The Potassium Permanganate Method

A Reliable Method for Differentiating Amyloid AA From Other Forms of Amyloid in Routine Laboratory Practice

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Alterations in affinity of amyloid for Congo red after incubation of tissue sections with potassium permanganate, as described by Wright et al, were studied. The affinity of amyloid for Congo red after incubation with potassium permanganate did not change in patients with myeloma-associated amyloidosis, familial amyloidotic polyneuropathy, medullary carcinoma of the thyroid, pancreatic island amyloid, and cerebral amyloidosis. Affinity for Congo red was lost after incubation with potassium permanganate in tissue sections from patients with secondary amyloidosis and amyloidosis complicating familial Mediterranean fever (consisting of amyloid AA). Patients with primary amyloidosis could be divided into two groups, one with potassium-permanganate-sensitive and one with potassium-permanganate-resistant amyloid deposits. These two groups correlated with the clinical classification in typical organ distribution (presenting with nephropathy) and atypical organ distribution (presenting with cardiomyopathy, nephropathy, and glossopathy) and the expected presence of amyloid AA or amyloid AL. Potassium permanganate sensitivity seems to be restricted to amyloid AA. The potassium permanganate method can be important in dividing the major forms of generalized amyloidosis in AA amyloid and non-AA amyloid. This can be used for differentiating early stages of the disease and cases otherwise difficult to classify. It is important to define patient groups properly, especially in evaluating the effect of therapeutic measures. (Am J Pathol 97:43–58, 1979)

AMYLOIDOSIS is a disease caused by extracellular deposition of protein fibrils. These fibrils are composed of polypeptide chains, arranged in the so-called beta-pleated sheet configuration, resulting in resistance to proteolytic digestion, insolubility under physiologic conditions, and green birefringence with polarized light after staining with Congo red.

The clinical classification of amyloidosis mostly used is a modification of the classification proposed by Reimann in 1935,[1] which has also been used by Kyle and Baird in their review of 236 cases of amyloidosis [2]:

1. Primary amyloidosis
2. Myeloma-associated amyloidosis
3. Secondary amyloidosis
4. Localized amyloid
5. Familial amyloidosis

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Since primary amyloidosis and familial amyloidosis are heterogeneous with regard to their clinical picture, further classification has been attempted. Differentiation based on histologic localization in perireticular and pericollagenic amyloidosis [3] has never become generally accepted. Differentiation based on the pattern of organ distribution was proposed by King [4] in typical and atypical distribution and by Isobe and Osserman [5] in Pattern II and Pattern I. However, problems were incurred by the use of nearly all organs in which amyloid depositions can be found, many of them without clinical consequences. This is illustrated by the addition of a third, "mixed," pattern by the latter authors. In our experience, a simplified differentiation based on the clinically evident dysfunction of the kidneys, the heart, and the tongue is more useful. The acquired and idiopathic as well as the hereditary forms of generalized amyloidosis can be divided this way (Table 1).

A major step toward the understanding of pathophysiologic mechanisms in amyloidosis was made by the isolation and purification of several different amyloid protein components (Table 2). In clinical practice it seems important to differentiate types of generalized amyloidosis with regard to the major protein component. Where the clinical picture is not always clear, and since it is practically impossible to determine amino acid sequence in every patient with generalized amyloidosis, an easier method for differentiating would be useful.

Determination of the level of the serum component SAA (which is immunochemically related to protein AA) does not seem to provide a suitable diagnostic procedure.[20,21]

The study of biopsy material by immunofluorescence has been tried.[22]

Table 1—Classification of Amyloidosis by the Pattern of Major Organ Dysfunction

	Clinical pattern of organ dysfunction	
Type of Amyloidosis	Typical distribution (nephropathy without cardiomyopathy and glossopathy)	Atypical distribution (cardiomyopathy, glossopathy, and nephropathy)
Acquired	Secondary amyloidosis	Myeloma-associated amyloidosis
Idiopathic	Primary amyloidosis with typical distribution	Primary amyloidosis with atypical distribution
Hereditary	Familial Mediterranean fever	Neuropathic [7-9]
	Urticaria, deafness, and nephropathy[6]	Cardiopathic[10]
		Corneal lattice dystrophy and cranial neuropathy[11]
Localized	Amyloid "tumors"	
	Cutaneous amyloidosis	
	APUD amyloid	
	Cerebral and senile cardiac amyloid	

Table 2—Clinicopathologic Correlations in Amyloidosis According to Types of Amyloid Protein

Amyloid protein	Possible precursor	Disease	Organ distribution
$AL^{12,13}$	Variable part of Ig light chain	Myeloma-associated amyloidosis "Primary" amyloidosis	Heart, tongue kidneys, nerves joints (atypical distribution)
AA^{14-16}	Serum component SAA	Secondary amyloidosis "Primary" amyloidosis	Kidneys and blood vessels (typical distribution)
A_{MCT}^{17}	(Pro) calcitonine	Medullary carcinoma of the thyroid	Thyroid
A_{SCA}^{18}	?	Senile cardiac amyloidosis	Heart
A_{FAP}^{19}	Subunit of prealbumin	Familial amyloidotic polyneuropathy	Peripheral nerves, kidneys, heart

This method is, however, not ready for routine laboratory use. Recently Wright et al [23] described a simple method for differentiating between amyloid AA and other types of amyloid. This method is a simplification of a method described by Romhányi.[24] It shows that secondary amyloid looses its affinity for Congo red after incubation with potassium permanganate. We applied the method according to Wright on histologic material from our files in order to check its reproducibility in our hands and to evaluate the clinical implications.

Materials and Methods

Amyloid deposits in material from 29 autopsies and 37 biopsy specimens from 27 patients were studied for their Congo red affinity after incubation with potassium permanganate.

A simplification of the classification by King [4] in typical and atypical organ distribution was used. Patients were divided according to the clinically evident involvement of the kidneys, the heart, and the tongue in 1) *typical distribution* in the case of nephropathy without evidence of cardiomyopathy and glossopathy and 2) *atypical distribution* in the case of cardiomyopathy, glossopathy, and nephropathy.

Additional classification was made in 1) *acquired amyloidosis* in the case of generalized amyloidosis with underlying chronic disease (secondary amyloidosis) and generalized amyloidosis accompanying multiple myeloma (multiple myeloma was diagnosed according to Costa et al [25]), 2) *idiopathic amyloidosis* in the case of generalized amyloidosis without detectable underlying disease known to be associated with amyloidosis, 3) *hereditary amyloidosis*, and 4) *localized amyloid.*

The patient data listed in Tables 3 and 4 show that the organ distribution was typical in all cases of secondary amyloidosis, while in myeloma-associated amyloidosis the organ distribution was atypical. So there was no need for changing the familiar nomenclature, apart from adding "typical" or "atypical distribution" in the case of primary (idiopathic) amyloidosis.

The effect of the potassium permanganate method was also studied on localized amyloid deposits in medullary carcinoma of the thyroid (2 cases), senile cardiac amyloid (1 case), pancreatic island amyloid (1 case), cerebral amyloid (3 cases), and some hereditary forms of amyloidosis (1 case of familial Mediterranean fever, 2 cases of the neuropathic form of Andrade, and 1 case of Meretoja's form, a case already reported by Winkelman et al [26]).

Table 3—Clinical and Laboratory Data of Autopsy Patients

Patient	Age (yrs)	Sex (M/F)	Clinical classification	Concomitant disease	Monoclonal component (serum/urine)	Bone marrow examination	Proteinuria (g/24 hrs)	Renal function (serum creatinine, creatinine clearance)	Liver/spleen size (cm)	Cardiomyopathy	Glossopathy	Others
1	48	M	SA	Ankylosing spondylitis	−/−	No myeloma	4	55 ml/min	−/−	−	−	−
2	30	M	SA	Hodgkin's disease	−/−	ND	10–20	?	2/1	−	−	−
3	74	F	SA	Rheumatoid arthritis	−/−	ND	3	91 ml/min	−/−	−	−	−
4	55	F	SA	Rheumatoid arthritis	−/−	ND	alb: +++	318 μmol/l	−/2	−	−	−
5	32	M	SA	Hodgkin's disease	−/?	No myeloma	5	170 μmol/l	−/−	−	−	−
6	59	F	SA	Rheumatoid arthritis	−/−	No myeloma	2	2 ml/min	−/−	−	−	−
7	35	F	SA	Rheumatoid arthritis	−/−	No myeloma	5	61 ml/min	−/−	−	−	−
8	74	F	SA	Rheumatoid arthritis	−/−	ND	?	?	−/−	−	−	−
9	68	F	SA	Rheumatoid arthritis	−/−	ND	10–20	35 ml/min	−/−	−	−	−
10	63	F	SA	Tuberculosis	−/−	ND	5–10	20 ml/min	3/2	−	−	−
11	54	M	SA	Tuberculosis	−/−	ND	5	62 ml/min	2/−	−	−	−
12	20	F	SA	Crohn's disease	−/−	ND	5–20	?	−/−	−	−	−
13	50	F	SA	Rheumatoid arthritis	−/−	ND	5–20	44 μmol/l	−/−	−	−	−

14	67	M	SA	Recurrent pulmonary infection	?/?	ND	alb: +++	?	−/−	−	−	−
15	67	M	SA	Recurrent pulmonary infection	?/?	ND	?	?	−/−	−	−	−
16	63	F	SA	Rheumatoid arthritis	−/?	ND	1-2	5 ml/min	−/−	−	−	−
17	44	F	SA	Rheumatoid arthritis	IgGκ/−	No myeloma	5-15	270 μmol/l	2/1	−	−	−
18	54	F	PA/TD	Paraganglioma	−/−	No myeloma	5-15	660 μmol/l	2/−	−	−	−
19	62	F	PA/TD	Carcinoid	−/−	ND	alb: +++	580 μmol/l	−/−	−	−	−
20	73	F	MAA	Myeloma	−/?	Myeloma	1-2	80 ml/min	−/−	+	+	Neuropathy
21	53	F	MAA	Myeloma	IgG λ'IgG	Myeloma	1-2	60 ml/min	2/−	+	+	Carpal tunnel syndrome
22	49	F	MAA	Myeloma	BJκ/BJκ	Myeloma	4-7	70 ml/min	−/−	+	+	Amyloid arthropathy
23	61	F	MAA	Myeloma	IgAκ/BJκ	Myeloma	2-4	50 ml/min	3/2	+	+	−
24	54	M	MAA	Myeloma	BJλ/BJλ	Myeloma	5-10	90 ml/min	1/−	+	−	−
25	61	M	PA/AD	None	−/−	No myeloma	0-1	14 ml/min	4/1	+	+	−
26	56	M	PA/AD	None	−/−	No myeloma	?	24 ml/min	1/1	+	+	Ocular neuropathy
27	69	M	PA/AD	None	−/−	No myeloma	4-5	56 ml/min	2/−	+	+	−
28		M	PA/AD	None	−/−	No myeloma	?	?	1/−	+	+	−
29	58	M	PA/AD	None	−/BJκ+λ	No myeloma	2	10 ml/min	−/−	+	+	Neuropathy

SA = secondary amyloidosis; PA = primary amyloidosis; TD = typical distribution; MAA = myeloma associated amyloidosis; AD = atypical distribution.

BJ = Bence-Jones protein.

ND = not done.

Table 4—Clinical and Laboratory Data of Biopsy Patients

Patient	Age (yrs)	Sex (M/F)	Clinical classification	Concomitant disease	Monoclonal component (serum/urine)	Bone marrow examination	Proteinuria (g/24 hrs)	Renal function (serum creatinine, creatinine clearance)	Liver/spleen size (cm)	Cardiomyopathy	Glossopathy	Others
30	60	M	SA	Ankylosing spondylitis	?/?	ND	alb: +++	55 ml/min	−/−	−	−	−
31	28	F	SA	Crohn's disease	−/−	No myeloma	10-20	19 ml/min	2/1	−	−	−
32	54	F	SA	Rheumatoid arthritis	−/?	ND	—	106 μmol/l	−/−	−	−	−
33	37	M	SA	Rheumatoid arthritis	−/?	No myeloma	—	70 ml/min	−/−	−	−	−
34	53	F	SA	Rheumatoid arthritis	−/−	No myeloma	5-10	5 ml/min	−/−	−	−	−
35	60	F	SA	Rheumatoid arthritis	−/−	No myeloma	5-10	5 ml/min	2/1	−	−	−
36	34	M	SA	Ankylosing spondylitis	−/?	No myeloma	2	64 ml/min	−/−	−	−	−
37	47	M	SA	Agammaglobulinemia Recurrent pulmonary infection	−/−	No myeloma	10-20	10 ml/min	−/−	−	−	−
38	47	M	SA	Osteomyelitis	−/?	No myeloma	5	5 ml/min	−/−	−	−	−
39	71	F	SA	Rheumatoid arthritis	−/−	No myeloma	3-9	20 ml/min	1/1	−	−	−
40	27	F	SA	Rheumatoid arthritis	−/−	5	45 ml/min		3/2	−	−	−
41	44	M	SA	Recurrent pulmonary infection	−/−	No myeloma	1-4	166 ml/min	−/−	−	−	−
42	61	F	SA	Rheumatoid arthritis	−/−	ND	2	18 ml/min	2/−	−	−	−
43	71	M	SA	Recurrent pulmonary infection	−/−	ND	2-7	25 ml/min	−/−	−	−	−
44	53	M	SA	Recurrent pulmonary infection	−/?	No myeloma	5-10	10 ml/min	−/−	−	−	−
45	59	M	SA	Rheumatoid arthritis	−/?	ND	15	5 ml/min	1/−	−	−	−
46	70	F	SA	Recurrent pulmonary infection	−/?	ND	5-10	40 ml/min	−/−	−	−	−
47	71	F	SA	Rheumatoid arthritis	−/?	ND	1-2	3 ml/min	−/−	−	−	−
48	70	M	PA/TD	None	−/−	No myeloma	5-10	94 ml/min	−/1	−	−	−
49	67	F	PA/TD	None	−/−	No myeloma	5	5 ml/min	−/−	−	−	−
50	50	M	PA/TD	None	−/−	No myeloma	5-10	20 ml/min	−/−	−	−	−
51	39	F	PA/TD	None	−/−	No myeloma	5	8 ml/min	1/2	−	−	−
52	73	M	PA/TD	None	−/−	No myeloma	3-5	30 ml/min	−/−	−	−	−
53	57	F	MAA	Myeloma	IgG λ/IgG λ	Myeloma	5-15	30 ml/min	1/2	+	−	Neuropathy
54	60	M	MAA	Myeloma	IgG κ/IgG κ	Myeloma	12	45 ml/min	−/−	+	−	−
55	49	M	MAA	Myeloma	BJ λ/BJ λ	Myeloma	10	92 ml/min	−/−	+	+	−
56	53	F	PA/AD	None	−/BJ λ		2	60 ml/min	2/1	+	+	−

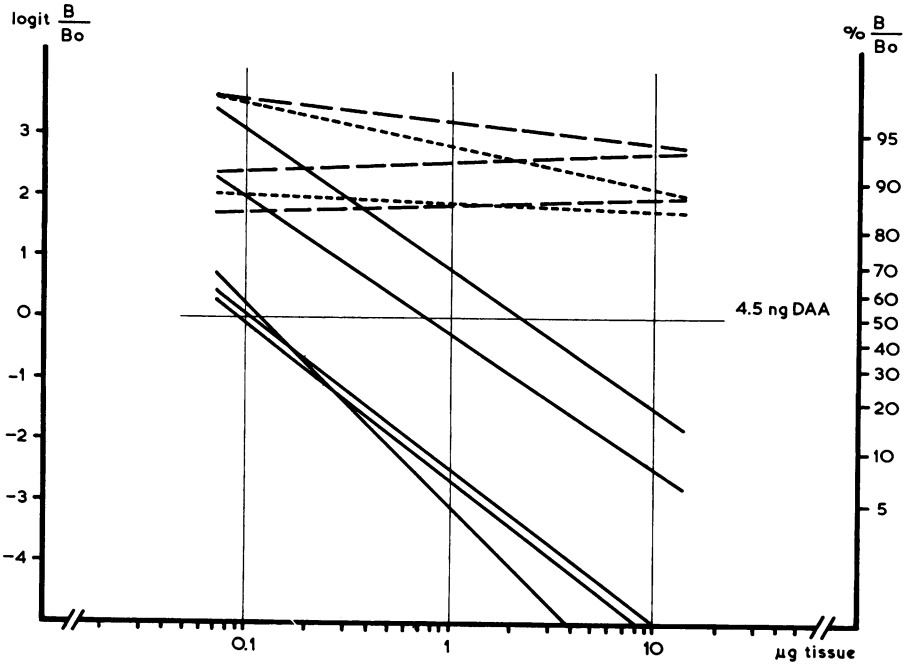
SA = secondary amyloidosis; PA = primary amyloidosis; TD = typical distribution; MAA = myeloma associated amyloidosis; AD = atypical distribution. BJ = Bence-Jones protein. ND = not done.

Autopsy material and biopsies of the rectal mucosa were fixed in 8% neutral formalin. Liver biopsies were fixed in Bouin's solution (Hollande) and kidney biopsies in Tellyesnizcky's solution (acetic acid–alcohol–formalin). The tissues were embedded in paraffin. Three kidney biopsies were embedded in methylacrylate after Burkhardt fixation (5 ml formalin and 10 ml glucose–menthanol solution).[27]

Sections were cut at 4 μ, incubated for 3 minutes with equal volumes of 5% potassium permanganate and 0.3% sulfuric acid, decolorized with 5% oxalic acid, washed twice in distilled water, and stained with alkaline Congo red according to Puchtler.[28] Serial sections were stained with Congo red and served as a control group. With the different fixatives and embedding procedures, the results of the potassium permanganate method were the same. The sections were studied by light microscope and polarizing microscope. Materials from autopsies were also studied with the fluorescence microscope. Both methods were comparable with regard to the affinity of amyloid deposits for Congo red.

Amyloid deposits were considered as potassium-permanganate–sensitive, when 90% or more of the deposits lost their affinity for Congo red after incubation with potassium permanganate.

In a radioimmunoassay,[29] protein AA content was determined in the amyloid-containing tissue of 5 patients with secondary amyloidosis, 2 patients with primary amyloidosis and



TEXT-FIGURE 1—The binding of alkali-degraded protein AA (DAA) to anti-DAA antibody is shown for normal non-amyloid-containing tissue (— — — — —), amyloid-containing tissue from 2 patients with primary amyloidosis with atypical distribution (- - - - - - - - -), and amyloid-containing tissue from 5 patients with secondary amyloidosis (————). Maximal binding (B_0) is obtained in the presence of ^{125}I-DAA only. Duplicate values of competitive binding (B) are obtained by adding three different amounts of alkali-degraded tissue (0.1, 1, 10 μg). The standard (B/B_0 = 50%) is obtained by adding 4.5 ng of purified DAA. Logit transformation results in a linearization of the curve. The crossing of the lines drawn through the three B/B_0 values obtained and the standard indicate the amount of tissue that should have yielded a B/B_0 value of 50% (corresponding with 4.5 ng DAA).

Table 5—The Effect of Potassium Permanganate Incubation on the Binding of Congo Red in the Major Types of Generalized Amyloidosis

	Autopsies and biopsies potassium permanganate	
	Sensitive	Resistant
Secondary amyloidosis	35	
Myeloma-associated amyloidosis		8
Primary amyloidosis		
Typical distribution	7	
Atypical distribution		6

atypical organ distribution, and in the tissue of 2 patients without amyloidosis (Text-figure 1). The potassium permanganate method was also used with this tissue.

Results

The results obtained by the potassium permanganate method are summarized in Table 5.

Amyloid deposits in patients with secondary amyloidosis were potassium-permanganate–sensitive. In one patient (Patient 9, Table 3), however, large interstitial resistant amyloid deposits were found in the heart without clinical evidence of cardiomyopathy.

Potassium-permanganate–sensitive amyloid deposits were also found in the patients classified as having primary amyloidosis with typical distribution. Two patients (Patients 18 and 19, Table 3) had tumors not known to be associated with generalized amyloidosis: paraganglioma and bronchial carcinoid.

All the patients with myeloma-associated amyloidosis and patients with primary amyloidosis with atypical distribution showed potassium-permanganate–resistant amyloid deposits.

Although the effect of the potassium permanganate incubation on the affinity for Congo red was identical in all the organs of the individual patient (with exception of Patient 9, Table 3, mentioned above), small potassium-permanganate–resistant amyloid deposits (less than 10%) were found in the vessel walls of different organs in most patients with secondary amyloidosis and primary amyloidosis with typical distribution.

Amyloid deposits in medullary carcinoma of the thyroid, senile cardiac amyloidosis, pancreatic island amyloid, and amyloid deposits in cerebral amyloidosis were potassium-permanganate–resistant.

The reaction to potassium permanganate in the familial types of amyloidosis was not uniform. The patient with familial Mediterranean fever exhibited sensitive amyloid deposits in the kidney biopsy, whereas 2 patients with the neuropathic form of Andrade and the patient with the

familial neuropathic form with corneal lattice dystrophy (as described by Meretoja) showed resistant deposits.

Amyloid-containing tissues which showed a high content of protein AA (as determined by radioimmunoassay) appeared to be potassium-permanganate–sensitive, whereas amyloid-containing tissues without detectable protein AA were potassium-permanganate–resistant.

Thus, the potassium permanganate sensitivity of the amyloid deposits seems to be related to their protein AA content.

Discussion

The results obtained with Wright's potassium permanganate method show a consistent pattern (Table 5). Recent investigations have revealed that secondary amyloid and amyloid in familial Mediterranean fever are mainly composed of amyloid AA.[14,15,16] In our series all the patients having these types of amyloidosis had potassium-permanganate–sensitive amyloid deposits. The finding of minimal resistant deposits in vessel walls can possibly be attributed to the additional deposition of non-AA amyloid.[30] Patient 9 (Table 3), with a long-standing rheumatoid arthritis, had large potassium-permanganate–resistant foci in the interstitium of the heart, without clinical evidence of cardiomyopathy. Amyloid deposits in the other organs, however, were sensitive. It seems reasonable to suppose a senile cardiac amyloidosis in this patient.

In sharp contrast to the potassium-permanganate–sensitive amyloid deposits in patients with secondary amyloidosis and familial Mediterranean fever, all the patients with amyloid deposits known to consist of amyloid AL,[12,13] APUD amyloid,[17] senile cardiac amyloid,[18] and the prealbumin-derived amyloid in familial amyloidotic polyneuropathy,[19] had potassium-permanganate–resistant deposits. Also resistant were amyloid deposits in cerebral amyloidosis and amyloid in the familial form of Meretoja. The chemical composition of these types of amyloid is still unknown. Therefore the results of the potassium permanganate method were in accordance with the expected composition of amyloid.

Primary amyloidosis offers diagnostic problems. Based on the major clinical presentation, we divided our patients in one group with a typical pattern and another group with an atypical pattern of organ distribution. In the typical group, the potassium permanganate method showed a sensitive reaction, whereas the atypical group showed a resistance to potassium permanganate. These results confirm the heterogeneity of so-called primary amyloidosis. The sharp differentiation of primary generalized amyloidosis is in contrast with the results mentioned by Wright. He

did not find this difference and introduced a "mixed pattern" apart from a group with resistant deposits. This discrepancy remains unexplained.

In view of the consistent reaction of amyloid AA to potassium permanganate, our findings indicate that at least in some of the cases of primary amyloidosis, we are dealing with amyloid AA. This is supported by the results of Cornwell [22] and Natvig,[31] who found amyloid AA in the tissue of some patients classified as having primary amyloidosis. The results in the other patients (myeloma-associated amyloidosis and secondary amyloidosis) are in accordance with the results of Wright [23] and of Romhanyi.[24] They are supported by the preliminary findings of a correlation between the results of the potassium permanganate method and the content of protein AA, as determined by radioimmunoassay (Text-figure 1).

It is remarkable that the potassium permanganate method strictly follows the clinical classification, based on clinical signs and symptoms of organ involvement. This implies that this clinical classification can be used in practical medicine. However, in early as well in end-stage amyloidosis, it can be difficult to classify a patient correctly. In these cases the potassium permanganate method is very useful.

The question remains as to why only amyloid AA loses affinity for Congo red after incubation with potassium permanganate.

Since binding with Congo red and typical green birefringence with polarized light are properties of the beta-pleated sheet structure,[32] an alteration in this structure after potassium permanganate incubation is obvious. It is highly probable that the high content of arginine in amyloid AA is responsible for the susceptibility to potassium permanganate, as suggested by Wright.[33] Recent investigations of the amino acid sequence of amyloid in medullary carcinoma of the thyroid and in senile cardiac amyloidosis [17,18] are compatible with this suggestion.

We can conclude from our results that Wright's method provides a very simple procedure for differentiating between amyloid AA and other forms of amyloid, of which amyloid AL is the most important. By this method the pathologist and the clinician in every laboratory and clinic can gain insight into the chemical characteristics of the amyloid of the individual patient. This is of particular interest for the proper classification of amyloid patients. This proper classification is necessary in the evaluation of the effect of a treatment with, eg, dimethylsulfoxide [34,35,36,37] or colchicine.[38]

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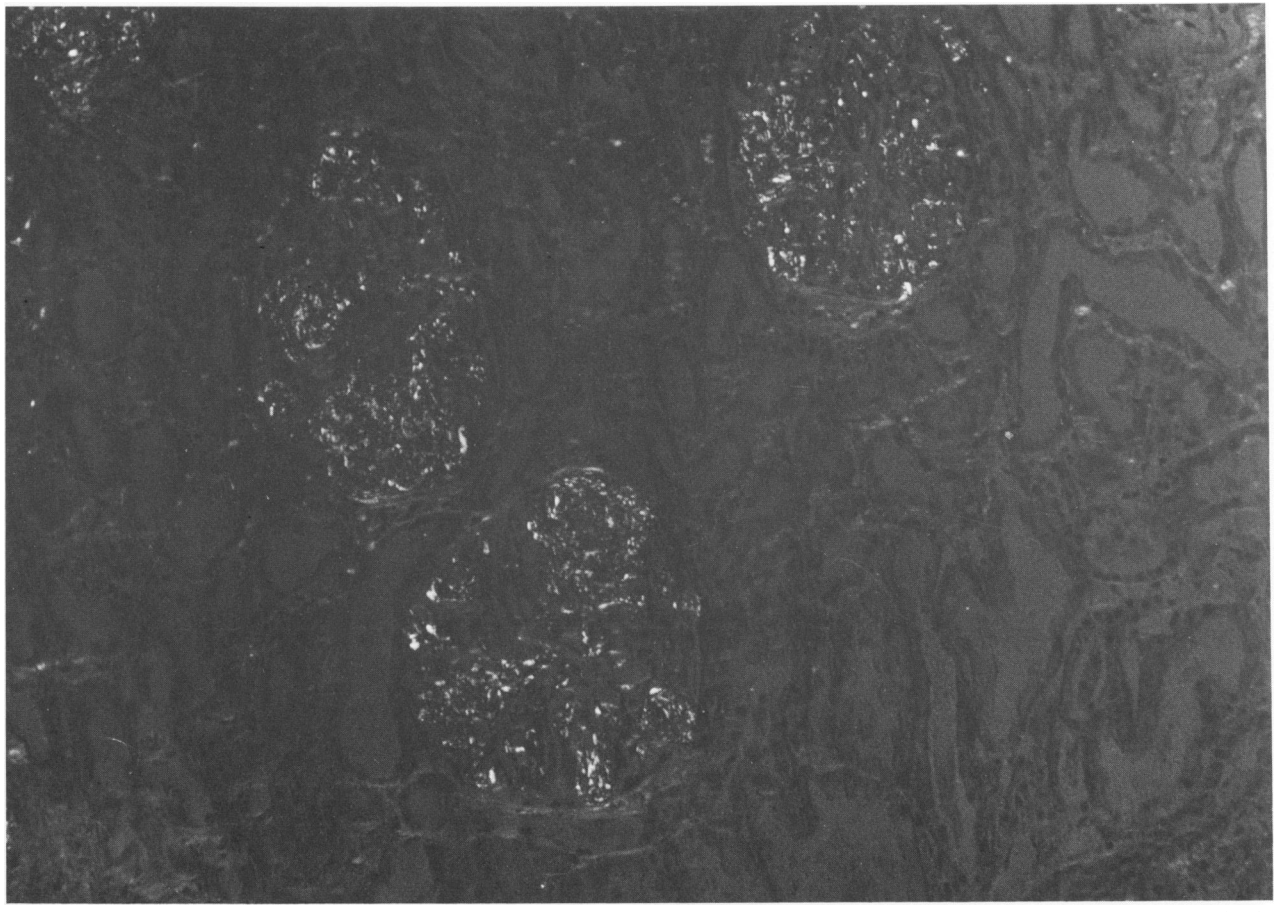
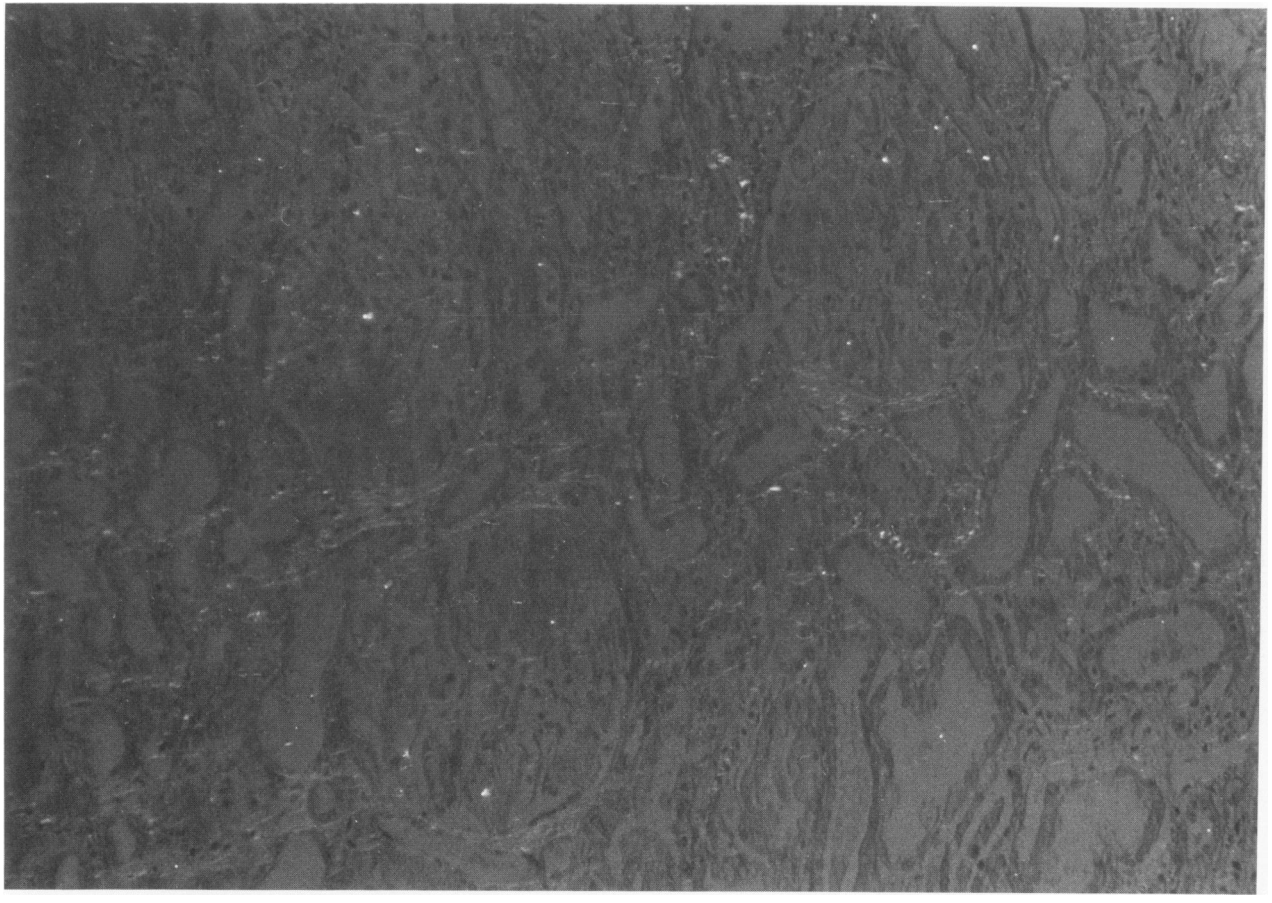


Figure 1—Potassium permanganate reaction in a kidney biopsy specimen from a patient with long-standing rheumatoid arthritis and generalized amyloidosis. **A**—The bright white foci represent typical green birefringence in three glomeruli.



B—The same three glomeruli in a serial section after potassium permanganate incubation show a loss of green birefringence. (Alkaline Congo red, polarized light, ×140)

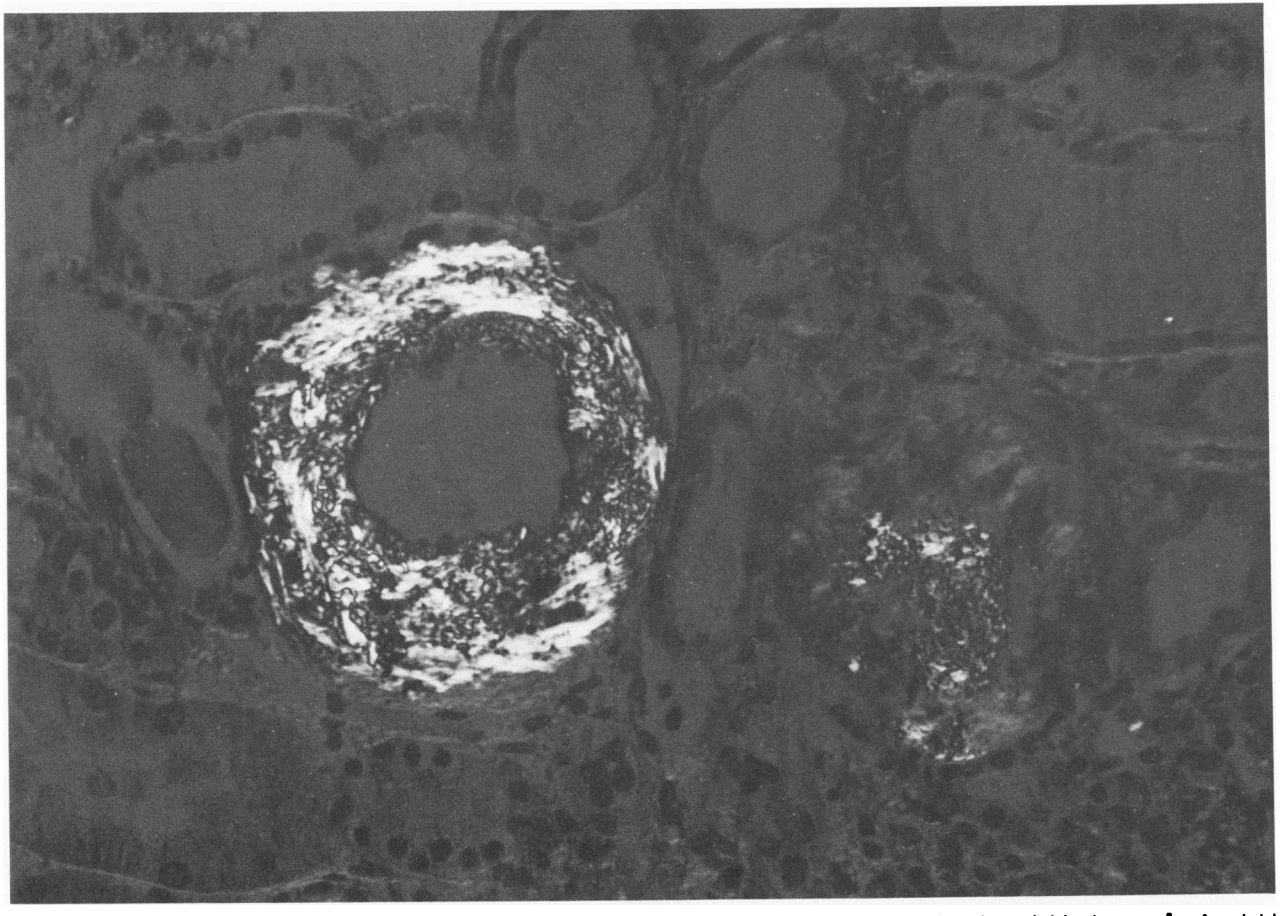
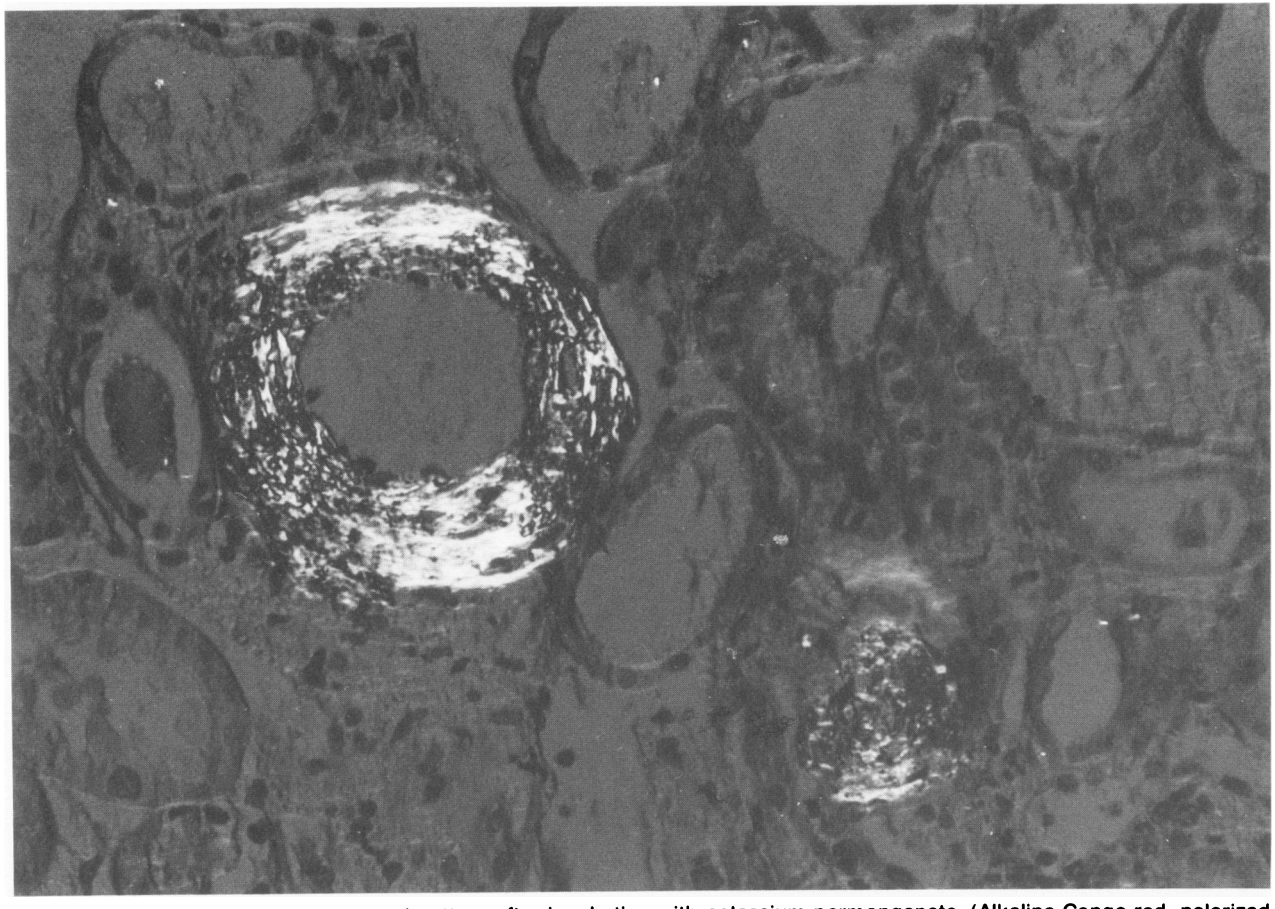


Figure 2—Potassium permanganate reaction in a kidney biopsy of a patient with myeloma associated amyloidosis. **A**—Amyloid deposits in a glomerulus and an artery.



B—A serial section shows an unchanged pattern after incubation with potassium permanganate. (Alkaline Congo red, polarized light, ×350)