animals were analysed. One kidney from each of 4 pigs of the same sex and weighing from 36–40 kg were used as controls.

All kidneys were fixed by in vivo vascular perfusion through the aorta (Maunsbach 1966; Elling et al. 1977) using 1% glutaraldehyde in a modified Tyrode solution (Maunsbach 1966). About 25 tissue cylinders were taken at random from 1 mm thick transverse slices of kidney tissue (see below) by means of a 0.8 mm gauge cannula and embedded in Epon with a random orientation. One μm thick sections were cut from successive blocks until 5 sections, each containing cortical structures such as proximal convolutions or glomeruli, were at hand. The sections were stained with toluidine blue.

The kidneys were weighed immediately following perfusion fixation and after the extrarenal pelvis had been removed. Subsequently, the kidneys were cut by transverse sections into slices of alternating thickness, every second slice having a thickness of 1 mm. The thickness of the other slices depended on the size of the kidney. Thus, in relatively large kidneys the thickness of the slices was 5 mm, whereas a thickness of 3 mm was used in relatively small kidneys. Thus approximately the same number of transverse slices was obtained in each case. The 1 mm slices were used for random sampling of specimens for light microscopic analyses. The thick slices were used for the determination of the cortex fraction by point-counting technique. The cortex fraction was obtained as the number of points falling on cortical areas divided by the number of points falling on the entire renal tissue including the intrarenal pelvic tissue. The cortex was defined macroscopically as the zone situated between the renal capsule and the arcuate arteries and thus included the medullary rays.

All stereological analyses were performed at the light microscope level by projecting microscopic fields on a test frame provided with a square lattice. In each of five sections 6 microscopic fields were counted successively, beginning in the upper left corner of the section. Thus, a total of 30 microscopic fields were analysed in each kidney. The length densities (L_v) of proximal tubules and peritubular capillaries, respectively, were assessed by counting the number of tubular or capillary profiles within the test frame according to the principles given by Gundersen (1977). The formula: $L_v = 2\,N$, where N is the number of profiles per test area, was used (Weibel 1979). Only tubules showing brush border projections were considered as proximal tubules. The total length of proximal tubules or capillaries per kidney was obtained as $L_v \times V_v$ cortex $\times$ kidney weight. The surface density of capillaries was determined by intersection counting using the formula: $S_v = 2\,I$ (Weibel 1979), where I is the number of intersections between test lines and capillary walls and L_t the total length of test lines covering the test area. The volume density of cortical interstitium was determined by point counting.

Estimation of the number of glomeruli. Estimation of the numerical density of particles in a given probe on the basis of common stereological methods (point counting) requires that the particles are of about equal size. This is usually assumed to be the case in normal kidney tissue. In chronically diseased kidneys, however, the size of the glomeruli is variable. A stereological method for counting particles of unequal size has been given by Sterio (1984). For this purpose a so-called "disector" is used, which is composed of a planar, unbiased counting frame of known area and a parallel plane of known distance away. This distance should be less than the minimum diameter of the particles to be counted. Counting by the disector method is performed on 2 subsequent sections (1 and 2) for which the thickness of 1 is known. The number of particles within the reference space can be obtained by counting the particles in section 1 which are no longer present in section 2. The major problem of this counting procedure is correct assessment of section thickness.

Unstained sections cut on a vibratome from random blocks of perfusion fixed renal cortical tissue were used. The tissue blocks, approximately 6×6 mm in size, were taken from the 3 or 5 mm thick slices of kidney tissue. Each specimen was mounted on a plastic block (15×25 mm) using a special glue (Loctite, Loctite Corp. Ltd., Dublin). The mounted tissue block was then cut into a square with 5 mm sides. This was done with a razor blade under a stereo microscope provided with an ocular micrometer. The block was then placed in a vibratome (Oxford® vibratome, Oxford Lab., USA) and trimmed down until the surface of the block was exactly parallel with the plane of sectioning. Subsequently, the specimen (tissue block and plastic block) was weighed with an accuracy of 0.1 mg (W1) using the mean of 3 weight determinations. Ten sections, each approximately 50 μm thick, were then cut on the vibratome. Two times two successive sections (for example sections 2–3 and 7–8) were picked up on microscope slides and covered by cover glasses using Aquamount. After sectioning the specimen was weighed again (W2) and the weight of individual sections (W) was obtained as $(W1 - W2)/10$. The mean thickness of individual sections was finally determined as W/A, where A is the surface area of the sections ($=25$ mm^2). A specific gravity of 1 was used for both atrophic and control kidneys (Seyer-Hansen et al. 1980).

From each pair of unstained sections a montage was made from overlapping microphotographs. In these glomerular structures could be easily identified (Fig. 1). Due to "drop out" of glomerular tufts, which were not fixed in the section at the

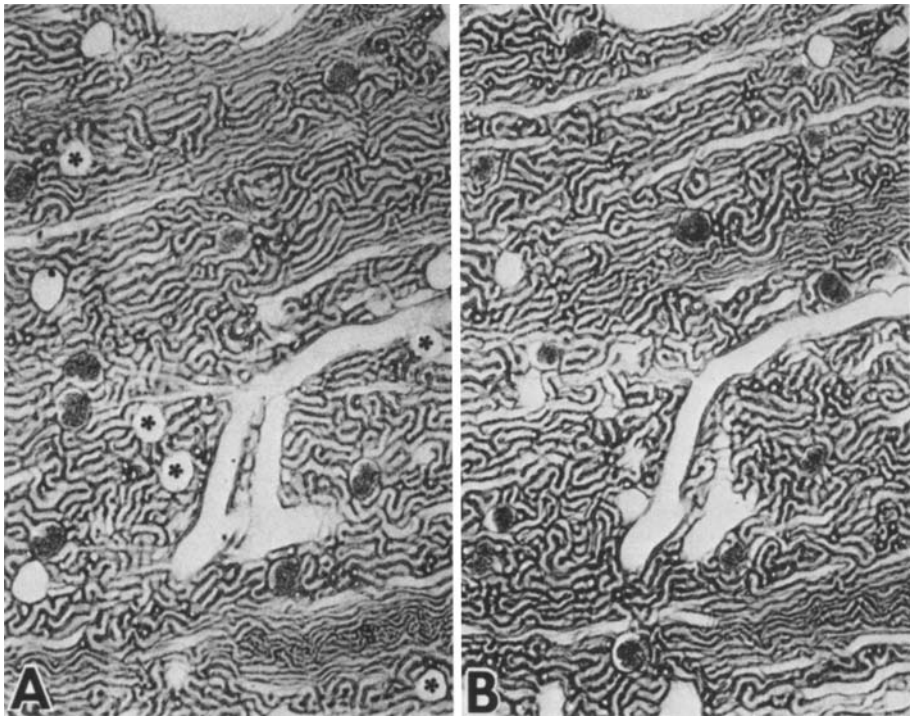

Fig. 1 shows light micrographs of 2 subsequent sections (**A** and **B**), approximately 50 μm in thickness, used for counting of glomeruli. Due to "drop out" some glomeruli are represented exclusively by their capsule. The determination of glomerular density is based on the number of glomerular structures in section **A** (asterixes) which can no longer be observed in section **B** (For further explanation see text)

Table 1. Numerical density and total number of glomeruli in kidneys of controls and hydronephrosis subgroups[a]

	Controls	Subgroup I	Subgroup II
N	4	9	5
Cortical interstitium, mean vol%	9.65±0.77 (8.5–10.1)	17.2±4.9 (9.9–25.4)	44.5±13 (31.0–59.6)
Kidney weight (g)	106±7.9	127±15*	114±17
Volume fraction of kidney cortex (%)	87.7±1.1	92.2±1.1*	89.1±10
Numerical density of glomeruli (N/mm^3)	9.50±1.0	8.46±0.92	9.14±1.7
Number of glomeruli per kidney ($\times 10^5$)	9.03±1.6	9.91±1.6	9.02±1.5

[a] Values are means±SD
* Indicates significant difference from control values ($2P<0.05$)

vascular poles, some glomeruli had to be identified by their capsule. This introduced no essential possibility of error, since the only structures which might imitate glomerular capsules were small vessels, which could be traced to the subsequent section and thus, according to the counting rules, should be disregarded. All glomeruli present in section 1 but not in section 2 were counted within an unbiased counting frame representing an area of approximately 17 mm^2 in the sections. A total of 6 paired sections was investigated for each kidney. The number of counted glomeruli was from 83–104 (mean 95.2) in controls and from 75–132 (mean 91.0) in ureter obstructed kidneys.

Definition of subgroups. The experimental group was divided arbitrarily into two subgroups, one (subgroup I, $n=9$) representing kidneys with a volume fraction of cortical interstitium $<30\%$ and one (subgroup II, $n=5$) representing kidneys with a volume fraction of cortical interstitium $>30\%$. Each subgroup was compared with the control group for statistical difference using a non-parametric rank-sum test (Wilcoxon).

Results

Table 1 shows that both mean kidney weight and volume fraction of kidney cortex increased significantly in subgroup I, whereas the same variables showed no significant changes from control values in subgroup II.

The numerical density as well as the total number of glomeruli per kidney showed no significant differences between controls and hydronephrosis subgroups.

Table 2 shows that the length density of proximal tubules in subgroup I was not significantly different from that of control proximal tubules. In subgroup II, however, the mean length density was reduced to about 60% of control values. The

Table 2. Proximal tubular length per unit volume cortical tissue, per kidney and per glomerulus in controls and hydronephrosis subgroups[a]

	Controls	Subgroup I	Subgroup II
N	4	9	5
Cortical interstitium, mean vol%	9.65±0.77 (8.5–10.1)	17.2±4.9 (9.9–25.4)	44.5±13 (31.0–59.6)
Length density of proximal tubules ($\mu m/\mu m^3$) ($\times 10^{-4}$)	2.55±0.13	2.31±0.20	1.53±0.69*
Total length of proximal tubules per kidney (km)	23.8±3.8	27.1±3.7	15.4±0.42*
Length of proximal tubules per glomerulus (cm)	2.63±0.14	2.76±0.33	1.66±0.68*

[a] Values are means±SD
* Indicates significant difference from control values ($2P<0.05$)

Table 3. Peritubular capillary length and capillary surface area per unit volume cortical tissue, per kidney and per glomerulus in controls and hydronephrosis subgroups[a]

	Controls	Subgroup I	Subgroup II
N	4	9	5
Cortical interstitium, mean vol%	9.65±0.77 (8.5–10.1)	17.2±4.9 (9.9–25.4)	44.5±13 (31.0–59.6)
Length density of cortical capillaries ($\mu m/\mu m^3$) ($\times 10^{-4}$)	7.08±0.22	6.83±0.30	5.08±1.6*
Total length of cortical capillaries per kidney (km)	66.5±3.3	78.0±10	45.2±12*
Length of cortical capillaries per glomerulus (cm)	7.50±1.1	7.90±0.70	4.98±0.86*
Surface density of cortical capillaries ($\mu m^2/\mu m^3$) ($\times 10^{-2}$)	1.58±0.05	1.48±0.81	1.04±0.34*
Total surface area of cortical capillaries per kidney (m^2)	1.53±0.13	1.72±0.17	1.04±0.39*
Surface area of cortical capillaries per glomerulus (mm^2)	1.70±0.22	1.74±0.21	1.14±0.34*

[a] Values are means±SD
* Indicates significant difference from control values ($2P<0.05$)

total length of proximal tubules also showed a significant reduction in subgroup II. The same was the case for proximal tubular length per glomerulus.

Table 3 illustrates that the length and surface area of cortical capillaries followed a pattern of changes similar to that of the proximal tubules. Thus, in subgroup I both capillary length and surface area were not significantly different from control values when determined per volume unit, for whole kidneys or per glomerulus. In subgroup II both capillary length and surface area were significantly reduced.

Discussion

Two major conclusions can be drawn from the present results. Firstly, in chronic hydronephrosis there appears to be no reduction in the number of glomeruli even at advanced stages of chronic nephropathy. Secondly, the length of the proximal tubules as well as the length and surface area of cortical capillaries show only minor and insignificant changes except in case of severe degrees of cortical interstitial fibrosis.

Estimates of the number of glomeruli per kidney have been achieved by different techniques, which probably accounts for the discrepancies between results reported in previous literature. Analyses of the human kidney have demonstrated that the number of glomeruli per kidney is close to 1 million (Dunnill and Halley 1973; Tryggvason and Kouvalainen 1975) which is in line with data reported by Vimtrup (1928). The present study shows that the number of nephrons in the normal pig kidney is slightly lower than in the human kid-

ney. Similar observations were made by Kunkel (1930).

The present observation that even severely hydronephrotic kidneys have normal numbers of glomeruli agrees with the experiences from diagnostic pathology, where it is often realized that the glomeruli may be surprisingly well preserved also in cases with marked tubular and interstitial changes. Corresponding observations have been made in experimental hydronephrosis (Hinman 1945; Strong 1940; Kinn and Bohman 1983). One may therefore suggest that in chronic obstructive nephropathy there is no reduction in the number of nephrons, at least not within the range of interstitial changes covered by the present series. In agreement with this Kramp et al. (1973) on the basis of microdissection analyses found that in kidneys with chronic renal damage "there was little evidence of physical disappearance of nephrons from the kidneys". The same investigators demonstrated that changes of the tubules procede in a heterogenous way and lead to a mixture of atrophic and hypertrophic units. The finding in the present subgroup I of an unchanged mean length of the proximal tubules migth therefore be the result of a decrease in length (atrophy) of some tubules and an increased length (hypertrophy) of other tubules. However, neither studies of human (Møller et al. 1984) nor experimentally induced (Møller et al. 1986) obstructive nephropathy have provided evidence that tubular hypertrophy takes place to any significant degree in this type of chronic renal disease.

An increase in kidney weight as observed here for kidneys of subgroup I is a well recognized, temporary feature of obstructive nephropathy (Kinn and Bohman 1983; Schubert et al. 1975), which may be due to accumulation of fluid in dilated tubules and vessels and in the interstitial space. A certain dilatation of the tubules may explain the observed tendency (Table 2) towards decreased length density of the proximal tubules (i.e. decrease in tubular length per volume unit tissue) in subgroup I. Thus, increase in the diameters of the tubules will tend to reduce the number of tubular profiles which can be counted per area unit tissue.

In a previous study it was demonstrated that the normally abundant mitochondria and greatly folded basolateral cell membranes of the proximal tubular cells become significantly reduced at an early stage of tubulo-interstitial disease, whereas the luminal and peritubular diameters of cross-sectioned tubules are reduced only in advanced degrees of chronic hydronephrosis (Møller et al. 1986). The present study adds to these findings by showing that the mean length of the proximal tubules is also unchanged in slight to moderate degrees of cortical interstitial fibrosis. It thus appears that the reduction in cell organelles associated with tubular atrophy to some degree precedes the alterations in the dimensions of the tubules.

The observation that severe increase in cortical interstitial volume is associated with reductions in the length of the proximal tubules agrees with findings in lithium-induced nephropathy (Ottosen et al. 1984).

Electron microscopic studies of atrophic proximal tubules have provided some evidence that the reduction of proximal tubular dimensions is at least partially due to decreased volumes of the tubular cells (Møller et al. 1986). It has also been suggested that this shrinkage of the cells and other morphological signs of atrophy might be pathogenetically related to a decreased transport across the tubulocapillary barrier subsequent to increased distances between tubules and capillaries (Møller and Skriver 1985). The present observations indicate that reduction in length or surface area of the peritubular capillaries is of no significance to the structural changes of the proximal tubules in slightly and moderately severe degrees of hydronephrotic kidney atrophy. However, in the case of advanced chronic nephropathy with a 3–4 fold increase in cortical interstitial tissue a reduction in the dimensions of the peritubular capillaries may have an additional effect on the structural involution of the tubules by further interfering with the blood supply of the tubular cells.

Some investigators have attributed the increased cortical interstitium of chronic renal disease with a direct compressive (Bohle et al. 1981) or contractile (Nagle et al. 1973) effect on the cortical microcirculation. However, it is not obvious by which means increase in cortical interstitium per se could reduce the length of the capillaries. Furthermore, quantitative electron microscopic analyses have shown that in cortical areas comprising proximal convolutions increase of the interstitium is not tantamount to decreased volume fraction of the cortical capillaries (Møller et al. 1985). Thus, it is possible that the reduced capillary length of advanced chronic nephropathy is due to mechanisms similar to those responsible for the decrease in tubular length.

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