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Morphological and Clinical Features of Renal Amyloidosis

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Summary. The morphological and clinical findings in 122 patients with renal amyloidosis proved by renal biopsy were studied. A higher incidence of amyloidosis occurred in males than females. Coexisting disorders in addition to amyloidosis were present in 80% of the patients. The nephrotic syndrome was a main clinical feature. No significant differences were observed in the manifestations of amyloid renal disease among patients with primary and secondary amyloidosis. Amyloid deposition was present in the mesangium and along the basement membrane. The glomerular basement membrane was damaged by the accumulation of amyloid. The amounts of amyloid in the glomeruli correlated roughly with the clinical manifestations of the disease. The formation of new basement membrane-like substance and the occurrence of granulomatous reaction against amyloid deposits were discussed in relation to the course of the disease.

Key words: Renal Amyloidosis — Nephrotic Syndrome — Morphology.

Renal involvement in systemic amyloidosis occupies a position of prime importance in the development of the disease. Renal insufficiency is a major cause of death in systemic amyloidosis (Teilum and Lindahl, 1954; Briggs, 1961; Sohar *et al.*, 1967; Brandt *et al.*, 1968). The information presently available on the relation between the structural and functional disturbances of the kidneys in systemic amyloidosis is conflicting in certain respects, mainly due to the fact that most reports have been based on the analysis of autopsy cases (Auerbach and Stemmerman, 1944; Mathews, 1954; Zuckerbrod *et al.*, 1956; Bero, 1957; Zollinger, 1966; Beneke, 1971). The development of the biopsy technique has made possible the study of *early stages of amyloidosis* so that the clinical and morphological features of this disease can be investigated during life. The availability of a large sample of patients with biopsy-proved renal amyloidosis provided this opportunity to examine the relationship between the structural alterations of the kidneys and the clinical manifestations of renal amyloidosis.

Materials and Methods

The records of 122 cases with biopsy-proved renal amyloidosis gathered between January, 1968 and February, 1974 were used.

The renal biopsy was performed with hematoxylin and eosin, periodic acid-Schiff (PAS), Goldner trichrome and van Gieson stains. The presence of amyloid was established by the positive green birefrigence under the polarized light in sections stained with Congo red. In addition, 0.5 μ thick metachrylate embedded semi-ultrathin sections stained by the Movat's silver method were used to evaluate the extent and location of amyloid deposits in the tissue.

The *extent of amyloid* deposits was *graded* from 0 to 4 in a random sample of at least 6 glomeruli in each patient: grade 0 = no obvious amyloid; grade 1 = amyloid deposits

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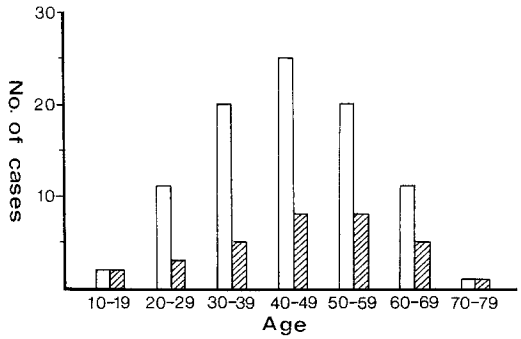

Fig. 1. Age distribution of the cases with renal amyloidosis. ▭ Male; ▨ female

affecting only part (less than one third) of the glomerulus; grade 2 = moderate amyloid deposits between grades 1 and 3; grade 3 = amyloid deposits affecting almost entire part (more than two thirds) of the glomerulus but leaving several patent capillaries; grade 4 = amyloid deposits completely affecting the glomerulus with or without increased amounts of fibrous elements. The final grade assigned to a patient's biopsy was the average of all glomeruli studied. The extent of amyloid deposits in the small arteries and arterioles was simultaneously graded from 0 to 3, namely, absent, slight, moderate or severe amyloid deposition, based on the amount present and the number of vessels affected.

Results

Fig. 1 shows the distribution of sex and ages of the patients. There were 90 males and 32 females ranging in age from 14 to 74 years. About 70% of the cases were between the ages of 30 and 59. Table 1 presents the frequency of disorders other than clinical features characteristics of amyloidosis observed in the patients. The majority of coexisting disorders were chronic suppurative conditions of the bone and the respiratory tract, rheumatoid arthritis and tuberculosis. Mediterranean fever was present in 5 cases under the age of 30. The conventional classification of the patients into primary and secondary amyloidosis on the basis of the absence or presence of coexisting disorders failed to disclose any significant differences in the morphology of the kidneys and clinical manifestations of the disease.

Histological Findings. Amyloid deposits in the glomeruli were noted in practically all cases. Deposition of amyloid usually occurred in many parts of the tufts and predominantly affected the mesangial region (Figs. 2, 3, 4). Occasionally, deposition was conspicuous near the vascular pole affecting the space under "Capillargrundhäutchen" (Bohle, 1955). Amyloid deposits on the inner surface of the capillary tufts were apparent in almost all cases either adjacent to the mesangium or more peripherally (Figs. 3, 4). Deposition on the epithelial side of the basement membrane was distinct but less conspicuous (Figs. 3, 4). Crescent- or comb-shaped subepithelial deposits were found in association with either mesangial or subendothelial deposits (Fig. 3).

Table 1. The frequency of coexisting disorders in patients with renal amyloidosis

	Male (83)	Female (32)
Cases with coexisting disorders	67	23
Type of disorder		
Chronic suppurative disorders		
Bone	18	2
Upper respiratory tract	7	3
Bronchus	5	1
Lung and pleura	5	1
Urinary tract	2	1
Small intestine (Crohn's disease)	1	1
Large intestine (Ulcerative colitis)	2	1
Miscellaneous	8	1
Rheumatoid arthritis	9	12
Tuberculosis	11	4
Mediterranean fever	4	1
Hodgkin's disease	2	0
Agammaglobulinemia	0	1

Total Number of cases appears in parenthesis.

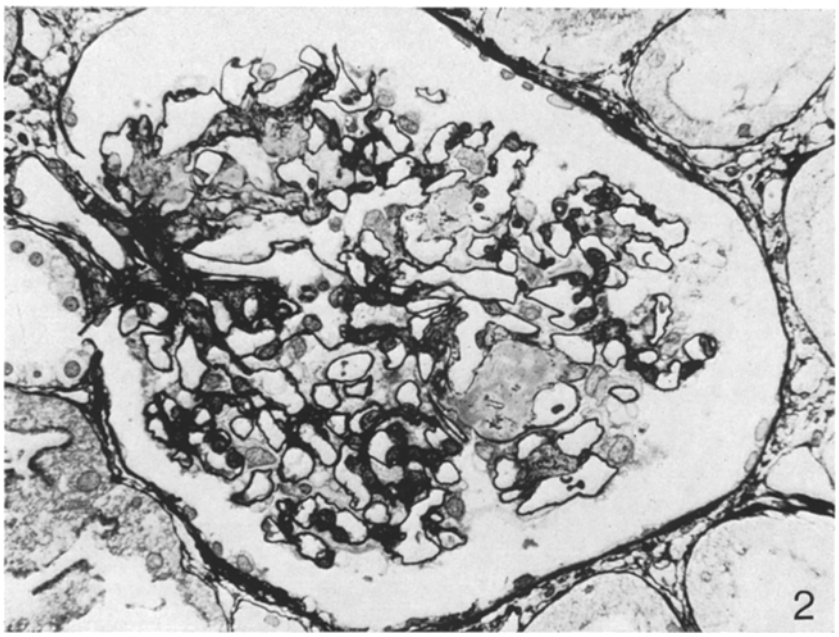

Fig. 2. Glomerulus from a 48 year old man with amyloidosis and osteomyelitis showing mild deposition of amyloid in the mesangial region. Movat's silver staining. Magnification ×400

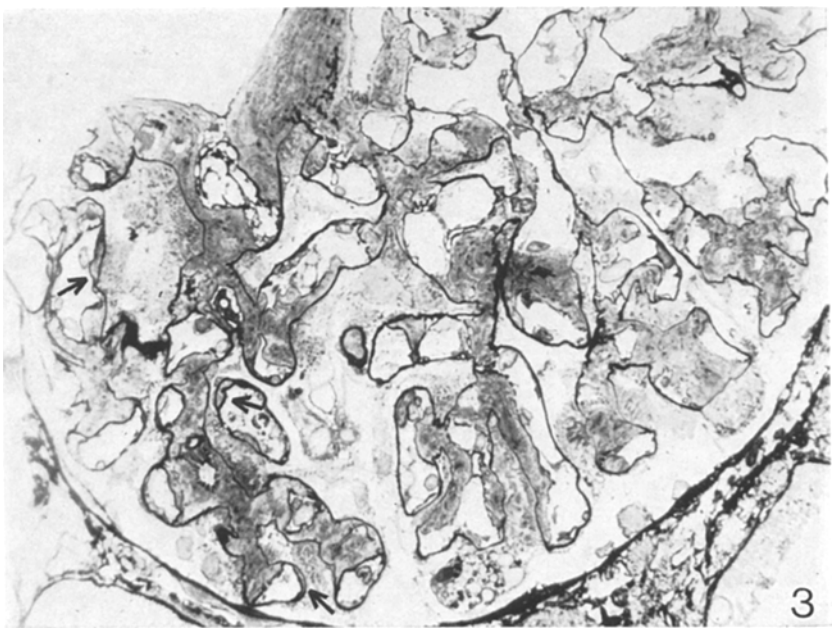

Fig. 3. Glomerulus from a 43 year old woman with amyloidosis and no coexisting disorders showing amyloid extending along the basement membrane. New formation of argyrophilic membrane is noted on the endothelial surface of the amyloid (arrows). Part of the basement membrane (lower right) shows focal decrease of argyrophilia in relation to deposition of amyloid on both sides of it. Movat's silver staining. Magnification ×520

The extension of the glomerular lesions appeared to occur in two ways. First, the deposits became larger and nodular accumulation of amyloid developed in the mesangium compressing the peripheral part of the tuft (Fig. 4). In some cases, argyrophilic fibers or chains of grains were fairly abundant in these amyloid nodules (Fig. 5). Second, amyloid appeared early in the subendothelial space, most probably in a form of "lateral progression" from the mesangium, and finally encircled and obliterated the capillary lumen (Fig. 3). In advanced cases, the glomeruli showed diffusely ramifying thickening of the mesangial region and capillary walls. Most cases showed both types of extension of the glomerular lesions. Subepithelial deposits occurred in both types of glomerular changes, but were more conspicuous in the second type described.

Thickening or abrupt breakdown of the basement membrane was not observed. The basement membrane could be outlined even in the places where it was embedded in a large mass of amyloid. Occasionally, it appeared to be frayed by the accumulation of amyloid and to have lost its affinity for silver compounds (Fig. 3, 4). Discrete foci of decreased argyrophilia commonly could be demonstrated in some portion of the basement membrane even in cases with mild glomerular changes. Formation of argyrophilic membrane was sometimes noted on the endothelial or epithelial surface of the amyloid (Fig. 3, 4).

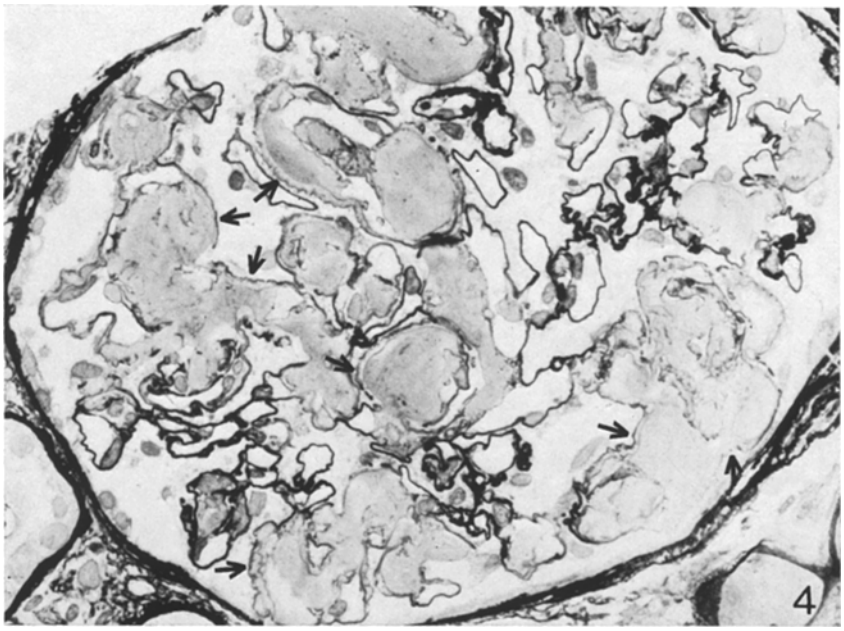

Fig. 4. Glomerulus from a 35 year old man with amyloidosis and tuberculosis showing nodular accumulation of amyloid in the mesangial region with extension along the basement membrane. The basement membrane embedded in the amyloid shows widespread loss of argyrophilia. New formation of argyrophilic membrane is distinct on the epithelial surface of the amyloid (arrows). Movat's silver staining. Magnification ×520

Cellular reaction against amyloid deposits was uncommon. In one patient, however, accumulation of cells with large irregularly indented vesicular nuclei was conspicuous within amyloid deposits of the glomeruli (Fig. 6). Fragmentation of amyloid mass was seen surrounded by the cytoplasmic projections of the cells. Furthermore, some giant cells containing PAS positive substance were observed (Fig. 6b, 6c). Simultaneous proliferation of capsular epithelial cells, with occasional formation of epithelial crescents, was also prominent in these glomeruli.

The relationship between amyloid in the glomeruli and renal arteries in the patients is shown in Table 2. Involvement of the arterial system (interlobular arteries and arterioles) was observed in 97 cases. All except two cases belonged to the perireticulin type amyloidosis (Missmahl, 1959; 1964). Glomerular involvement was invariably observed in all cases while the degree of amyloid accumulation in the arterial system typically was more modest than in the glomeruli. In addition, amyloid accumulated mainly in the media between the individual muscle cells or in the intima, while the adventitia appeared to be almost spared.

Amyloid deposition around the tubules and in the vasa recta was apparent in 57 cases. Massive deposits of amyloid were present in the interstitium in 12 of these cases. Amyloid also accumulated in the walls of the vasa recta and inside the tubular basement membrane. Occasionally this caused focal disappearance of the basement membrane. Amyloid deposition also occurred much more fre-

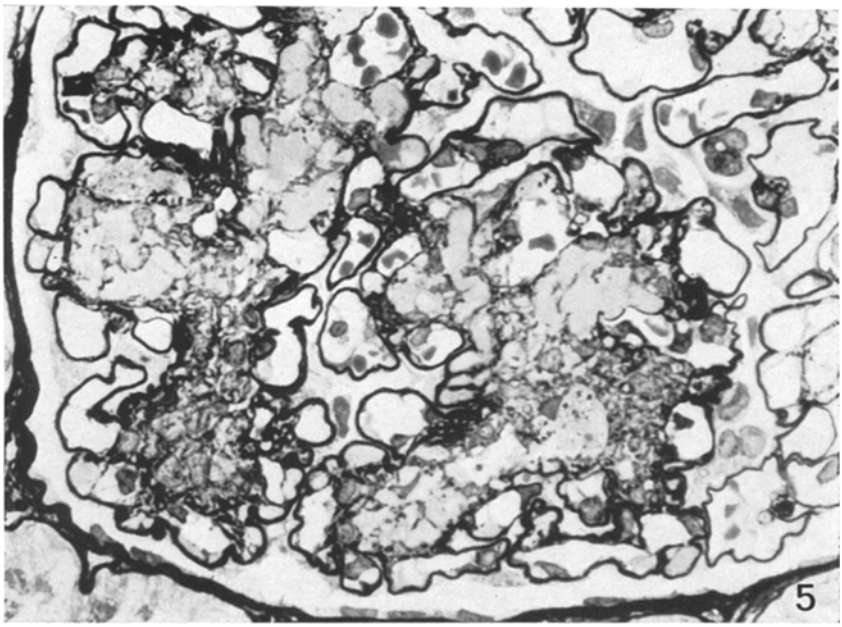

Fig. 5. Amyloid nodules from a 44 year old man with amyloidosis and osteomyelitis. The argyrophilic fibers or grains are linked together. Movat's silver staining. Magnification ×720

quently around the collecting tubules and Henle's loop in the renal medulla than in the proximal tubules.

Hyaline-droplet formation in the tubular epithelium was seen in approximately 45% of the cases. The change was frequently associated with fatty or vacuolar changes of the tubular epithelium. Foam cells were noted in 23 cases. They were present in the interstitium usually in groups or in clusters. Atrophy or loss of tubules followed by proliferation of connective tissue usually occurred in the cases with advanced amyloid deposition.

Relationship between Histology and Clinical Features. The most common clinical feature was proteinuria, which was frequently the reason for performing the biopsy. The quantitative value of proteinuria in 103 cases ranged from 0.5 to $45^0/_{00}$. Daily fluctuations in protein excretion in the patients were considerable. Consequently, a detailed assessment of the correlation between the morphologic severity of amyloidosis revealed by the biopsy and the level of proteinuria was not carried out. Suffice it to say, however, that massive proteinuria was observed in cases with mild as well as cases with severe glomerular changes.

The nephrotic syndrome was shown by three-fourths of the patients (82 out of 108) at the time of biopsy or shortly before it. As shown in Table 2, the nephrotic syndrome developed in more than half the cases with mild (grade 1) glomerular changes and 93% of the cases with moderately severe (grade 3) glomerular lesions. A tendency toward regression of the nephrotic syndrome appeared in grade 4 cases.

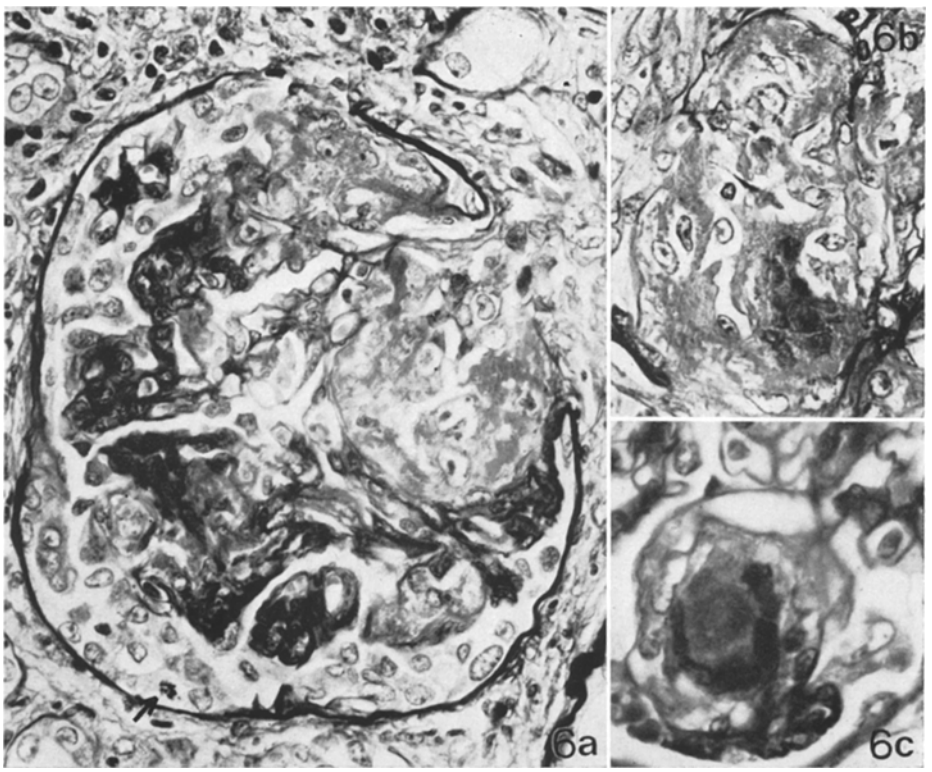

Fig. 6 a—c. Glomeruli from a 33 year old woman with amyloidosis and rheumatoid arthritis showing granulomatous reaction against amyloid. (a) Amyloid nodules infiltrated by large mononuclear cells with vesicular nuclei. Proliferation of capsular epithelial cells is conspicuous. Arrow shows a mitotic figure. PAS staining. Magnification ×440. (b) Proliferation of similar cells with formation of a multinucleate giant cell. Part of an epithelial crescent is seen at upper left. PAS staining. Magnification ×520. (c) A multinucleate giant cell containing PAS positive substance in the cytoplasm. PAS staining. Magnification ×720

Microscopic hematuria was present at biopsy in 53 out of 88 cases. It was found in all but one of 12 patients with severe grade 4 glomerular lesions while no major differences were noted in the frequency of hematuria among patients with grade 1, 2, or 3 glomerular lesions.

The serum creatinine level was determined in 99 cases. The majority of glomerular lesions was related to the level of serum creatinine; high creatinine levels were more frequent in cases with grade 3 and 4 than in grade 1 and 2 glomerular changes (Table 2). Moderate or advanced azotemia (serum creatinine more than 3.0 mg/dl) occurred in 4.5, 5.7, 33.3 and 66.7% of the cases with grade 1, 2, 3 and 4 glomerular lesions, respectively.

Hypertension, defined as a blood pressure over 150 mmHg systolic or 90 mmHg diastolic, was present in 21 of 102 cases in which blood pressure was measured at the time of the renal biopsy. Four of the hypertensive cases were less than 40 years old, 7 were between 40 and 50 years of age and 10 were more

Table 2. The relationship between the grade of glomerular involvement by amyloid and the arterial changes, nephrotic syndrome, serum creatinine level, and incidence of hypertension in patients with renal amyloidosis

		Glomerular involvement by amyloid (Grade)			
		1	2	3	4
Arterial involvement by amyloid (Grade)	0	10	7	4	0
	1	19	24	15	4
	2	2	7	8	5
	3	1	2	3	7
Incidence of nephrotic syndrome		16 ($n=28$)	30 ($n=37$)	25 ($n=27$)	11 ($n=16$)
Serum creatinine (mg/dl)	0.0–1.6	18	28	9	1
	1.7–3.0	3	5	9	4
	3.1–6.0	1	1	5	5
	over 6.0	0	1	4	5
Incidence of hypertension		3 ($n=26$)	6 ($n=35$)	3 ($n=26$)	9 ($n=15$)

Number of cases shown in table.

than 50 years old. The occurrence of hypertension was consistently associated with grade 4 glomerular lesions (Table 2). Nine of the hypertensive cases showed moderate or severe arterial changes.

Polyuria exceeding 2000 ml/day was described in 5 cases, all of which showed grade 3 or 4 glomerular involvement by amyloid accompanied by retention of creatinine in the blood. Amyloid deposition around the wall of vasa recta and tubules was absent or slight in these 5 patients.

Erythrocyte sedimentation rate was recorded in 84 patients. It was more than 100 mm/hour in 34 patients, over 50 mm/hour in 28 cases, over 20 mm/hour in 14 cases and less than 20 mm/hour in 8. There was no reliable correlation between the severity of the glomerular lesion and the erythrocyte sedimentation rate.

Discussion

The present investigation has revealed a number of features that appear to be characteristic of the perireticulin type amyloidosis (Missmahl, 1959; 1964). As pointed out by Letterer (1949), males are affected three times as frequent as females, but this sex difference varies according to the coexisting disorders. Proteinuria is the most constant abnormality and is frequently associated with the nephrotic syndrome which was observed in 76% of the present series. The occurrence of the nephrotic syndrome correlates roughly with the amount of amyloid in the glomeruli. The syndrome was most frequently observed in the cases with fully developed glomerular lesions. Our observations are similar to those of Auerbach and Stemmerman (1944), Allen (1962), and Missmahl (1971) but at variance with those of Bell (1933), Heptinstall and Joekes (1960) and

Martin *et al.* (1966) who found no close relationship between the degree of proteinuria or the nephrotic syndrome and the extent of amyloid deposition in the kidneys.

We found that the mesangium is most likely the earliest and primary site of amyloid deposition. The present observations are generally in agreement with recent studies on glomerular amyloidosis that also have revealed amyloid accumulation primarily in the mesangium and along the basement membrane (Suzuki *et al.*, 1963; Shimamura and Sorenson, 1965; Shirahama and Cohen, 1967; Jao and Pirani, 1972).

The presence of amyloid extending along the basement membrane was invariably observed in all of the present cases. Such deposition of amyloid may cause severe metabolic and structural damage to the basement membrane, as represented by decreased argyrophilia or the findings by electron microscope that the amyloid fibrils are traversing across the lamina densa of this structure (Movat, 1960; Bergstrand and Bucht, 1961; Shirahama and Cohen, 1967; Jao and Pirani, 1972), and may contribute to the massive escape of protein into the urine.

Hypertension was present in 21 out of 105 cases (20%) in the present series. The condition was most consistently associated with advanced glomerular and arterial lesions, substantiating the findings of Bell (1933). It seems also worth mentioning that in most of the cases in which hypertension was present, contracted kidneys were found at autopsy, whereas in those cases that died of uremia associated with a normal blood pressure, the common autopsy finding was the large, pale kidney (Mark and Mosenthal, 1938; Allen, 1962; Zollinger, 1966).

It has been generally stated that hypertension is an unusual manifestation of amyloid disease of the kidneys (Rosenblatt, 1933; Dixon, 1934; Mark and Mosenthal, 1938; Altnow *et al.*, 1939; Auerbach and Stemmerman, 1944). Recent studies, however, suggest that the association of hypertension is more common than formerly appreciated. For example, Mathews (1954) found hypertension in 20% of cases with primary amyloidosis, Zuckerbrod *et al.* (1956) observed it in 21% of their cases with secondary amyloidosis, and Heptinstall and Joekes (1960) found hypertension in 54% of cases diagnosed by renal biopsy. The greater incidence of hypertension in recent investigations of amyloidosis might be related to the relatively good general condition of the patients compared with patients in the past, before the use of specific therapy for infection such as tuberculosis (Heptinstall and Joekes, 1960).

The prognosis of amyloidosis is poor. It is to be noted, however, that the rate of amyloid deposition can vary and a number of five-year survivals already have been recorded (Fraser and Kaye, 1961; Brandt *et al.*, 1968). The appearance of increased amounts of argyrophilic substance within the amyloid mass of the mesangium or formation of argyrophilic membrane on the surface of more peripherally located amyloid deposits might be a sign of slow deposition of amyloid, as in some of the present cases (Fig. 3, 4, 5). Moreover, clinical remission of hepatic (Waldenström, 1928; Parkins and Bywaters, 1959) and renal amyloidosis (Lindeman *et al.*, 1961; Kuhlbäck and Wegelius, 1966; Lowenstein and Gallo, 1970) following eradication of underlying inflammatory process has been described. In fact, the series of Waldenström included 3 cases in which the amyloid disappeared completely in serial liver biopsy.

Little is known, however, about the mode of regression in human amyloidosis. Phagocytosis might be an important mechanism responsible for the removal of amyloid under experimental conditions (Kuczynski, 1923; Morgenstern, 1926; Richter, 1954). Outstanding cellular proliferation with evidence of phagocytosis of amyloid was observed in the glomeruli of one patient in the present study (Fig. 6). This observation is basically consistent with the findings of animal experiments that indicate the possibility of resorption of amyloid. The occurrence of such granulomatous lesions is still quite unusual in human amyloidosis, but has been described already in the spleen and liver of a patient under clinical remission of amyloidosis associated with chronic osteomyelitis (Métraux, 1929).

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