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Cortical Interstitial Tissue and Sclerosed Glomeruli in the Normal Human Kidney, Related to Age and Sex

A Quantitative Study

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Summary. The relative amount of interstitial cortical tissue was measured by the point count method in kidney tissue from human individuals without renal disease. One series (54 kidneys) consisted of kidneys intended for transplantation and removed immediately after death from persons who died suddenly. The other series (69 kidneys) was obtained by autopsy. In both groups, the percentage of interstitial tissue was dependent on age and followed the equations of regression (1) $y=12.45+0.11\ x$ (donor series) and (2) $y=23.8+0{,}10\ x$ (autopsy series). The autopsy values were significantly greater than the donor-kidney-values. There was no difference due to sex. The relative number of sclerotic, obsolescent glomeruli was very small (0–1%) until the age of 40. Thereafter it increased, most markedly in the autopsy series, until it reached values of about 30% in persons more than 80 years old.

Key words: Renal cortical interstitium – Sclerosed glomeruli – Age-relation – Morphometry.

Introduction

Interest in the importance of the renal interstitium for kidney pathology (Bohle et al., 1977[1, 2, 3]; Fischbach et al., 1977; Jepsen and Mortensen, 1979; Hestbech et al., 1977) has increased during later years. Reliable normal values of the relative amount of cortical interstitial tissue in man for different age groups are, however, not available. The same applies to another important variable in kidney pathology, the relative number of obsolescent glomeruli.

We decided therefore to investigate these variables in order to obtain reference values for comparison with morphometric studies of pathological material. Ideally a study of this type should be performed on renal biopsies from human individuals without kidney disease. Since ethical considerations prevented us

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from making biopsies on normal persons we chose to use material from two other sources which á priori should be expected to reflect normality. These sources were: 1) tissue from cadaver kidneys aimed at transplantation or from normal nephrectomized kidneys, and 2) kidneys obtained by autopsy from patients which satisfied specified criteria of normal renal function.

Material and Methods

1. The Donor Group. (54 kidneys from 54 persons). Most of the kidneys (47) were removed with the aim of transplantation, but for various reasons they were not used for this purpose (no suitable recipient available, perfusion not judged to be optimal, the presence of abnormal arteries making anastomosis technical difficult, or lack of a surgical team at the time scheduled for transplantation). The potential donors were previously healthy apart from the acute condition which led to a sudden death (traumatic lesions due to traffic accidents, acute intracranial haemorrhages etc.). The criteria for selection of donor kidneys include negative urinanalysis for protein, se-creatinin below 1.3 mg%, no malignant disease present and no symptoms or signs of renal disease according to available information. For practical reasons we use the term "donor group" although only 47 out of 54 kidneys were from potential donors. Included in this group were 7 normal kidneys obtained by surgery from living patients at correction of dystopia or removed due to surgical complications comprising the renal vessels or ureter.

2. The Autopsy Group. (69 kidneys from 69 patients). These kidneys were selected from the autopsies performed at the Institute of Pathology, Kommunehospitalet, Aarhus during the period 1.9.1977–1.4.1978. Only kidneys from patients who fulfilled the following criteria were included: 1) No symptoms or signs of renal disease revealed during life according to the written report, 2) proteinuria and glucosuria excluded by investigation of the urine during his last stay at the hospital, 3) no diabetes mellitus present, 4) se-creatinine below or at 1.3 mg%, 5) normal blood pressure, 6) no focal macroscopical changes of the kidney at autopsy (cysts, tumors).

The distribution of the material according to age and sex appears in Table 1.

Histological Technique. Tissue was fixed by immersion for 24–28 hours in 4% aqueous formaldehyde buffered to pH 7.1 with phosphate. Dehydration with alcohol, embedding in paraffin following the standard procedure of the laboratory. Approximately 5 µm sections were stained for connective tissue with Picro-Sirius, a modification of the method of van Gieson using Sirius-red instead of Fuchsin.

Morphometry. The relative amount of interstitial fibrous tissue was measured by a point counting technique (Hestbech et al., 1977). The measuring 36-point ocular grid had a square corresponding

Table 1. Distribution by age and sex

Age (years)	Donor series		Autopsy series	
	Male	Female	Male	Female
9	–	1	–	–
10–19	5	4	–	–
20–29	12	2	–	–
30–39	5	1	1	6
40–49	8	4	4	2
50–59	5	4	5	2
60–69	1	1	3	11
70–79	1	–	13	12
80–89	–	–	3	4
90–98	–	–	2	1
	37	17	31	38

to 250 μ^2. The total magnification used was 400×. Counted was 15–25 fields (average: 20). The first measuring field was placed just subcortically, followed by consecutive fields across the cortex. Each point was registered as either "fibrous tissue" or "not fibrous tissue". Included in the first category were the basement membranes of the tubules but not the tubular cells and lumina, the renal corpuscles with their contents, vessels or perivascular tissue. The number of points on "fibrous tissue" divided by the total number of points constituted the per cent of interstitial fibrous tissue.

The relative number of totally sclerotic, obsolescent glomeruli were counted using a magnification of 100× and differential counting of 100 glomeruli. Partially sclerosed glomeruli were not included, but were rare. All investigations were done blindly, i.e. without the investigator knowing the age and sex of the patient.

Precision and Representativity. The precision of the point count method was determined from double measurements blindly performed on 10 specimens. There was a SD of 1.0 on values of interstitial fibrous tissue from 8.5% to 23.0% corresponding to deviations of the douple measurements between 4.3% and 11.8%.

We checked that results were uniform in different locations of the kidney. 10 specimens from one kidney were obtained, distributed from one pole to the other. There was a SD of 1.8 on values of interstitial fibrous tissue of 30.9–37.2%, i.e. a variation of 5.8%–4.8%.

Statistical Methods. A regression analysis for variables normally distributed around the regression line was used. For the calculation of a possible difference of regression lines the two relevant lines were compared with regard to inhomogeneity of variance (Geigy, Scientific Tables, 7. ed., Basel, pp. 174–179). Significance levels of 5% were used.

Results

Interstitial Fibrous Tissue

The measuring results together with the regression lines are displayed in Fig. 1. The equation of regression for the *donor series* is:

$$y = 12.45 + 0.11x$$

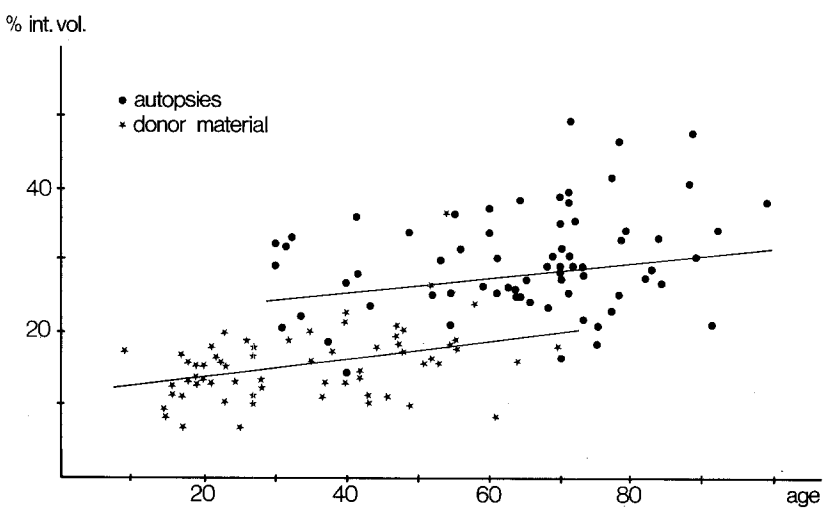

Fig. 1. Percent interstitial volume in renal cortex correlated to age

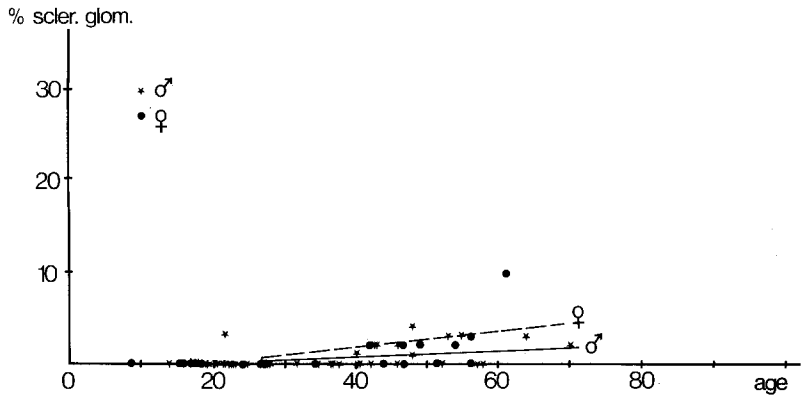

Fig. 2. Percent sclerosed glomeruli correlated to age donor-kidney series

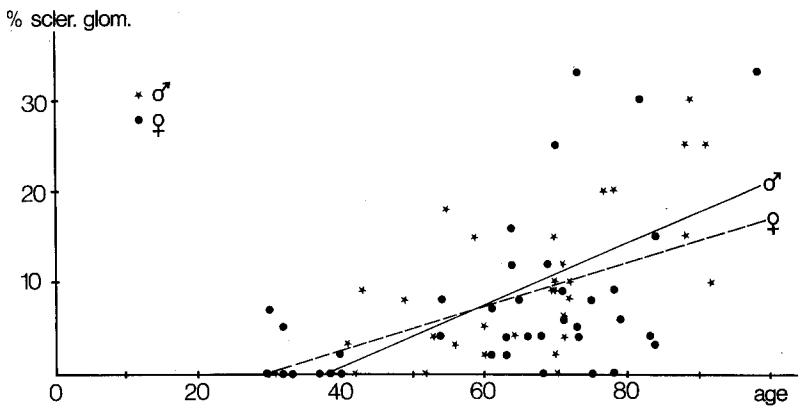

Fig. 3. Percent sclerosed glomeruli correlated to age autopsy series

with x as the age in years and y as the relative amount of interstitial fibrous tissue in the cortex.

For the *autopsy series* the corresponding equation is:

$$y = 23.8 + 0.10x.$$

The regression lines are significantly displaced ($2p < 0.001$), i.e. for a given age, the relative amount of interstitial tissue is greater in autopsy specimens than in donor tissue. The dependency on age is significant (donor material: $2p = 0.022$; autopsy material: $2p = 0.04$). There was no difference between the slopes of the regression lines ($2p = 12.5$). There is no significant difference between the regression lines of males versus females.

Glomerular Sclerosis

The relative number of sclerotic glomeruli appears from Figs. 2 and 3. The equation of regression for the *donor series* is:

$$y = -1.20 + 0.06 \cdot x.$$

Table 2. Reported values of the relative amount of renal cortical interstitial fibrous tissue in man

Author	Number of kidneys	Age range	Relative amount of interstitial tissue	Correlation to age
Dunnill and Halley (1973)	17	1 d–73 y	<36 y: $11.7\% \pm 5.5\%$ >36 y: $15.7\% \pm 3.0\%$	n.s.
Bohle et al. (1977)	20	?	mean: 9.0% range: 7–11%	?
Hestbech et al. (1977)	13	33–65	mean: 13.6% range: 6.3%–21.3%	n.s.
Present study	donor ser.		range: 7.1%–37.1%	
	54	9–70	regr. lin.: $11.9\% + 0.12$ x age	yes
	autop. ser.		range: 14.8%–47.9%	
	69	30–98	regr. lin.: $23.8\% + 0.1$ x age	yes

The correlation with age is significant ($2p < 0.01$). For the *autopsy series*, the corresponding equation is:

$$y = -9.62 + 0.62 \cdot x.$$

The correlation with age is significant ($2p < 0.001$).

The differences between the regression lines of males and females are not significant.

Discussion

The Relative Amount of Interstitial Fibrous Tissue

Previous quantitative studies of this variable in human renal tissue are scarce and performed on small series. The main results are shown in Table 2. The investigations of Dunnill and Halley (1973), and the present authors were done on whole kidneys, those of Bohle et al. (1977) on needle biopsies from living persons and those of Hestbech et al. (1978) on needle "biopsies" from forensic autopsies and donor kidneys. There is a rather good correspondance between the results of the three preceding investigators and the values of the donor series from the present study. Our results from the donor series are slightly higher than previous results which might be explained by the fact that we counted the tubular basement membranes together with the interstitium. This was not the case in the study of Hestbech et al. (1977) and it is not known if it was done in the other two studies. Our reason for doing this was that we felt that the decision of fibrous tissue versus tubule was easier (and the measurements therefore more precise) if the tubular basement membranes were counted together with fibrous tissue.

The significant increase of fibrosis with age demonstrated by the present study was not apparent from the two previous reports, which contained data relevant to the problem. Although the average percentage of interstitial fibrous tissue was greater in Dunnill's older group than in his younger, the difference

was not significant. We consider these negative results to be due to the small size of these series.

The systematic difference between our donor series and autopsy series is interesting. As appears from Fig. 1 the regression line is situated at a higher level for the autopsy series than for the donor series. This is evident also in the age-interval in which the two series overlap. One explanation for this result might be that the kidneys of the autopsy series are not so "normal" as those of the donor material. The former are kidneys which might have been subject to influences from suffering sustained in a severe illness eventually leading to death. This influence might have led to some diffuse nephron atrophy with accompanying interstitial fibrosis, without being detected by the relatively crude investigations made in order to exclude renal disease.

Whatever the cause may be it appears to us that this result is of importance for the selection of normal controls for comparison with specimens investigated for possible pathological alterations. The controls should of course be age matched as is evident from our demonstration of age-dependent interstitial fibrosis. It is, however, also important not to compare test material obtained from biopsies with normal material from autopsy kidneys.

Glomerular Sclerosis

The demonstration of an increase in the number of sclerosed glomeruli with advancing age is consistent with the general experience and is a common statement in textbooks. Nevertheless the quantitative study of Howell and Piggot (1948) failed to reveal a correlation with age, but the number of subjects was small (20). On the other hand, Kaplan et al. (1975) found that the number of sclerotic glomeruli in kidneys removed at autopsy was small until the age of 40. After this age there was an increase and a broad scatter of the values. The present study shows that the relative number of totally sclerosed glomeruli is very small until the age of 40. From this age there is an increase to a level of about 30% in persons more than 70 years old. The tendency is much more marked in the autopsy series also in the age-interval common for the two series. This difference between the series may be due to the same cause, suggested above for interstitial fibrosis. The x-axis is cut by the regression lines at about 20 and 33 years indicating that physiological glomerular obsolescence begin at this age.

It has been reported [Shock (1946)[1, 2], Davies and Shock (1950), Olbrich et al. (1950)] that glomerular filtration rate (GFR) (measured as inulin clearance) tubular function (measured as T max of Diodrast) and renal plasma flow (measured as the clearance of hippurate) undergo a decrease of 40–50% between 40 and 80–90 years. The weight of the total renal mass and of the cortical tissue mass is reduced to about half of the value at 40 years over the same time span. The reduction of the GFR is partly explained by the reduction of the number of glomeruli due to glomerular sclerosis, but total disappearance of glomeruli cannot be excluded. More precise calculation, demanding counting of the absolute number of glomeruli and taking into consideration the diminished mass of cortical tissue is necessary to clarify this point.

The reduction of the tubular function should be considered to be a function of the reduction of total mass of renal tissue (Wald, 1937; Arataki, 1926; Dunnill and Halley, 1973) together with the increase in relative amount of interstitial tissue. More precise calculations can only be performed if all spatial compartments of the kidney are measured and compared with total renal mass.

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