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Amyloid Casts Within Renal Tubules: a Singular Finding in Myelomatosis

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Summary. This study was carried out in order to investigate a possible relationship between multiple myeloma and the occurrence of material exhibiting the properties of amyloid within renal tubules.

Two groups of autopsied patients, with myelomatosis and benign monoclonal gammopathy were examined for the presence of amyloid deposits in renal and extra-renal sites. Urines were analysed for the presence and amount of Bence Jones protein and the pattern of the associated proteinuria was characterized.

Renal tubular casts exhibiting the histochemical characteristics of immuno-amyloid were found exclusively in myeloma patients with Bence Jones proteinuria but without the renal lesions classically described as "myeloma kidney".

This finding was independent of the occurrence of immuno-amyloid deposits in other renal and extra-renal sites, suggesting involvement of local factors in the pathogenesis of amyloid formation and deposition within renal tubular lumina.

The results of present study suggest the conclusion that the presence of amyloid intratubular casts is to be regarded as a peculiar finding in myelomatosis.

Key words: Amyloidosis – Monoclonal gammopathy – Multiple myeloma – Bence Jones protein – Kidney tubules.

Introduction

The vascular structure of the kidney is frequently affected by amyloid deposition in patients with cardiovascular amyloidosis of "primary" and "senile" type.

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"Secondary" amyloidosis, associated with a variety of chronic inflammatory, infectious and neoplastic diseases, is also known to involve the kidney as well as other parenchymal organs.

In all these conditions both the renal vascular structures – including glomerular capillaries – and the interstitium can be affected by amyloid deposits exhibiting the histochemical characteristics of "immuno-amyloid" (Pearse et al., 1972).

A further morphological finding, consisting of the presence within renal tubules of material having staining, histochemical and ultrastructural characteristics of amyloid, appears to occur in myeloma patients. Possible inter-relationships between amyloidosis, myelomatosis and tubular "paraproteinaceous" casts (Paraprotein-cylindern) has been suggested by Randerath (1947).

In an attempt to contribute further to the elucidation of the relationship between tubular immuno-amyloid and myelomatosis, a series of patients with plasma cell dyscrasias has been investigated for the presence of amyloid deposits in renal and extra-renal sites.

Material and Methods

The case material consisted of 30 autopsied patients with monoclonal gammopathies. Patient selection was made only on the basis of the availability of complete clinical, laboratory and immunological investigations.

The *first group* (Table 1) consisted of 15 patients ranging in age from 59 to 87 years (mean 72) who were diagnosed as having multiple myeloma according to accepted criteria (Commitee of the Chronic Leukemia – Myeloma Task Force, 1973). Patients were classified according to the clinical staging system described by Durie and Salmon (1975) and subclassified A and B on the absence or presence of impaired renal function.

The *second group* consisted of 15 patients from 55 to 99 years of age (mean 78) who were diagnosed as having benign monoclonal gammopathy. These patients were followed up for at least 3 years before death without evidence of myelomatosis or related B-cell malignancies. This was documented by negative results of repeated bone marrow and skeletal axis X-ray examinations, the relatively low and steady concentration of the serum paraproteins, the absence of Bence Jones proteinuria and of significantly decreased serum levels of immunoglobulins other than the paraproteins throughout the time of observation. The relevant autopsy findings in these cases are listed in Table 2.

All serum and concentrated urine samples were studied by agarose gel electrophoresis as described by Johansson (1972) and by immunoelectrophoresis (Scheidegger, 1955) performed in 1.5% agar gel in the same electrophoresis buffer. Anti-whole human serum and monospecific sera anti-heavy and light chains of immunoglobulins were obtained from commercial sources (Dakopatts, Copenhagen and Behringwerke, Marburg Lahn).

Total protein contents in the urines were estimated by the biuret method (Bell and Baron, 1968) and turbidimetrically with 3% sulphosalicilic acid (Poortmans and van Kerchove, 1963).

The search for Bence Jones proteinuria was performed by electrophoresis, followed by immunoelectrophoresis, of fresh urines after concentration (up to 400 times or more) by dialysis against polyethylene glycol (mean molecular weight 35,000) in 18/32″ Visking tubing. The amount of Bence Jones proteinuria was quantitated on electrophoresis by densitometric estimation of the monoclonal band and then expressed in g/24 h.

The classification of proteinuria as glomerular and tubular and the evaluation of the selectivity pattern of glomerular proteinuria were made by visual comparison of the electrophoretic and immunoelectrophoretic separations of the serum and concentrated urine samples (Manuel and Revillard, 1970).

The histological study was performed on tissue material from kidney, heart, pancreas, lung and liver obtained at autopsy and immediately fixed in 10% buffered formalin. From paraffin

Table 1. Patients with myelomatosis

Case No.	Autopsy No.	Sex (M/F)	Age (yr)	Serum paraprotein		Clinical stage	Autopsy findings
				class	type		
1	543/75	F	59	IgG	k	III A	Bronchopneumonia, generalized arteriosclerosis, myeloid metaplasia of the liver
2	579/75	F	75	IgA	λ	I A	Cerebral infarction
3	1687/75	M	61	–	–[a]	III B	Pulmonary thromboembolism
4	218/77	F	74	IgG	λ	III B	Generalized haemorrhagic syndrome
5	229/77	F	74	IgG	k	II A	Bronchopneumonia, bleeding from multiple acute gastric ulcers
6	1113/77	F	80	IgG	λ	I A	Bronchopneumonia, generalized arteriosclerosis
7	1210/77	F	87	IgA	λ	III B	Cardiomegaly in patient with pace-maker
8	696/78	F	64	IgG	k	III B	Bronchopneumonia, fibrinous pericarditis
9	717/78	F	76	IgG	k	III A	Pulmonary infarction
10	837/78	M	62	IgG	k	III A	Bronchopneumonia, vertebral collapse, spinal cord compression
11	1293/78	F	78	IgA	k	III B	Severe pulmonary emphysema, chronic hepatitis
12	2156/78	F	78	IgG	k	III B	Pulmonary thromboembolism
13	2388/78	M	58	IgA	k	III A	Severe fatty liver
14	2531/78	M	72	IgA	k	II A	Myocardial infarction
15	411/79	F	84	IgA	λ	III A	Hepatocellular carcinoma, adenocarcinoma of the rectum

[a] k light chain disease

embedded specimens, serial sections were cut at 5 μ and processed for light microscopy with routine staining methods (hematoxilin-eosin, Van Gieson and Periodic Acid Schiff methods).

After staining with Congo Red in alkaline-alcoholic solution according to Puchtler et al. (1962), sections were examined under polarized light. All tissue materials exhibiting the typical birefringence and yellow-green dichroism were further studied as follows:

- staining for tyrosine according to Glenner and Lillie (1959);
- staining for tryptophan (DMAB method) according to Pearse (1968);
- search for autofluorescence under U.V. light;
- trypsin digestion methods (according to Romhanyi, 1972) and potassium permanganate method (according to Wright et al., 1977).

Table 2. Patients with benign monoclonal gammopathy

Case No.	Autopsy No.	Sex (M/F)	Age (yr)	Serum paraprotein		Autopsy findings
				class	type	
16	1836/75	F	73	IgG	k	Generalized arteriosclerosis, myocardial infarction
17	1779/76	M	55	IgG	k	Bronchopneumonia, cirrhosis of the liver
18	1844/76	F	72	IgG	k	Bronchopneumonia, cirrhosis of the liver
19	2148/76	M	69	IgG	k	Bronchopneumonia, acute myocardial infarction
20	2197/76	M	74	IgG	λ	Primary liver carcinoma in cirrhosis, neoplastic thrombosis of portal vein and upper cava vein
21	594/77	F	67	IgG	λ	Haemoperitoneum in cirrhosis of the liver
22	978/78	M	86	IgG	λ	Cerebral infarction, bronchopneumonia
23	385/78	F	89	IgG	λ	Bronchopneumonia, generalized arteriosclerosis
24	590/78	M	87	IgG	λ	Bronchopneumonia, generalized arteriosclerosis
25	1183/78	F	72	IgG	k	Cerebral infarction, renal infarction, chronic hepatitis
26	1797/78	F	83	IgA	k	Cerebral infarction, pulmonary thromboembolism
27	2255/78	M	75	IgG	k	Bronchopneumonia, generalized arteriosclerosis
28	2318/78	F	89	IgG	k	Cerebral infarction, bronchopneumonia
29	2395/78	M	84	IgA	k	Cerebral infarction, bronchopneumonia
30	268/79	F	99	IgG	λ	Severe cardiac amyloidosis, cerebral arteriosclerosis

Results

The patterns of amyloid deposition found in the individual cases examined and the data concerning the presence and amount of Bence Jones proteinuria and the pattern of associated proteinuria are presented in Tables 3 and 4.

By accepted histochemical methods, pancreatic insular deposits were recognized as of the Apud type of Pearse et al. (1972). In all other sites the deposits were found to be composed of immuno-amyloid as exhibiting positivity for tryptophan and tyrosine staining and autofluorescence under U.V. light. Both Apud and Immuno-amyloid deposits were trypsin and potassium permanganate resistant.

Amyloid intratubular casts (AIC) were found exclusively in myeloma patients without evidence of any immuno-amyloid deposition in the kidney and the other organs studied (Table 3).

Table 3. Patients with myelomatosis

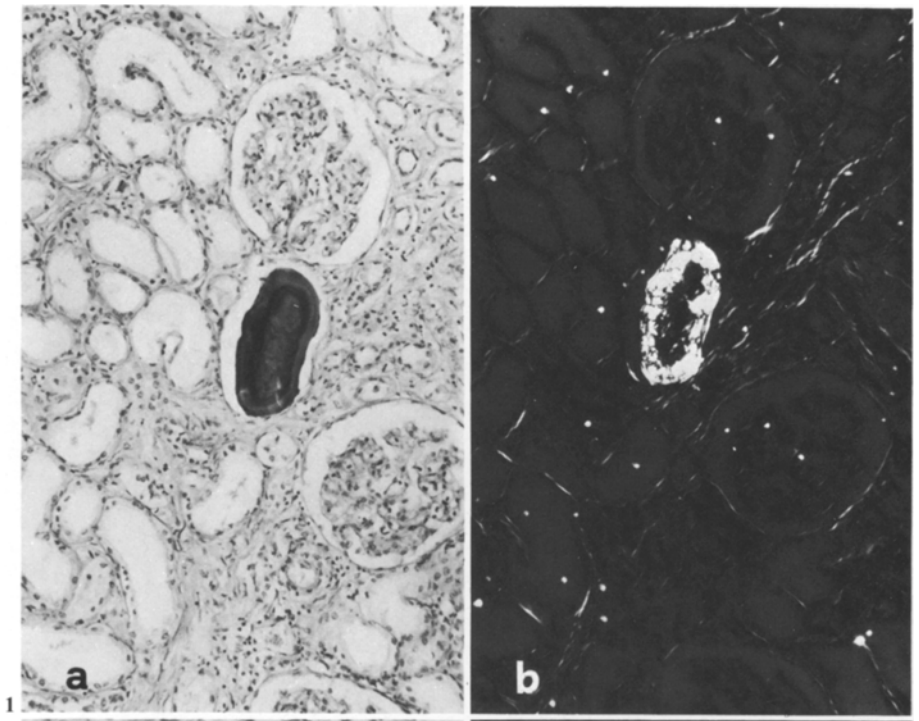
Case No.	Bence Jones proteinuria		Proteinuria			Serum creatinine (mg/dl)	Amyloid Deposits[b]											
	type	g/24 h	Total protein (g/24 h)	Pattern[a]			Heart		Pancreas		Liver		Lung		Kidney			
				Glomerular	Tubular		V	Int	V	Is	V	Int	V	S	V	Glom	Tub	Int
1	k	1.60	2.20	–	+	1.5	–	–	–	+	–	–	–	–	–	–	+	–
2	λ	0.07	1.00	MS	–	0.9	–	–	–	–	–	–	–	–	–	–	–	–
3	k	0.50	2.50	S	+	2.5	+	+	+	–	+	–	+	–	+	+	–	–
4	λ	0.37	2.80	PS	+	2.2	+	+	–	+	+	–	+	–	+	–	–	+
5	k	0.02	0.15	MS	+	1.6	–	–	–	+	–	–	–	–	–	–	–	–
6	λ	0.10	1.60	PS	–	0.7	–	–	–	–	–	–	–	–	–	–	–	–
7	λ	6.60	9.80	–	+	4.9	–	–	–	–	–	–	–	–	–	–	+	–
8	k	3.40	9.80	PS	+	12.4	–	–	–	–	–	–	–	–	–	–	–	–
9	k	0.04	0.80	PS	–	0.8	–	–	–	–	–	–	–	–	–	–	–	–
10	k	0.74	1.50	–	+	1.5	–	–	–	–	–	–	–	–	–	–	+	–
11	k	2.00	3.00	–	+	3.1	–	–	–	–	–	–	–	–	–	–	–	–
12	k	1.10	2.10	S	+	2.4	–	–	–	+	–	–	–	–	–	–	+	–
13	k	0.20	1.60	S	–	1.0	–	–	–	–	–	–	–	–	–	–	–	–
14	k	0.02	0.16	S	–	1.0	–	–	–	–	–	–	–	–	–	–	–	–
15	λ	0.02	0.12	S	+	1.2	–	–	–	–	–	–	+	+	–	–	–	–

[a] S = selective; MS = moderately selective; PS = poorly selective

[b] V = vessels; Int = interstitium; Is = islets of Langerhans; S = septa; Glom = glomeruli; Tub = tubules

Table 4. Patients with benign monoclonal gammopathy.

Case No.	Bence Jones proteinuria		Proteinuria			Serum creatinine (mg/dl)	Amyloid deposits[b]											
	type	g/24 h	Total protein (g/24 h)	Pattern[a]			Heart		Pancreas		Liver		Lung		Kidney			
				Glomerular	Tubular		V	Int	V	Is	V	Int	V	S	V	Glom	Tub	Int
16	–	–	0.08	MS	–	1.0	–	–	–	+	–	–	+	–	–	–	–	–
17	–	–	0.08	S	–	0.8	–	–	–	–	–	–	–	–	–	–	–	–
18	–	–	0.15	S	–	2.2	–	–	–	–	–	–	–	–	–	–	–	–
19	–	–	0.20	MS	–	2.5	–	–	–	–	–	–	–	–	–	–	–	–
20	–	–	0.15	S	–	1.2	–	–	–	–	–	–	–	–	–	–	–	–
21	–	–	0.20	S	–	1.6	–	–	–	+	–	–	–	–	–	–	–	–
22	–	–	0.09	MS	–	0.9	–	–	–	–	–	–	–	–	–	–	–	–
23	–	–	0.14	PS	–	1.4	–	–	–	–	–	–	–	–	–	–	–	–
24	–	–	0.08	S	+	1.4	–	–	–	–	–	–	+	–	–	–	–	–
25	–	–	0.30	MS	+	1.0	–	–	–	+	–	–	–	–	–	–	–	–
26	–	–	0.60	PS	–	1.5	–	–	–	+	–	–	+	–	–	–	–	–
27	–	–	0.25	S	+	2.3	–	–	–	–	–	–	–	–	–	–	–	–
28	–	–	0.08	S	–	1.1	–	–	–	–	–	–	–	–	–	–	–	–
29	–	–	0.12	S	–	1.3	–	–	–	–	–	–	+	+	–	–	–	–
30	–	–	0.13	PS	+	2.5	+	–	–	–	+	–	+	+	+	–	–	–



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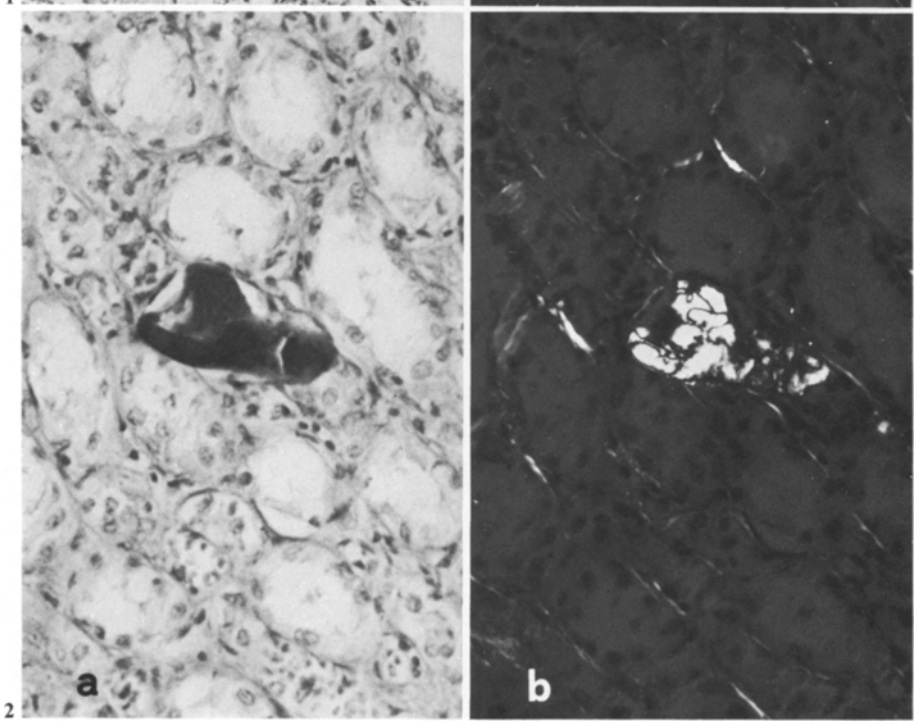

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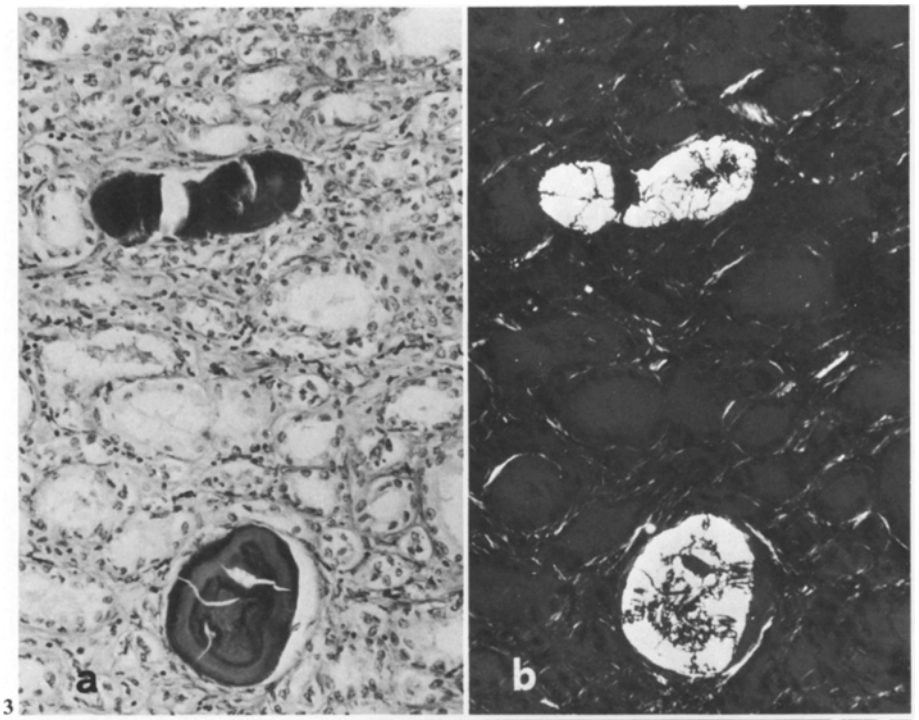
Fig. 1a and b. Single amyloid intratubular cast. Note the absence of glomerular involvement by amyloid deposition. **a** Congo red stain (×40). **b** Congo red stain – polarized light (×40)

Fig. 2a and b. Small broken homogeneous amyloid intratubular cast. **a** Congo red stain (×100). **b** Congo red stain-polarized light (×100)



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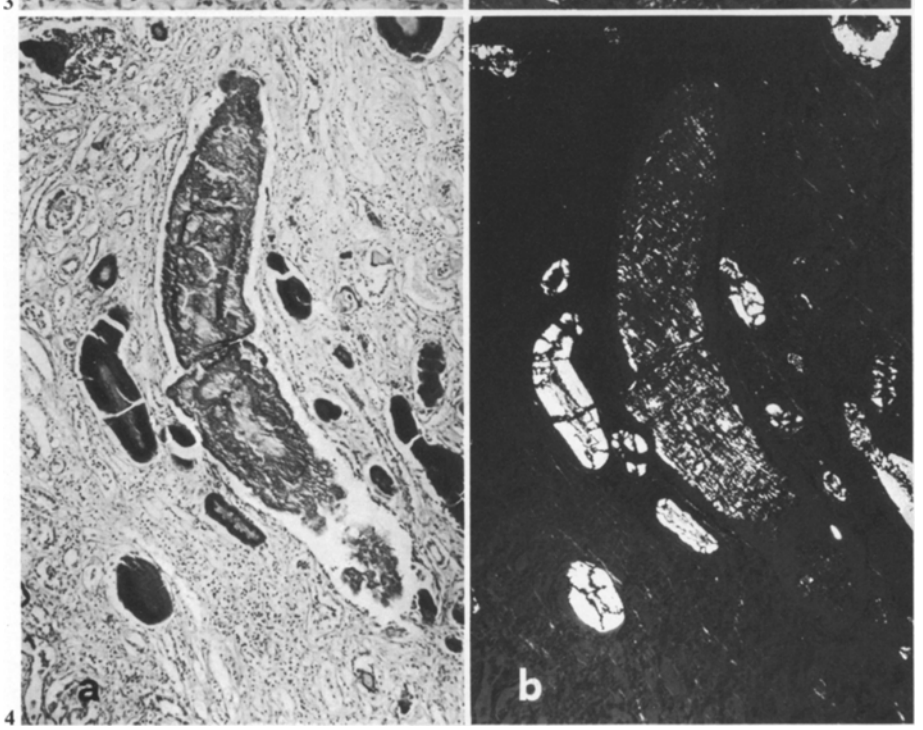

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Fig. 3a and b. Simultaneous occurrence in the same section of two amyloid intratubular casts exhibiting the homogeneous and laminated pattern respectively. **a** Congo red stain (×63). **b** Congo red stain – polarized light (×63)

Fig. 4a and b. **a** Section of kidney with multiple intratubular casts of various size and appearance (Congo red, ×25). **b** Same field viewed under polarized light (Congo red, ×25). Most of the casts exhibit characteristics of amyloid. Both homogeneous and laminated patterns are recognizable

When examined by routine histological methods, the appearance of these casts was indistinguishable from that of the concurrent proteinaceous casts. Only some casts showed the characteristics of amyloid when the Congo red stained sections were observed under polarized light. Their appearance was generally homogeneous, but a laminated pattern was also observed (Figs. 1, 2, 3, 4). All these casts exhibited positivity after staining for tryptophan and for tyrosine.

AIC were found to be resistant to trypsin digestion and potassium permanganate oxydation. In two cases, however, after such treatments AIC were shown to exhibit some tendency to break into fragments, probably in relation to lack of tissue support and/or the presence of foreign material.

Discussion

In the last few years increasing evidence has been accumulated which points out the association of amyloidosis with monoclonal immunoglobulin abnormalities.

A number of data now support the conclusion that the major constituent of amyloid deposits associated with multiple myeloma is a fibrillar protein derived primarily from fragments of homogeneous immunoglobulin light chains (Glenner et al., 1971 a; Glenner et al., 1971 b; Skinner and Cohen, 1971; Iserski et al., 1972; Glenner et al., 1973; Linke et al., 1973; Shirahama et al., 1973; Terry et al., 1973; Epstein et al., 1974). Amyloidosis in association with well-documented multiple myeloma has been reported to occur with variable frequency depending upon the series, but rarely exceeding 20 per cent (Bayrd and Bennet, 1950; Magnus-Levy, 1952; Azzopardi and Lehner, 1966; Snapper and Kahn, 1971; Aly et al., 1972; Azar, 1973). It is possible that other types of amyloidosis, unrelated to myeloma per se, might also have contributed to previously reported figures.

Such a relatively low incidence could be explained by assuming that the "amyloidogenic" potential is inherent in the structure of only a limited number of monoclonal immunoglobulin light chains (Glenner et al., 1973). In addition, the frequency, extent and distribution of amyloid deposits do not seem to differ significantly in myeloma patients and in age-matched control population without myelomatosis (Limas et al., 1973).

Morphological evidence for a close relationship between myeloma and amyloidosis can be provided by the finding of amyloid deposits in intimate relation to myeloma cells within or outside the bone marrow (Dahlin and Dockerty, 1950; Azar, 1973). With the exception of this observation, the presence of amyloid deposits within renal tubules appears to be the only other morphological change by which amyloidosis and myelomatosis can be regarded as closely related.

The finding of material exhibiting characteristics of amyloid within renal tubules of myeloma patients has been reported previously. Vassar and Culling (1962) found a significant fluorescence with Thioflavine T within tubular casts in a high proportion of patients with "myeloma nephrosis". Azzopardi and Lehner (1966) reported the finding of amyloid deposits located mainly in the

outer laminae in some laminated tubular casts in "myeloma kidneys". Ultrastructural study of the kidney performed by Abrahams et al. (1966) in a myeloma patient identified fibrillar amyloid-like material within casts and tubular epithelium. The staining properties of amyloid have also been demonstrated in the tubular casts of two myeloma patients reported by Demmler (1969) and by Friman et al. (1970) respectively.

More recently, Limas et al. (1973) reported the finding of material exhibiting the tinctorial and ultrastructural characteristics of amyloid in the renal tubular casts of 15 myeloma patients, without apparent relationship to the presence or extent of amyloid deposition in other organs. The Authors emphasized the amyloid-rimmed aspect of some proteinaceous tubular casts as a distinguishing characteristic of myeloma patients. Similar peripheral rimming of tubular casts by material having amyloid-like staining properties was also found, in the absence of parenchymal amyloid deposits, by De Fronzo et al. (1975) in four myeloma patients with acute renal failure.

In our material amyloid intratubular casts were not found to be uniform in size and appearance. We observed, in fact, the simultaneous occurrence of small and large casts both homogeneous and laminated in appearance.

Tubular deposits appear to be independent of the occurrence of amyloid deposits in other renal and extra-renal sites, with the exception of pancreatic insular amyloidosis. The latter, however, exhibits histochemical characteristics by which it can be identified as Apudamyloid, whereas intratubular and cardiovascular deposits are invariably constituted of immuno-amyloid. The simultaneous occurrence, in the same patients, of both types of deposits can therefore be regarded as unrelated (Westermark, 1974; Antonutto et al., 1975).

The absence of other renal and extra-renal immuno-amyloid deposits in patients with AIC suggests that the phenomenon may result from local factors which seem to occur only in myelomatosis and not in benign monoclonal gammopathies. It is therefore reasonable to assume that Bence Jones proteinuria plays a major role in determinating AIC.

If results of more specific and sensitive investigations (such as electrophoresis and immunoelectrophoresis of adequately concentrated urine) had been available in previously reported series, Bence Jones proteinuria would probably have been detected in a higher proportion of patients, thus further supporting the relationship between AIC and urinary excretion of monoclonal immunoglobulin light chains.

At the present time, however, there are not sufficient data to substantiate precise correlations between presence (and extent) of amyloid intratubular deposition and immunochemical type, amount and duration of urinary excretion of Bence Jones protein.

Data from our series would indicate that the finding of AIC may not necessarily be associated with the largest outputs of Bence Jones protein and with the most severe impairment of renal function. On the other hand, the renal histological findings in patients with AIC were not those described classically as "myeloma kidney", whereas renal lesions consistent with "myeloma kidney" were observed in other myeloma patients without AIC.

The studies enabling the in vitro creation of amyloid-like fibrils from Bence Jones proteins by proteolysis (Glenner et al., 1971 a; Linke et al., 1973; Shirahama et al., 1973) raised the hypothesis suggesting direct involvement of lysosomes in amyloidogenesis (Glenner et al., 1973). Further insights into a possibly determinant role of lysosomes in the in vivo production of amyloid have been provided by the demonstration (Epstein et al., 1974) that digestion of human Bence Jones proteins of kappa and lambda type by naturally occurring lysosomal enzymes from human kidney can result in precipitates having the tinctorial and ultrastructural characteristics of amyloid fibrils.

The demonstration of amyloid within the renal tubules of myeloma patients without systemic amyloidosis suggests that a similar proteolytic process can occur also in vivo. However, this does not necessarily imply that the proteolytic event takes place at an intracellular level (i.e. within tubular epithelium) and that the presence of amyloid within tubular lumen results from extrusion of processed Bence Jones protein.

It is tempting to speculate on the possibility that conditions closely similar to those required for the in vitro creation of amyloid fibrils from Bence Jones proteins may occur in vivo within renal tubules of certain myeloma patients. Both special "amyloidogenic" characteristics inherent in the molecule of Bence Jones protein (Glenner et al., 1973) and the occurrence of functional disturbances of the renal tubule induced by the underlying disease (Martinez-Maldonado et al., 1971; Snapper and Kahn, 1971; De Fronzo et al., 1975) and/or by Bence Jones protein itself (Preuss et al., 1967; Maldonado et al., 1975; McGeoch et al., 1978) should be taken into account as factors which may contribute causally to the phenomenon.

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