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Quantitative ultrastructure of human proximal tubules and cortical interstitium in chronic renal disease (hydronephrosis) *

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Summary. Surgically removed perfusion-fixed human kidneys with chronic renal disease (hydronephrosis) were studied by electron microscopy in order to determine whether there is a quantitative relationship between ultrastructural changes in proximal tubules in atrophy and changes in the surrounding cortical interstitium. Morphometric techniques were applied to montages of electron micrographs each covering several tubular profiles in the cortical labyrinth and to montages representing cross-sections of individual proximal convoluted tubules at a higher magnification. In order to enable a quantification of the spatial relations between individual tubular cross-sections and adjacent peritubular capillaries a tubulo-capillary index (TCI) was defined. This index was based on the mean distances between individual tubular cross-sections and adjacent peritubular capillaries and on the fraction of tubular circumference facing capillaries. Normal tissue from similarly fixed human nephrectomy specimens, which had been removed mainly because of neoplastic disorders, served as control material. In the hydronephrotic kidneys the relative volume of cortical interstitium (excluding capillaries) covered a range from 19.2–70.3%. Inverse correlations were demonstrated between the relative volume of cortical interstitium and various structural variables of proximal convoluted tubules, including tubular wall volume, the volume of mitochondria and the surface area of basolateral membranes. The TCI showed positive correlations with these tubular variables. No significant correlation was found between the volume fractions of cortical interstitium and capillaries. Finally, it was found that an increase in the volume fraction of the cortical interstitium from 16.2% in controls to 24.7% in cortical areas of hydronephrotic kidneys was associated with a 40–50% reduction in the volume of mitochondria

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and in the surface area of basolateral membranes in proximal tubules. The results are consistent with a pathogenic interrelationship between tubular and interstitial changes. An important factor in this relationship might be disturbed topographic associations between tubules and blood capillaries caused by the increase in cortical interstitium. The results further show that even slight increases in the cortical interstitial volume are associated with significant quantitative changes in tubular fine structure suggesting impaired tubular functions.

Key words: Atrophy – Proximal tubule – Human nephropathy – Electron microscopy – Quantitative changes

Introduction

The results of structure-function studies in human chronic renal disease have indicated that a relative volume increase of cortical interstitium and tubular atrophy are of greater pathophysiological significance for the impairment of glomerular filtration than are glomerular changes (Bohle et al. 1977; Riemenschneider et al. 1980; Risdon et al. 1968; Schainuck et al. 1970; Sloper et al. 1980). This seeming paradox has stimulated interest in defining the tubular and interstitial changes and their mutual relationships in chronic renal disease.

In a previous investigation we have described the qualitative ultrastructural changes of proximal tubules and adjacent cortical interstitium, including the capillaries, in varying degrees of cortical atrophy (Møller et al. 1984). The aim of the present study was to extend these qualitative observations through quantitative analyses of proximal tubular and cortical interstitial changes in varying degrees of cortical atrophy. A particular aim was to investigate the relationship between changes in proximal tubular ultrastructure and changes in the spatial associations between tubules and peritubular capillaries.

Materials and methods

The present study is based on the same perfusion-fixed kidneys as our preceding qualitative study (Møller et al. 1984) in which further details regarding patients and preparatory methods are given.

Control kidneys. Kidney tissue from macroscopically and light microscopically normal parts of 8 human nephrectomy specimens was used as control material. The indication for nephrectomy was generally a tumour involving only part of the kidney or limited to the renal pelvis. In one case, however, the only pathological change in the kidney appeared to be a few simple cysts. The age of the patients varied from 46 to 86 years (mean age 61.9 years).

Hydronephrotic kidneys. Nine kidneys with various degrees of hydronephrosis were used for the study of tubular atrophy. The age of the patients was from 19 to 77 years (mean age 61.4 years).

Preparation for light and electron microscopy. The kidneys were perfusion-fixed within 15 min after interruption of the blood supply as previously described (Møller et al. 1982). The perfusion pressure applied was between 80 and 100 mm Hg. The fixative was 2% glutaraldehyde in

0.1 M cacodylate buffer to which was added 2% dextran T40 (Pharmacia, Uppsala, Sweden) (Bohman and Maunsbach 1970). The total osmolality of the fixative was approximately 400 mosm/kg H_2O. Tissue blocks from macroscopically well fixed cortical areas representing the entire cortical width were divided into two equal halves for light and electron microscopy, respectively. Paraffin sections were stained with haematoxylin and eosin. Specimens for electron microscopy were cut into 1 mm cubes, postfixed in 1% osmium tetroxide, stained *en bloc* with uranyl acetate and embedded in Epon with a random orientation. Semi-thin sections (about 1 μm thick) were stained with toluidine blue. Ultrathin sections were mounted on formvar-coated one-hole grids, stained with uranyl acetate and lead citrate and examined in a JEOL 100B electron microscope at 80 kV.

Selection of cortical areas and individual proximal tubules for morphometry. Even in generally well perfusion-fixed human nephrectomy specimens minor cortical areas may escape perfusion (Møller et al. 1982). A primary selection of cortical areas for electron microscopic morphometry was therefore carried out by light microscopy of the paraffin sections and the toluidine blue stained sections. The paraffin sections showed which parts of the cortical tissue were adequately fixed for further processing, the main criteria being patent tubular lumens and blood-free vessels. The final selection of cortical areas for ultramicrotomy and morphometry was carried out on the semi-thin sections originating from cortical areas immediately adjacent to those selected by the primary screening. The selected areas fulfilled the following criteria: (1) each selected area contained mainly proximal convoluted tubules and at least one proximal convoluted tubule cut at approximately right angle to its axis, (2) the area should be at least 200 × 300 μm and (3) the area occupied by glomerular structures had to be less than 10% of the entire selected area. Medullary rays were avoided as were large intrarenal vessels. In control kidneys, tissue with inflammatory cells was excluded and only occasional sclerotic glomeruli were permitted. Cortical tissue from hydronephrotic kidneys was accepted provided there were no polymorphonuclear leucocytes and only a few mononuclear leucocytes in the interstitium. In most cases only one cross-sectioned profile of a proximal convoluted tubule was available within the cortical area selected and sectioned for electron microscopy. No attempt was made to select any particular segment (S1 or S2) of the proximal convoluted tubules, since in atrophic proximal tubules these segments could no longer be safely identified. Tubular profiles totally lacking brush border were not included, since such profiles were not distinguishable from distal tubules.

Four cortical areas, each with a suitable proximal tubular cross-section, were analysed in each kidney. Thus, a total of 32 cortical areas were analysed in controls and 36 cortical areas in hydronephrotic kidneys.

Morphometric analyses. For each selected cortical area two montages were made from overlapping electron micrographs. One montage comprised the entire selected cortical area at a final magnification of 1900 × (magnification of negatives 830 ×) and one montage covered the selected proximal tubular cross-section at a final magnification of 10.000 × (magnification of negatives 3.300 ×). The low magnification cortical montages were used for the determination of the volume fractions of selected cortical areas occupied by proximal tubular epithelium (excluding brush border), proximal tubular lumens, epithelium and lumens from distal nephrons including distal tubules and cortical collecting ducts, interstitial connective tissue (including tubular basement membranes) and intertubular capillaries. This was accomplished by point-counting from a coherent double lattice test system (Weibel 1979) with a spacing between main lines of 30 mm (corresponding to 15.6 μm) and with a spacing between fine lines of 10 mm (corresponding to 5.2 μm). Points falling on glomerular structures or on larger vessels were excluded from the total count in each case.

The montages of individual, cross-sectioned proximal tubules were used for the determination of proximal tubular parameters. The smallest peritubular and luminal diameters were measured directly on the montages. The cell height was obtained as half the difference between the peritubular and luminal diameters. The tubular wall volume (μm^3/mm tubular length), the volume density (V_v) of mitochondria ($\mu m^3/\mu m^3$) and the surface density (S_v) of basolateral membranes ($\mu m^2/\mu m^3$) were determined by point and intersection counting, respectively, using a coherent double lattice test system. Due to the great variation in the density of the various

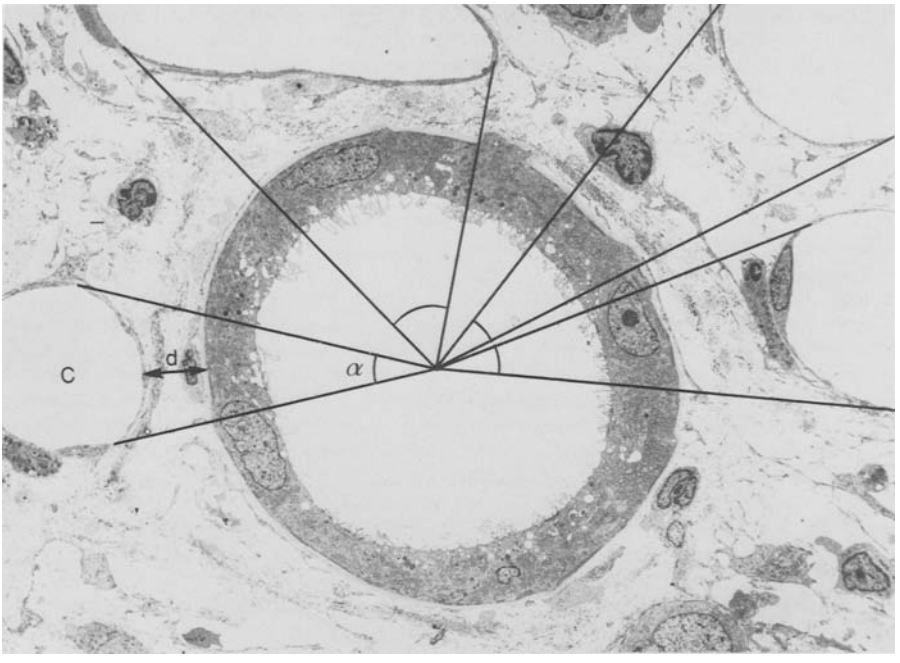

Fig. 1. Electron micrograph showing cross-sectioned atrophic proximal tubule and surrounding peritubular capillaries (*C*) and illustrating the variables used for determination of the tubulo-capillary index (TCI). As peritubular capillaries were included all capillaries from which a straight, unbroken line could be drawn to the center of the tubule. The topographical relationship between the tubule and each of the peritubular capillaries was characterized by (1) the fraction of the total tubular circumference facing the capillary (individual tubulo-capillary fraction, F) and (2) the distance between the tubule and the capillary (individual tubulo-capillary distance, D). F was determined as the tubulo-capillary angle (α in Fig. 1) in per cent of 360°. D was determined as the mean of 3–8 distances (d in Fig. 1) measured at regularly spaced intervals along the tubulo-capillary interface from the base of the tubular epithelium to the luminal surface of the capillary. The semiquantitative topographical relationship between the tubule and each peritubular capillary was then defined as F/D. The TCI was obtained as the sum of the F/D – values for all tubulo-capillary relationships. The total capillary fraction (TCF), which is the fraction of tubular circumference facing all surrounding capillaries, was obtained as the sum of individual F-values. The total capillary distance (TCD) was obtained as the mean value of all tubulo-capillary distances (*d*) measured for a given tubule

tubular characteristics to be measured in hydronephrotic kidneys two test systems with different spacing between lines had to be used. For control proximal tubules and slightly to moderately atrophic tubules a test system with 30 mm between the main lines (corresponding to 3.0 μm) and with 10 mm between the fine lines (corresponding to 1.0 μm) was used, while a test system with 25 mm and 5 mm between main and fine lines, respectively, was applied to severely atrophic tubules. The relative volume (in percent) and the absolute volume (in μm^3/mm tubular length) of mitochondria were derived from the values for mitochondrial volume density (V_v) and the tubular wall volume (TWV). The surface area of basolateral membranes (μm^2/mm tubular length) was obtained from the tubular wall volume and the value for the surface density (S_v) of basolateral membranes, which was determined from the formula: $S_v = 2N_i/L_t$, where N_i is the number of intersections between test lines and cell membranes and L_t the total length (in μm) of the test lines on the tubular wall. It should be noticed, however, that the prerequisite for the application of this stereological formula is random orientation

Table 1. Relative volumes of cortical structures in control and hydronephrotic human kidneys[a]

	Controls	Hydronephrotic kidneys		
		Subgroup I Interstitium <30%	Subgroup II Interstitium 30–45%	Subgroup III Interstitium >45%
Interstitium, mean volume % in groups	16.2±3.9	24.7±3.0	36.3±4.3	58.0±7.4
Proximal tubular epithelium, volume %	34.9±6.0	21.7±7.0***	16.4±4.4***	12.8±5.6***
Proximal tubular lumens, volume %	30.6±6.3	33.0±10	21.9±5.0***	16.1±8.1***
Distal nephron epithelium, volume %	6.58±5.9	6.09±4.9	8.12±4.3	2.5±2.6*
Distal nephron lumens, volume %	2.42±3.9	7.06±10	7.32±5.9	2.20±2.3
Unclassified tubules, volume %	0	0.185±0.67	1.79±2.9	2.92±3.0
Intertubular capillaries, volume %	9.30±3.4	7.33±2.4	8.24±3.7	5.51±2.4**
Number of cortical areas	32	13	12	11
Number of kidneys	8	6	7	5

[a] Values are means of cortical areas ±SD and are equivalent to volume densities (V_v) expressed in 10^{-2} $\mu m^3/\mu m^3$.

* $2p < 0.05$
** $2p < 0.01$
*** $2p < 0.001$

of the membranes. In oriented tubular cross-sections, as used here, this requirement may not be entirely fulfilled. However, at present the above formula represents the best approximation. The values obtained for surface density of basolateral membranes have therefore to be considered approximate. The thickness of the tubular basement membrane was measured at 8 locations at regularly spaced intervals along the tubular circumference and the result was recorded as the mean value.

To obtain a semi-quantitative estimate of the topographical relationship between individual cross-sectioned proximal tubules and adjacent peritubular capillaries a tubulo-capillary index (TCI) was defined as the ratio of the fraction (in per cent) of the total tubular circumference facing capillaries (tubulo-capillary fraction, TCF) to the average distance between the tubule and capillaries (tubulo-capillary distance, TCD; for further explanation see Fig. 1). This index has no immediate physiological correlate but is based on the assumption that the metabolic interaction between tubules and capillaries is proportional to the area of the tubulo-capillary interface and inversely related to the distance between the tubule and the peritubular capillaries. Thus, a high index is obtained when distances between tubules and capillaries are small and a large fraction of tubular circumference is facing capillaries and vice versa. The index was

Table 2. Quantitative structural analysis of cross-sectioned proximal tubules in control and hydronephrotic human kidneys[a]

	Controls	Hydronephrotic kidneys		
		Subgroup I Interstitium <30%	Subgroup II Interstitium 30–45%	Subgroup III Interstitium >45%
Interstitium, mean volume % in groups	16.2±3.9	24.7±3.0	36.3±4.3	58.0±7.4
Peritubular diameter, μm	56.9±6.6	52.6±9.8	46.7±11.0***	35.4±6.6***
Luminal diameter, μm	41.5±6.2	41.2±8.7	35.5±9.8	27.0±8.5***
Cell height, μm	7.72±1.2	5.68±0.9***	5.55±1.3***	4.22±1.6***
Tubular wall volume, 10^5 μm^3/mm tubular length	14.8±3.6	10.2±3.3***	8.35±2.7***	5.0±1.0***
Relative volume of mitochondria, % of tubular wall volume	16.3±2.3	10.7±3.5***	7.99±2.7***	5.41±1.7***
Absolute volume of mitochondria, 10^5 μm^3/mm tubular length	2.41±0.6	1.15±0.6***	0.690±0.34***	0.271±0.11***
Surface density (S_v) of basolateral cell membranes, μm^2/μm^3 tubular wall	0.963±0.20	0.815±0.20*	0.625±0.12***	0.570±0.20***
Surface area of basolateral cell-membranes, 10^5 μm^2/mm tubular length	14.3±3.6	8.17±3.3***	5.27±1.4***	2.66±0.9***
Basement membrane thickness, μm	0.641±0.27	0.785±0.36	0.975±0.32***	1.48±0.5***
Number of tubules	32	13	12	11

[a] Values are means of tubules ±SD

* $2p<0.05$
* $2p<0.01$
*** $2p<0.001$

assessed on the low-magnification montages for each of the selected proximal tubular cross-sections.

Definition of sub-groups in hydronephrosis. Since, in diagnostic practice, the amount of interstitial connective tissue is often used as a marker of the severity and progress of renal disease it was found important to determine the quantitative ultrastructural changes of proximal

Table 3. Semiquantitative topographical relationships between cross-sectioned proximal tubules and peritubular capillaries related to interstitial tissue volume[a]

	Controls	Hydronephrotic kidneys		
		Subgroup I Interstitium <30%	Subgroup II Interstitium 30–45%	Subgroup III Interstitium >45%
Interstitium mean volume % in groups	16.2±3.9	24.7±3.0	36.3±4.3	58.0±7.4
Tubulo-capillary index[b]	14.5±5.4	8.76±4.6*	7.09±3.3*	2.17±1.3*
Tubulo-capillary fraction[b], %	40.6±14	35.0±13	37.9±10	17.9±9.5*
Tubulo-capillary distance[b], μm	4.12±1.3	5.81±1.6*	7.35±2.3*	10.9±5.5*
Number of tubules	32	13	12	11

[a] Values are means of tubules ±SD
[b] for definitions see text

* $2p < 0.001$

tubules in cortical areas with a slight, a moderate and a severe increase in the volume fraction of cortical interstitium. Therefore, three hydronephrosis subgroups were arbitrarily defined according to the following ranges of interstitial tissue volume fractions: Subgroup I <30.0%, subgroup II 30.0–45.0% and subgroup III >45.0%. Each subgroup was then compared with the control group with respect to different cortical and proximal tubular variables and also as regards the individual components of the tubulo-capillary index. Due to regional variations as to the amount of interstitial tissue within the same kidney cortical areas from a hydronephrotic kidney were usually allocated to more than one subgroup. Thus, subgroups I, II and III comprised cortical tissue from 6, 7 and 5 hydronephrotic kidneys, respectively.

Statistical analyses. Each of the hydronephrosis subgroups were compared with the control group using t-tests for testing differences between means. Since each group contained cortical areas from the same kidney as well as cortical areas from different kidneys, it was first demonstrated that in the control group the inter-kidney variations were not significantly larger than the intra-kidney variations. This conclusion was also considered applicable to the hydronephrotic kidneys, since they contributed about equally to the different subgroups and since the variation within each subgroup was comparatively large. Furthermore, for each pair of variables in the hydronephrotic kidneys a regression analysis with random coefficients was performed to assess a possible relationship between the variables. If appropriate a transformation (logarithmic or reciprocal) of one or both of the variables was performed before the analysis. In the analysis a regression line was fitted for each kidney and an overall estimate of the slope, b, was obtained. Two different versions of the test statistic $t = b/SE(b)$ were used to evaluate the strength of the association. If the variation in the individual slopes was not significantly larger than expected when compared with the variation around the individual lines, the overall slope b was obtained as a weighted average of the individual slopes and the standard error of b was derived from the intra-individual variation. Otherwise b was obtained as a simple average of the individual slopes and the standard error was derived from the variation between the individual slope estimates. An overall coefficient of determination R^2 was determined as a weighted average of R^2 for each kidney.

The curves fitted to the data shown in the figures were not derived from the above statistical analyses, but were obtained using a standard program on a HP-85 desk computer not allowing for a possible correlation between measurements from the same kidney.

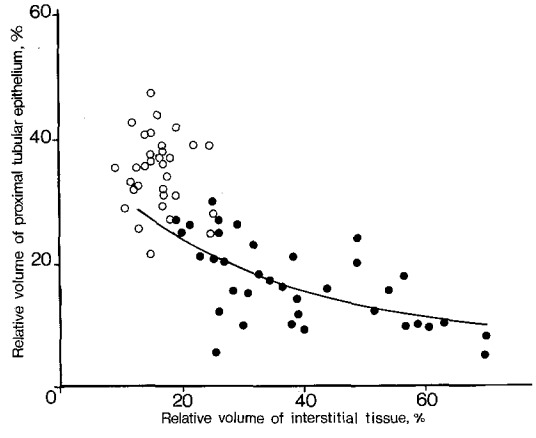

Fig. 2. Relative volume of proximal tubular epithelium related to relative volume of interstitial tissue. Controls (o) no correlation. Hydronephrosis (•) significant inverse correlation ($2p < 0.05$, $R^2 = 0.24$). Values originate from 8 control and 9 hydronephrotic kidneys (4 specimens per kidney)

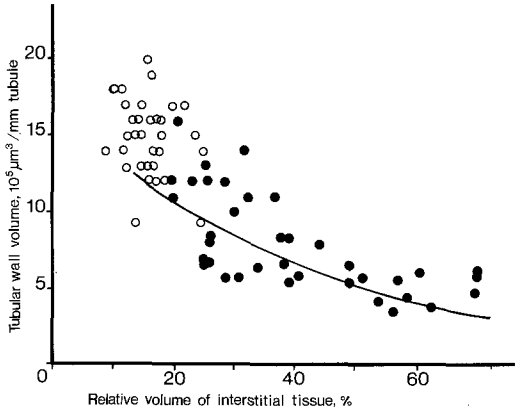

Fig. 3. Tubular wall volume of individual cross-sectioned proximal tubules related to relative volume of surrounding interstitial tissue in control and hydronephrotic kidneys. Controls (o) no correlation. Hydronephrosis (•) significant inverse correlation ($2p < 0.01$, $R^2 = 0.45$). Each point is derived from measurements on one cross-sectioned proximal tubule and its surrounding interstitium

Results

Relative volumes of cortical structures in control and hydronephrotic kidneys. The mean relative volumes for different cortical structures appear from Table 1. In control kidneys the relative volume of cortical interstitium showed a mean value of 16.2% (range: 8.9–25.2%). Hydronephrotic kidneys were characterized by an increase in relative volume of the cortical interstitium. When the cortical areas were divided into three subgroups on the basis of the relative volume of the interstitium significant differences were demonstrated between these subgroups and the control group with respect to other cortical variables. Thus, there was a significant decrease in the relative volume of proximal tubular epithelium in hydronephrosis subgroup I (mean interstitial volume % = 24.7), and this showed a further reduction in subgroup II (mean interstitial volume % = 36.3) and III (mean interstitial volume % = 58.0). The relative volume of proximal tubular lumens, having

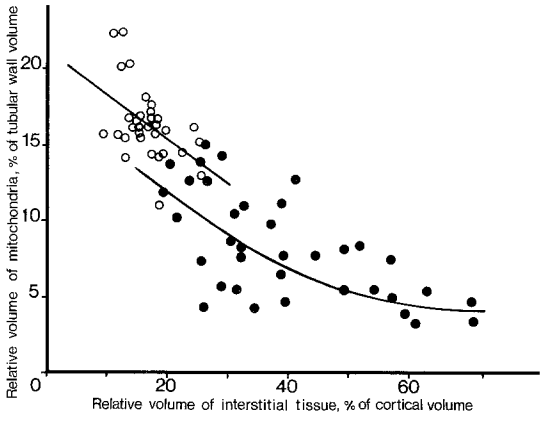

Fig. 4. Relative volume of mitochondria in individual cross-sectioned proximal tubules related to relative volume of interstitial tissue. Controls (o) significant inverse correlation ($2p < 0.01$, $R^2 = 0.41$). Hydronephrosis (•) significant inverse correlation ($2p < 0.02$, $R^2 = 0.40$)

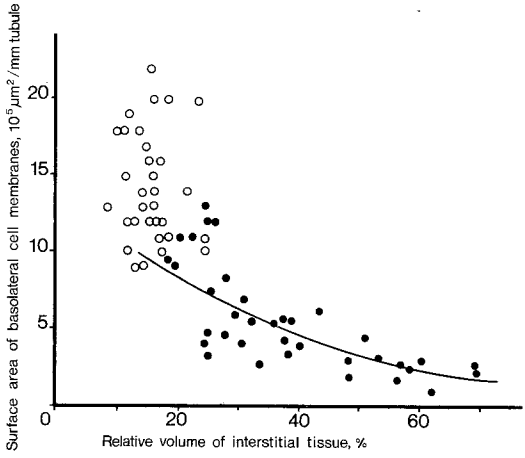

Fig. 5. Surface area of basolateral membranes in individual cross-sectioned proximal tubules related to relative volume of interstitial tissue. Controls (o) no correlation. Hydronephrosis (•) significant inverse correlation ($2p < 0.01$, $R^2 = 0.49$)

a mean value of 30.6% in controls, was not significantly changed in subgroup I, but a significant reduction appeared in subgroup II and a further reduction in subgroup III. The relative volume of distal nephron epithelium showed no significant changes from control values in any of the subgroups. The relative volumes of intertubular capillaries in subgroups I and II were not significantly different from control values, but in subgroup III the relative volume of cortical capillaries was significantly reduced.

Quantitative structure of cross-sectioned proximal tubules in control and hydronephrotic kidneys. Values for different proximal tubular variables are listed in Table 2. Increase in relative volume of cortical interstitium from a mean value of 16.2% in controls to 24.7% in hydronephrosis subgroup I resulted in insignificant changes in peritubular and luminal diameters of cross-sectioned proximal tubules. In subgroup III, however, there was a

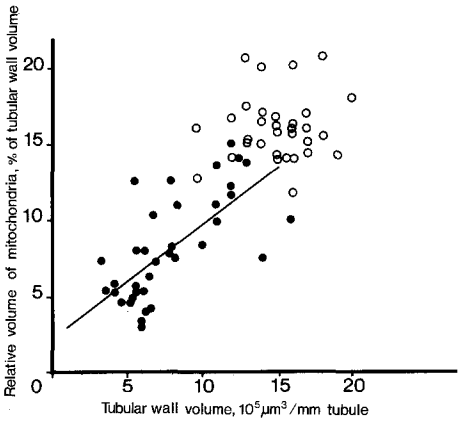

Fig. 6. Relative volume of mitochondria in individual cross-sectioned proximal tubules related to tubular wall volume. Controls (o) no correlation. Hydronephrosis (•) significant positive correlation ($2p < 0.02$, $R^2 = 0.72$)

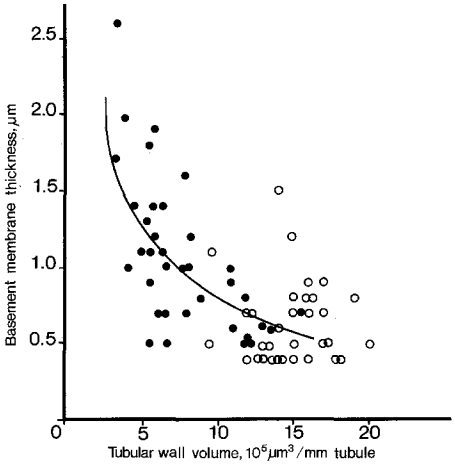

Fig. 7. Basement membrane thickness of individual cross-sectioned proximal tubules related to tubular wall volume. Controls (o) no correlation. Hydronephrosis (•) significant inverse correlation ($2p < 0.05$, $R^2 = 0.21$)

significant reduction in proximal tubular luminal diameters. Both proximal tubular cell height and tubular wall volume showed a significant decrease in subgroup I. Furthermore, in subgroup I the volume (relative and absolute) of mitochondria was only about half that of control values and the surface area of basolateral membranes was reduced to about 40%. In subgroups II and III this tendency was further accentuated. The thickness of tubular basement membranes showed a gradual increase in all subgroups.

Relationship between proximal tubules and peritubular capillaries in different subgroups. The tubulo-capillary index (TCI), which in a semi-quantitative manner describes the topographical relationships between tubules and capillaries, showed significant reductions in all subgroups when compared with control values (Table 3). This reduction was attributable mainly to changes in the distances between tubules and capillaries, which appeared significantly increased in subgroup I, whereas the fraction of tubular circumference facing capillaries (TCF) was not significantly reduced except for subgroup III.

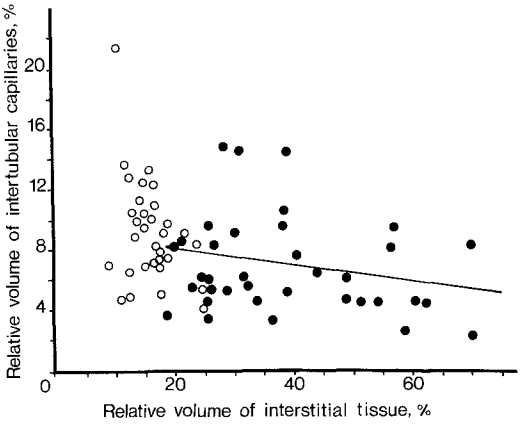

Fig. 8. Relative volume of intertubular capillaries related to relative volume of interstitial tissue. No correlation ($0.20 < 2p < 0.30$)

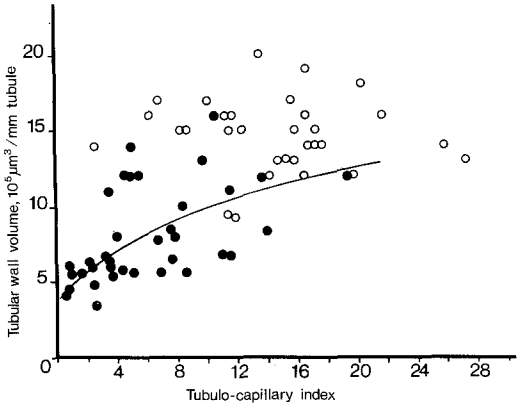

Fig. 9. Tubular wall volume of individual cross-sectioned proximal tubules related to tubulo-capillary index. Controls (○) no correlation. Hydronephrosis (●) significant positive correlation ($2p < 0.05$, $R^2 = 0.19$)

Correlations between proximal tubular and interstitial variables. In these analyses values for individual proximal tubules and cortical areas from control and hydronephrotic kidneys were used without prior subclassification into groups. In hydronephrotic kidneys the statistical analyses showed several significant correlations between proximal tubular variables and cortical interstitium as well as between different proximal tubular variables mutually. Thus, the relative volume of proximal tubular epithelium was inversely correlated with the relative volume of cortical interstitium (Fig. 2). The tubular wall volume per mm tubular length, the relative volume of mitochondria and the surface area of basolateral cell membranes all showed inverse correlations with the relative volume of interstitium (Figs. 3–5). Furthermore, there was a positive correlation ($2p < 0.01$, $R^2 = 0.68$) between the thickness of the tubular basement membrane and the relative volume of interstitial tissue. Finally, it was demonstrated that in controls there is a significant positive correlation between the relative volume of mitochondria in per cent of tubular wall volume and the relative volume of interstitial tissue

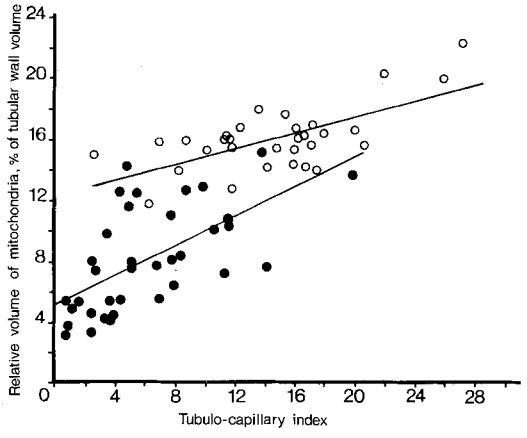

Fig. 10. Relative volume of mitochondria in individual cross-sectioned proximal tubules related to tubulo-capillary index. Controls (o) significant positive correlation ($2p < 0.01$, $R^2 = 0.61$). Hydronephrosis (•) significant positive correlation ($2p < 0.02$, $R^2 = 0.41$)

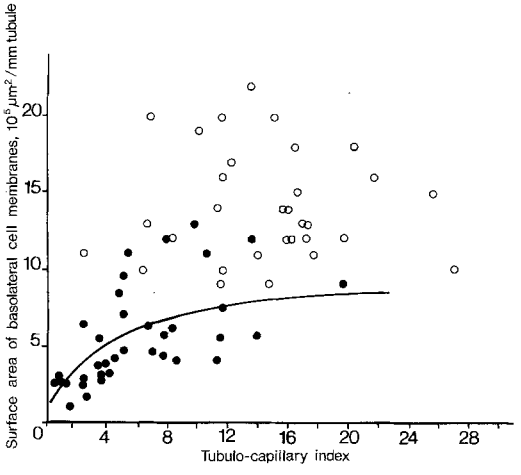

Fig. 11. Surface area of basolateral membranes of individual cross-sectioned proximal tubules related to tubulo-capillary index. Controls (o) no correlation. Hydronephrosis (•) significant positive correlation ($2p < 0.05$, $R^2 = 0.19$)

(Fig. 4). The tubular wall volume per mm tubular length showed positive correlations with the relative volume of mitochondria (Fig. 6) and with the surface density of basolateral membranes ($2p < 0.01$, $R^2 = 0.68$). In addition, there was an inverse correlation between the thickness of the tubular basement membrane and the tubular wall volume (Fig. 7). The relative volume of cortical capillaries showed no significant correlations ($0.05 < 2p < 0.10$) with the various proximal tubular variables or with the relative volume of cortical interstitium (Fig. 8).

Correlations between tubular variables and the tubulo-capillary index (TCI). In hydronephrotic kidneys the tubular wall volume was positively correlated with the TCI (Fig. 9). The relative volume of mitochondria showed positive correlations with the TCI not only in hydronephrotic kidneys but also in controls (Fig. 10). There was also a significant positive correlation between the surface area of basolateral membranes per mm tubular length and the TCI (Fig. 11).

Discussion

The present investigation demonstrates that in human hydronephrosis there are significant correlations between quantitative changes in proximal tubular ultrastructure and changes in the surrounding interstitium, including the relationships between tubules and capillaries. Furthermore, it shows that an increase in relative volume of cortical interstitial tissue from 16.2% in controls to 24.7% in cortical areas of hydronephrotic kidneys is associated with marked reductions in proximal tubular wall volume, volume of mitochondria and surface area of basolateral membranes. In the following we shall consider the representativity of the control material and the sampling problems involved in the study. Furthermore, we shall discuss the implications of the results for the pathogenesis of proximal tubular atrophy and for glomerular function in the light of prevailing theories.

Representativity of control material. In the present study the relative volume of cortical interstitium was used as a basis for the comparison of quantitative changes of proximal tubules in atrophy. It is therefore important to compare the value for this variable in control tissue with data derived from previous investigations of non-diseased human kidneys, in particular as the present control kidneys were in part the seat of pathological changes. The cortical interstitium of the present control group on the average constituted 16.2 vol.% of analysed cortical areas. This value is somewhat less than the value of 19.3 vol.%, which is calculated from the data given by Kappel and Olsen (1980) for non-diseased, formalin-fixed human kidneys from individuals with a mean age similar to that of the present control group (61.9 years). On the other hand the present value is somewhat higher than that of 13.6 vol.% reported by Hestbech et al. (1977) for formalin-fixed kidneys from individuals with a slightly lower mean age (50.5 years). However, these minor discrepancies may be due not only to differences in the definition of the compartment "interstitium" as pointed out by Kappel and Olsen (1980) but also to differences in tissue preparation and in evaluation by light and electron microscopy. Therefore, in spite of the limitations of comparisons with previously published data, it is concluded that the values for relative volume of cortical interstitium found in the present control kidneys may be considered as being within the normal range.

Identification of tubules. In the normal kidney cortex a distinction between the convoluted and straight parts of the proximal tubules is fairly easy, whereas the modifications of ultrastructure, which take place in the proximal tubule in atrophy (Møller et al. 1984) and the general disorganization of the kidney cortex in chronic renal disease may render this distinction less obvious. However, in the present series it was found that identification of the convoluted part of the proximal tubule was difficult only in cases of severe cortical interstitial fibrosis (i.e. relative volume of interstitium $>50\%$), where both the structural characteristics of the tubules and the general cortical architecture were considerably changed. In slight and mod-

erate degrees of cortical interstitial fibrosis, however, cortical architecture was usually sufficiently well maintained to allow a distinction between the convoluted and straight parts partly because of the parallel course of the latter in the medullary rays.

The observed variations in proximal tubular variables in the control group probably in part reflect differences between various segments (S1 and S2) of the proximal convoluted tubules (Maunsbach 1973). In different stages of hydronephrotic cortical atrophy, in addition, the variation might also be due to a heterogenous morphological change of the tubules in the development of atrophy.

The influence of age on the ultrastructure of human proximal tubules is not known, but according to observations in rat kidneys increasing age does not significantly alter such proximal tubular variables as relative volumes of mitochondria, lysosomes and endocytic vacuoles (Christensen and Madsen 1978).

Quantitative relationship between tubular and interstitial parameters. The present observations confirm the results of previous light microscopic investigations (Mackensen-Haen et al. 1981) in showing that there is a significant inverse correlation between quantitative changes in cortical interstitium and proximal tubular epithelium. These observations are further extended by the demonstration of close correlations between increase in cortical interstitium and decrease in various structural constituents of proximal convoluted tubules, which is in agreement with our previous findings in a study of the qualitative changes of proximal tubules in atrophy (Møller et al. 1984). The quantitative changes from control values recorded in subgroup I indicate that even at this low level of cortical interstitial increase (about 25 vol.%) there may be considerable reductions in functionally important constituents of the proximal tubules, such as mitochondria and basolateral membranes. The reduction in the surface area of basolateral membranes corresponds with the qualitative observation that in early atrophy there is a reduced number of interdigitations between cell membranes of adjacent tubular cells (Møller et al. 1984). Concerning the mitochondria there was a reduction not only in absolute but also in relative volume, which means that the decrease in mitochondria in tubular atrophy is proportionally larger than the decrease in tubular wall volume.

In previous light-microscopic analyses of the quantitative relationship between changes in cortical interstitium and capillaries in chronic renal disease it has been demonstrated that with an increasing interstitium there is a reduction in the number and area of postglomerular capillaries (Bohle et al. 1981). Such a relationship was not observed in the present study. In contrast, and in agreement with findings in lithium-induced nephropathy (Christensen et al. 1982) this study showed that the relative volume of cortical capillaries remain unchanged, unless the cortical interstitium is greatly increased in volume (i.e. exceeds 40 vol.%).

In spite of the observed inverse correlation between the tubular wall volume and the thickness of the tubular basement membrane the latter

often showed inconsistent quantitative changes. Thus, proximal tubules, which according to other ultrastructural criteria were severely atrophic, often showed insignificant changes in basement membrane thickness. In addition, control tubules occasionally had a basement membrane thickness corresponding to that of severely atrophic tubules. Therefore, the basement membrane thickness is less valuable as an indicator of tubular atrophy than are other ultrastructural parameters.

Tubulo-capillary index (TCI) related to tubular ultrastructure. Attempts to determine the spatial relationship between tubules and peritubular capillaries have been carried out in a few previous studies (Gise et al. 1981; Sloper et al. 1980). In these only the distances between tubules and capillaries were taken into account. The evaluation of a tubulo-capillary index (TCI) as used here takes into account not only mean tubulo-capillary distances but also the fraction of tubular circumference facing capillaries, which may be equally important for the interaction between tubules and capillaries (see Materials and methods). Additionally, the use of this latter variable combined with the mean tubulo-capillary distance minimizes the problem encountered in previous studies as to whether or not remote capillaries should be included in the evaluations (Sloper et al. 1980). It should be pointed out, however, that the principles applied here for the assessment of the TCI do not fulfill all criteria for stereological analysis and the present method should therefore be considered a semiquantitative evaluation rather than a strictly stereological one.

Pathogenic mechanisms in tubular atrophy. The present observation that several structural characteristics of the proximal tubules showed significant inverse correlations with the relative volume of cortical interstitium suggests that there is a pathogenic interrelationship between the increase in the interstitium and proximal tubular atrophy. On account of the demonstrated relationship between TCI and proximal tubular variables it seems reasonable to assume that the increase in cortical interstitium may influence proximal tubular ultrastructure by altering the spatial associations and consequently metabolic interactions between tubules and capillaries. Such a mechanism is consistent with the observation that the functional capacity of proximal tubules for protein absorption becomes reduced, when tubulo-capillary distances increase (Gise et al. 1981). The possibility that increased interstitium may compress peritubular capillaries and thereby reduce tubular blood-supply appears unlikely, since there was no correlation between the relative volumes of cortical capillaries and interstitium, respectively.

Whether or not changes in proximal intratubular pressure may have pathogenic significance in tubular atrophy in hydronephrosis is difficult to evaluate. Observations in rats have shown that ureteral obstruction is followed by increased proximal intratubular pressure in both acute (Gottschalk and Mylle 1956) and chronic (Wilson 1972) hydronephrosis. However, a corresponding morphological change in the proximal tubules, i.e. a dilatation of the tubular lumens, appears to be an inconsistent finding

in experimental hydronephrosis. In rabbits, for example, ureteral obstruction may even result in collapse of the proximal tubules (Kinn and Bohman 1983; Nagle et al. 1973). In the present hydronephrosis group proximal tubules showed no increase in luminal size despite reduction in cell height. The reason for this may be that proximal tubular dilatation is an early and transitory phenomenon, which is no longer present in the stages of hydronephrosis represented in this material.

It has been demonstrated that acute ureteral obstruction is followed by a significant drop in cortical capillary blood-flow (Huland et al. 1980) and in glomerular filtration rate (Harris and Gill 1981), which has been attributed to a release of vasoactive substances such as renin (Vaughan et al. 1970) and prostaglandin (Huland and Gonnermann 1983). It is therefore possible that part of the structural changes in the proximal tubules represent an adaptive response to a reduced functional load, which precedes alterations of the interstitium and tubulo-capillary relationships.

Potential effects of tubular and interstitial changes on glomerular filtration rate (GFR). Two main hypotheses have been put forward concerning a possible relationship between alterations in the tubulo-interstitial compartment and a reduced GFR. One hypothesis favours the role of the interstitial changes in supposing that an increase in the interstitial connective tissue may reduce GFR by means of constriction of the post-glomerular capillaries (Bohle et al. 1977). Support for this theory has been provided by the demonstration of significant correlations between relative volume of interstitial tissue, number and area of post-glomerular capillaries and serum-creatinine in chronic renal disease (Bohle et al. 1981). The present results are not immediately consistent with this concept, since they show that changes in cortical interstitium and capillaries are unrelated within a large range of interstitial volume changes. According to another hypothesis a reduced uptake and transport of sodium chloride by pathologically changed proximal tubular cells may reduce GFR by way of a negative feed-back mechanism triggered by an increased load of sodium chloride on the macula densa region of the distal tubules (Bohle and Thurau 1974). In our previous qualitative study of proximal tubular atrophy (Møller et al. 1984) it was suggested that the reduction of basolateral membranes and mitochondria in particular represented a possible structural correlate to the functional impairment implied by this so-called Thurau mechanism. The present quantitative findings are in support of this view by showing that even in supposedly early stages of atrophy there is a 40–50% reduction in basolateral membranes and mitochondria.

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