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The Influence of Glomerular and Interstitial Factors on the Serum Creatinine Concentration in Renal Amyloidosis

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Summary. 48 cases of renal amyloidosis (grade I–IV) were investigated morphologically (point-counting method). Statistically significant positive correlations exist between relative interstitial volume and serum creatinine concentration at the time of biopsy. In amyloidosis there are furthermore connections (positive rank correlations) between the extent of glomerular amyloidosis and renal insufficiency, although single cases of grade III show normal serum creatinine concentrations if the interstitium is not enlarged. With regard to interstitial alterations only fibrosis, not amyloid masses, seems to influence renal function, especially in the case of predamaged glomeruli (grade II to IV). The diminished renal function – reduction of the glom. filtr. rate – may be explained in the case of interstitial fibrosis by slowing of renal blood flow caused by a decreasing cross-sectional area of postglomerular vessels.

Renal insufficiency seems to depend upon both the glomerular and the interstitial factor, but in grades II–IV the interstitial fibrosis is thought to be of more importance for renal function.

Key words: Renal insufficiency – Interstitium – Glomerular involvement – Interstitial fibrosis – Renal amyloidosis.

Introduction

In perimembranous, membranoproliferative, mesangioproliferative and endocapillary (acute) glomerulonephritis (GN) it has been shown that whilst there are no significant correlations between serum creatinine concentration and the severity of glomerular lesions, those between serum creatinine concentration and the degree of enlargement of the renal interstitium are good (Bohle et al., 1977; Fischbach et al., 1977).

This paper will attempt to determine whether in renal perireticular amyloidosis, in which the amyloid masses are localized primarily in the glomerular tuft, the serum creatinine concentration is influenced mainly by the extent of these deposits or rather by the enlargement of the interstitium.

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Materials and Methods

48 biopsies of renal amyloidosis of different grades of severity containing at least 5 glomeruli were investigated. 5 grades of amyloid deposits are differentiated, using the following criteria.

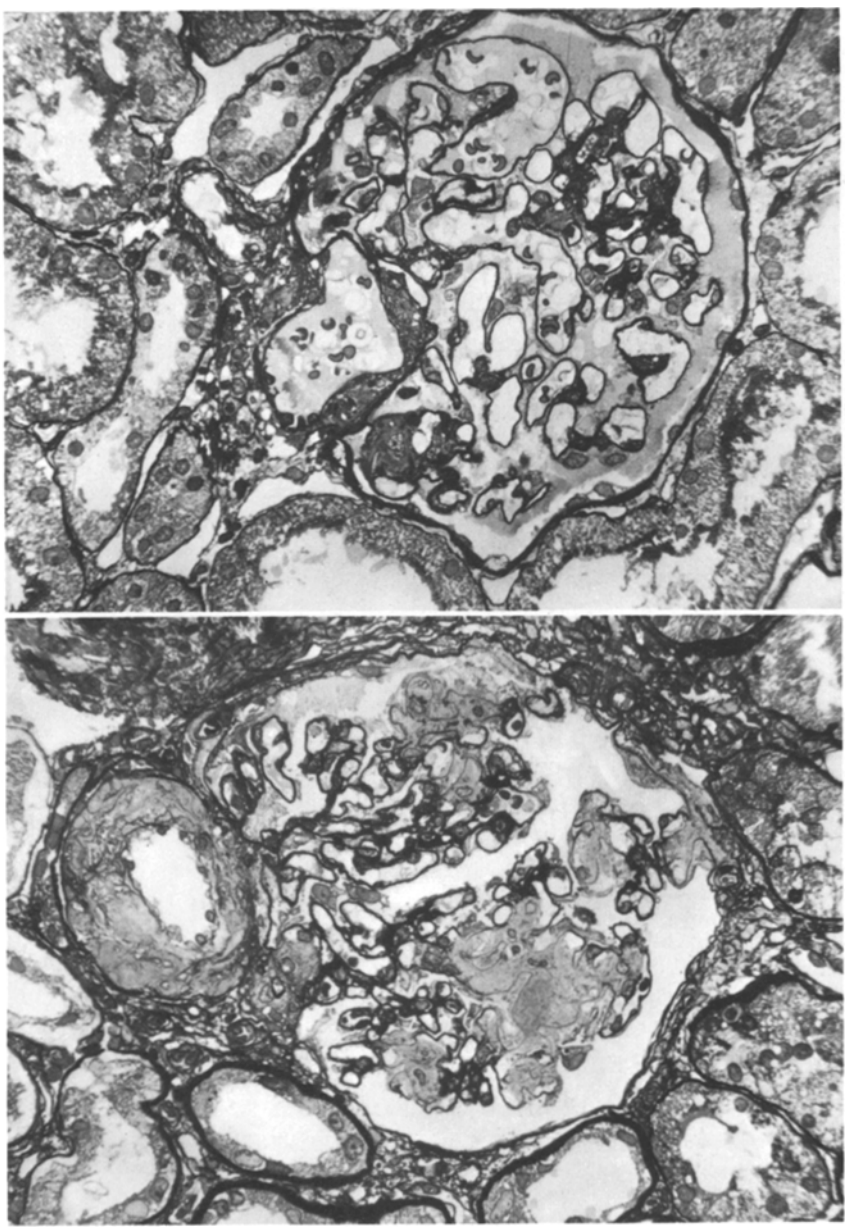
Grade I: Discrete mesangial and subendothelial deposit of amyloid in single lobules of the glomerular tuft.

Grade II: Deposit in up to 50% of the glomerular tuft in all glomeruli.

Grade III: Deposit in up to 70% of the glomerular tuft in all glomeruli.

Grade IV: Deposit of amyloid in almost all capillaries of the glomeruli.

Grade V: Glomeruli almost without nuclei (cells) looking like hyaline spheres.



a

1b

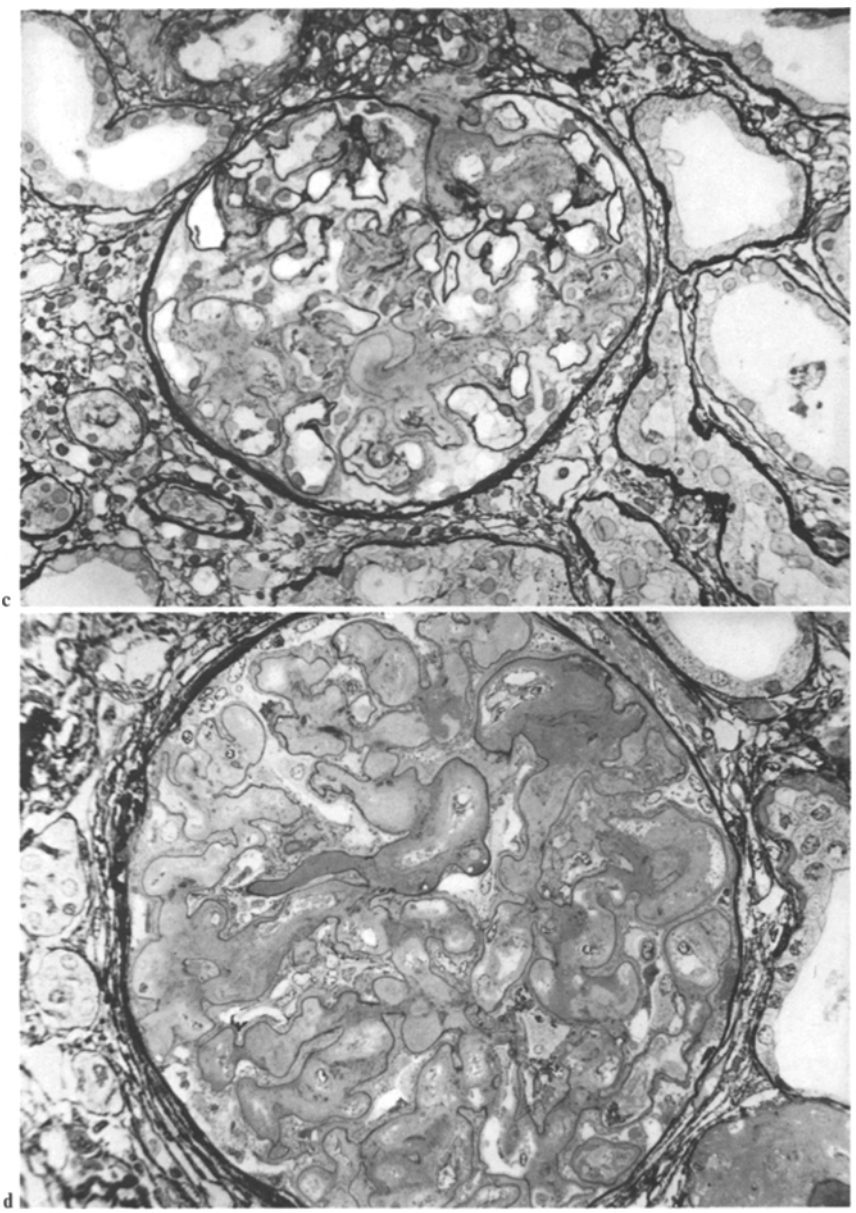


Fig. 1a–d. Glomerular amyloidosis. **a** Grade I (7051186): Focal amyloid deposits in the glomerular sinus and the mesangium of a peripheral capillary. **b** Grade II (703567): Focal amyloid deposits at both sides of the basement membrane and in the wall of the afferent arteriole.
c Grade III (K 5038/70): Very widespread amyloid deposits at both sides of the basement membrane and in the mesangium of nearly all glomerular capillaries.
d Grade IV (7311875): Amyloid masses in the whole glomerular tuft

There is a visible decrease in silver impregnation of the basement membrane – when surrounded by amyloid masses – from grade I to grade IV. All glomeruli silver impregnation after MOVAT, 360:1.

In this paper only grades I–IV were investigated (Fig. 1 a–d). In all these grades amyloid masses were found on both sides of the glomerular basement membrane. In grades III and IV the numbers of mesangial and endothelial cells are diminished.

Biopsy specimens were fixed in formalin (4%, pH 7,4) and embedded in paraffin. Sections, 5 to 10 µm thick, were stained with PAS, Goldner-Trichrome and Kongo-red and were examined by light-microscope. Additional methacrylate-fixed semithin sections (silver impregnation after Movat) of all biopsies were available.

In all sections point-counting to discriminate between interstitium, epithelium and lumina was carried out with a Reichert-Visopan (objective 10/0,2, magnification 125:1). Under a lattice of 1 cm five projection fields of the renal cortex were examined per kidney, neglecting large vessels and glomeruli. The results of point-counting – relative interstitial volumes – were correlated with the values of the serum creatinine concentration at the time of biopsy. We determined the correlation-coefficient r, the error probability α (from the t-test) and the equations of regression for linear, parabolic and exponential functions. 20 normal kidneys served as controls. In all cases we tried to determine – using Spearman's rank correlation (r_s) –, whether there were correlations between the severity of deposits of amyloid masses in the glomeruli and the relative interstitial volume or the serum creatinine concentration at the time of biopsy respective.

Results

In glomerular amyloidosis there is a significant positive correlation between the increase of the serum creatinine concentration and the relative interstitial volume (Fig. 2, Table 1).

In grade I of glomerular amyloidosis the relative interstitial volume never exceeds normal values (Fig. 3). In grade II and III the relative volume of the

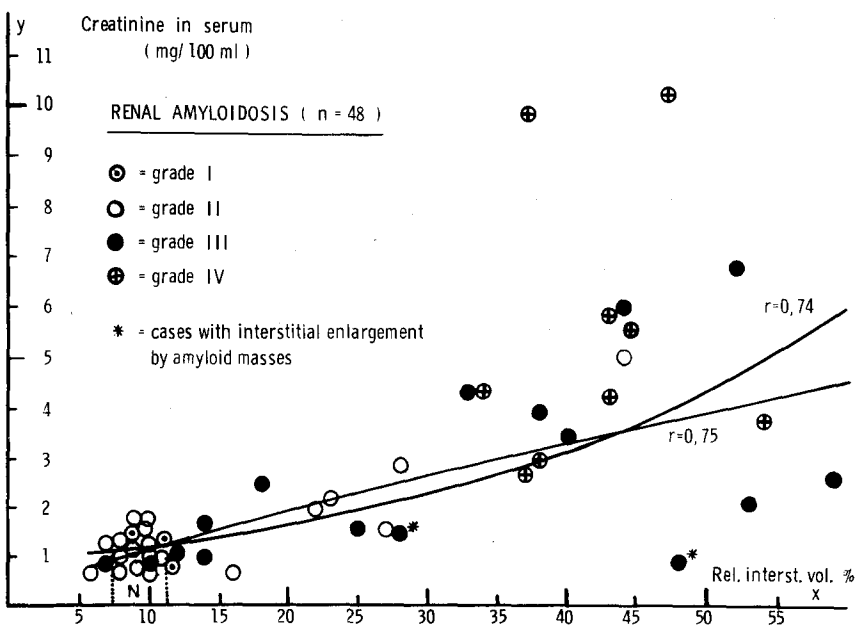


Fig. 2. Correlations between relative interstitial volume (x-axis) and serum creatinine concentration (y-axis) at the time of biopsy. *N* Range of normal kidneys

Table 1. Analysis of regressions and correlations between relative interstitial volume and serum creatinine concentration at the time of biopsy in renal amyloidosis

x/y	Function	Correl. coeff. r	Error prob. α
Linear (lin/lin)	$y=0.42+0.088x$	0.65	
Parabolic I	$y=0.53+0.19x-0{,}0018x^2$	0.67	all
Exponential (lin/log)	$y=0.89\cdot e^{0.031x}$	0.74	$\leqq 0.0001$
Parabolic II (power log/log)	$y=0.23\cdot x^{0.728}$	0.75	

Means: Interstitium (x) $23{,}8 \pm 16.4$ Vol%. Creatinine (y) $2{,}5 \pm 2.2$ mg/100 ml

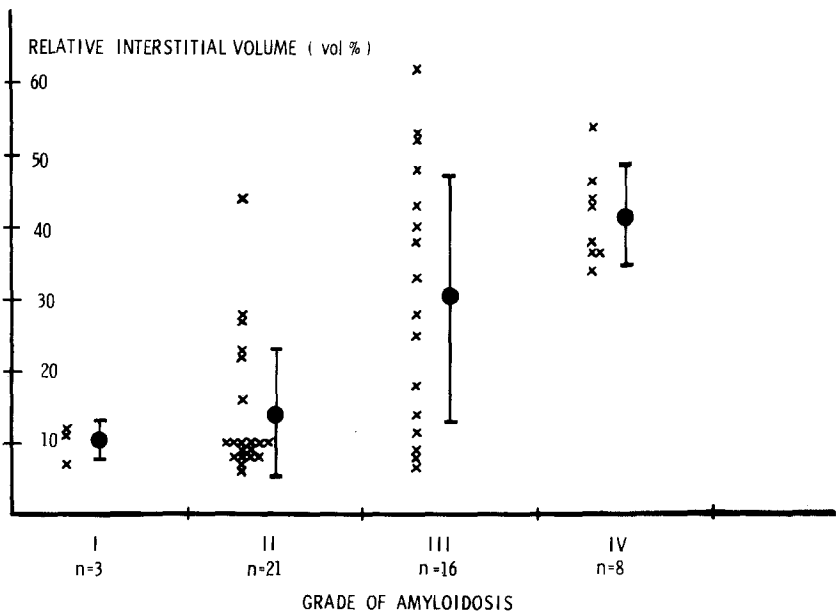


Fig. 3. Statistical analysis: Grade of glomerular amyloidosis and relative interstitial volume. Significance (t-Test, $P \leqq 0.05$) between all groups, except I to II I to III and III to IV

interstitium varies enormously. There is a significant enlargement in grade III and IV compared with grade II, but no significant difference between grade III and IV.

In those cases in grade III, where the interstitium is not enlarged (Fig. 2, Fig. 3), the serum creatinine concentration is normal (Fig. 6a). A broadening of the interstitium by amyloid deposits alone – even if considerable – seems not to lead to renal insufficiency in amyloidosis grade II and III (s. Figs. 2 and 5).

Spearman's rank correlations between grade of amyloidosis and relative interstitial volume are significantly positive ($r_s=0{,}62$; $t=5{,}22$; $P<0.0001$).

The enlargement of the relative interstitial volume in all cases of amyloidosis grade IV is considerable, even when there are only small masses of amyloid.

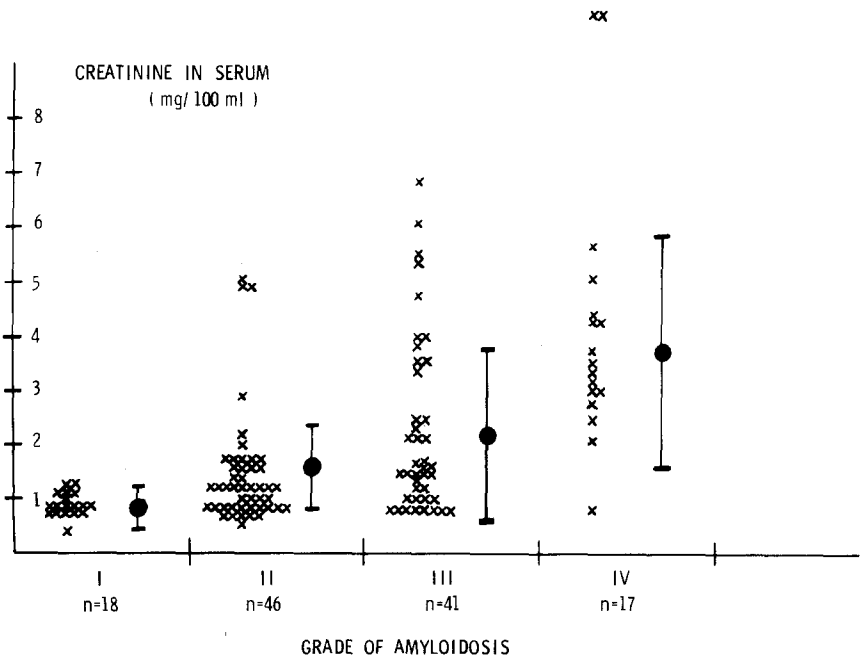


Fig. 4. Statistical analysis. Grade of glomerular amyloidosis and serum creatinine concentration at the time of biopsy. No significance between group I and II, all other couples show significant differences. For this figure all cases of glom. amyloidosis were plotted if clinical data could be obtained neglecting the quality of the sections

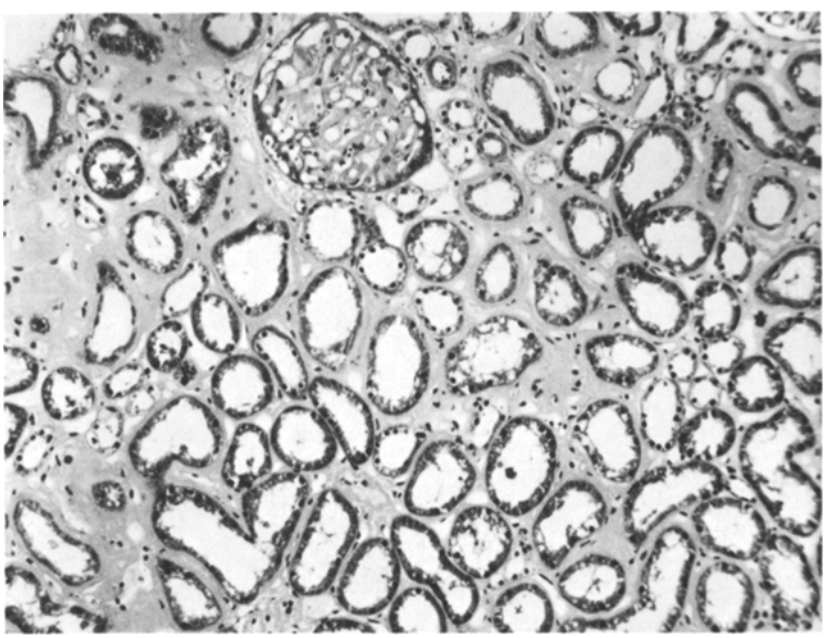


Fig. 5. (696185): Glomerular amyloidosis grade III (see Fig. 2) combined with an enlargement of the interstitium by almost diffuse amyloid deposits; ser. crea 0.9 mg/100 ml ser. crea. clearance 121 ml/min; rel. interstitial volume 48%. Goldner-trichrome, 144:1

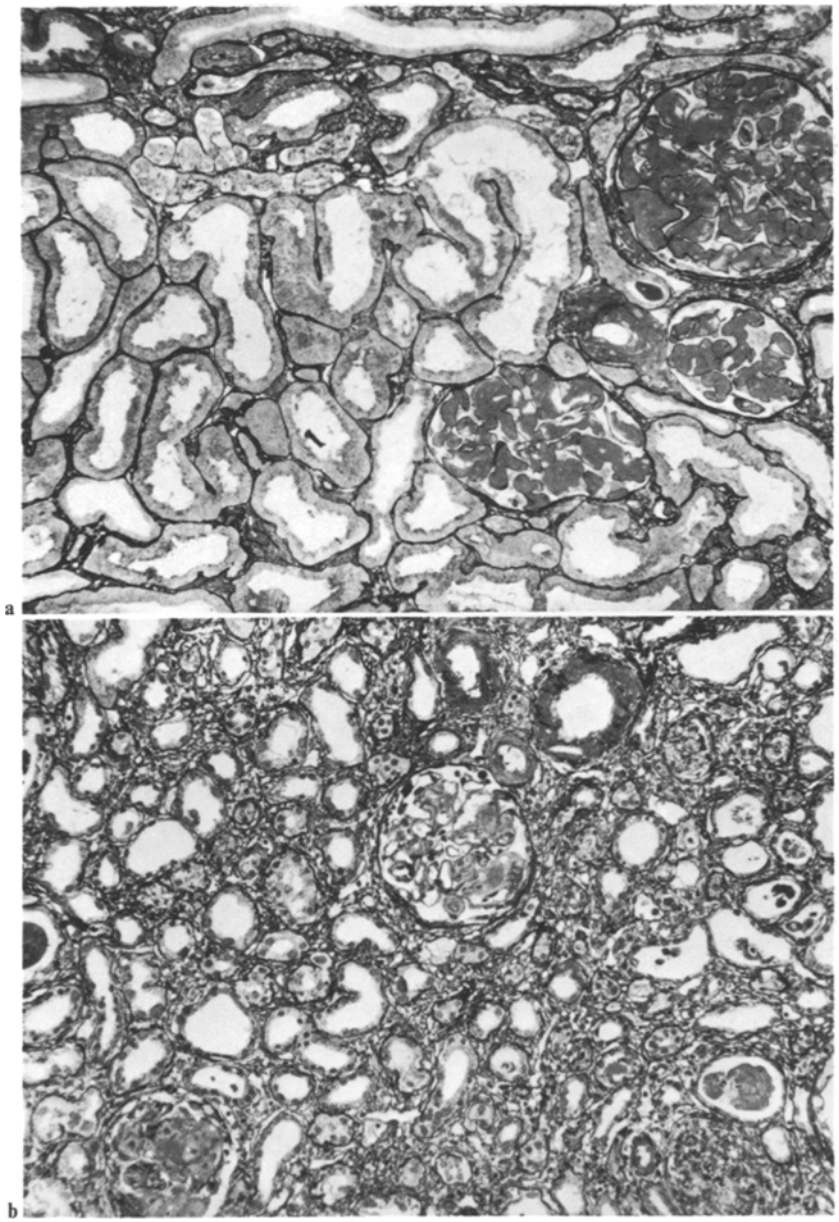


Fig. 6. a (748114) Amyloidosis grade III. Interstitium not essentially enlarged (<15 Vol.%). Ser. crea. 0.8 mg/100 ml. Silver inpregnation after MOVAT, 144:1. **b** K 5038/70 Amyloidosis grade III. Almost diff. enlargement of the interstitium by fibrosis and lymphocytic infiltration. Rel. interst. vol. 52%; ser. crea. 6.9 mg/100 ml; Silver impregnation after MOVAT, 144:1

The mean serum creatinine concentration increases with the severity of glomerular lesions, the difference between grade III and IV being significant (Fig. 4). Similar results were obtained computing the rank-correlations ($r_s = 0.46$; $t = 3.45$; $P < 0.002$).

Discussion

As in glomerulonephritides (perimembranous, membranoproliferative, mesangioproliferative and endocapillary (acute) GN) so in glomerular amyloidosis, significant correlations between the serum creatinine concentration at the time of biopsy and the relative interstitial volume have been established.

In contrast to the GN's, however, additionally significant correlations exist between the severity of glomerular amyloidosis and the relative interstitial volume with regard to the creatinine concentration. Nevertheless these rank correlations are of less relevance than the regression equations for the relation between serum creatinine and interstitial volume. Analysis of the single biopsies shows that enlargement of the interstitium by deposits of amyloid does not influence the serum creatinine concentration in the same way as fibrosis (Fig. 5). Thus the regression curve shows a less pronounced rise above 25% relative interstitial volume than the curves for perimembranous and membranoproliferative GN (Bohle et al., 1977; Fischbach et al., 1977). In grade II to IV, in which a great part of the glomerular tuft is occupied by amyloid fibrils, glomerular involvement seems to be an additional factor in the increase in serum creatinine. In amyloidosis grade II and III, however, the serum creatinine concentration is found to be normal, provided that the interstitium is not enlarged by fibrosis (Fig. 6).

In all, the shape of the curve seems to be influenced more by the interstitial alterations – deposits of amyloid lead to a flattening, fibrosis to a moderate rise – than by glomerular amyloidosis.

The discrepancy between structure and function in severe glomerular amyloidosis was recognized by Theodor Fahr (1925). Further authors maintained that even in severe glomerular amyloidosis renal function – measured by creatinine clearance and max. urinary concentration – is not disturbed (Muerhcke et al., 1955; Heptinstall and Joekes, 1966; Watanabe and Saniter, 1975).

The loss of protein was, however, observed to increase with enlarging amyloid masses in the glomerulus (Muerhcke et al., 1955). Nephrotic syndrome was found in 93% of patients with amyloidosis grade III and in 68% of those with grade IV, even if the serum creatinine concentration was elevated (Watanabe and Saniter, 1975). This phenomenon could be explained by the enormous reserve capacity of the glomerulus, which, as shown by the experiments of Brenner et al. (1976), under normal conditions uses only a part of the capillary surface for filtration. This surface is said to be augmented enormously compared to the corresponding sphere (Plate, 1976).

If the interstitium is not broadened by fibrosis, this reserve capacity is sufficient to maintain renal function untill grade III. In contrast, additional interstitial fibrosis leads to renal decompensation.

These findings might be explained by a reduction of the cross-sectional area of the postglomerular vessels by interstitial fibrosis. In consequence, the slowing of renal blood flow may lead to a diminished clearance of creatinine by predamaged glomeruli. Renal insufficiency is therefore more often observed in amyloidosis grade IV, where both glomerular and interstitial involvement play a role.

Diffuse interstitial fibrosis combined with tubular atrophy was observed in most of our cases. Focal interstitial alterations were found predominately in grade IV, however, where some glomeruli were completely hyalinized. Less involved nephrons showed compensatory hypertrophy.

Enlargement of interstitial tissue in renal amyloidosis is an old observation (Fahr, 1925; Muerhcke et al., 1955; Zollinger, 1966; Sohar et al., 1967; Brandt et al., 1968; Walker et al., 1971; Brod, 1973). Serial biopsies (Muerhcke et al., 1955) in one patient showed no connection between diminution of renal function and progression of glomerular amyloidosis, but interstitial fibrosis was reported to have increased. Possible correlations between increasing interstitial fibrosis and rising serum creatinine concentration are not mentioned by these authors; they attribute the renal insufficiency primarily to amyloid deposits in the glomeruli Zollinger, 1966; Heptinstall, 1966; Reubi, 1970. Zollinger discusses "glomerular occlusions", Reubi a "diminishing of renal blood flow by glomerular amyloid masses". This may be relevant for grade V, or perhaps IV. In most cases of amyloidosis grade II–IV, however, renal insufficiency seems to be caused by both glomerular and interstitial factors. Interstitial fibrosis is thought to be more important.

References

Bohle, A., Grund, K.E., Mackensen, S., Tolon, M.: Correlations between renal interstitium and level of serum creatinine. Morphometric investigations of biopsies in perimembranous glomerulonephritis. Virchows Arch. A Path. Anat. and Histol. **373**, 15–22 (1977)

Bohle, A., Bader, R., Grund, K.E., Mackensen, S., Neunhoeffer, .: Serum Creatinine Level and Renal Interstitial Volume. Analysis of Correlations in Acute Endocapillary Glomerulonephritis and in Moderately Severe Mesangioproliferative Glomerulonephritis. 1977 Virchows Arch. A Path. Anat. and Histol. (in press)

Brandt, K., Cathcart, E.S., Cohen, A.S.: A Clinical Analysis of the Course and Prognosis of Fourty-Two Patients with Amyloidosis. Am. J. Med. **44**, 955–969 (1968)

Brenner, B.M., Baylis, Ch., Deen, W.M.: Transport of Molecules across Renal Glomerular Capillaries. Physiological Rev. **56**, 502–533 (1976)

Brod, J.: The Kidney., pp. 387. London: Butterworths & Co. 1973

Fahr, Th.: Handbuch der speziellen pathologischen Anatomie und Histologie, Bd. VI, Teil I, S. 231 u. 234. Berlin: Springer 1925

Fischbach, H., Mackensen, S., Grund, K.E., Kellner, A., Bohle, A.: Relationship between glomerular lesions, serum creatinine and interstitial volume in membranoproliferative glomerulonephritis. Klin. Wschr. **55**, 603–608 (1977)

Heptinstall, R.H.: Pathology of the Kidney, pp. 571–589. Boston: Little Brown and Co. 1966

Heptinstall, R.H., Joekes, A.M.: Renal amyloid. A report on 11 cases proven by renal biopsy. Ann. Rheum. Dis. **19**, 126 (1960)

Muehrcke, R.C., Pirani, C.L., Pollack, U.E., Kark, R.M.: Primary renal amyloidosis with the nephrotic syndrome studied by serial biopsies of the kidney. Guy's hosp. rep. **104**, 295–310 (1955)

Plate, W.R.: Untersuchungen zur Struktur des Glomerulum. Rekonstruktion eines menschlichen Glomerulum an 0.5 µ dicken Serienschnitten. Inaugural-Dissertation Tübingen 1976
Reubi, F.: Nierenkrankheiten, pp. 309–315. Berlin-Stuttgart-Wien: Huber 1970
Sohar, E., Gafni, J., Mordehai, P., Heller, H.: Familial mediterranean fever. A survey of 470 cases and review of the literature. Am. J. Med. **43**, 227–253 (1967)
Watanabe, T., Saniter, T.: Morphological and Clinical features of Renal Amyloidosis. Virchows Arch. A Path. Anat. and Histol. **366**, 125–135 (1975)
Zollinger, H.: Niere und ableitende Harnwege. In: Doerr, Uehlinger, Spezielle pathologische Anatomie, pp. 207–216. Berlin-Heidelberg-New York: Springer 1966

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