MEDICAL GRAND ROUNDS

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AMYLOIDOSIS

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I. Historical Aspects

Few entities in medicine are surrounded by as much confusion and controversy as amyloidosis. Prior to the mid-19th century, this disorder was classified with other "waxy" or "lardaceous" degenerations of the liver. In 1842, Rokitansky described the liver in this condition as enlarged, indurated and waxy; he also noted similar changes in the spleen and other organs. Virchow (1855) coined the term "amyloid" because this translucent, hyaline substance stained with iodine in a manner similar to starch. Virchow also was the first to describe the intimate relation of amyloid to blood vessels and to postulate the presence of a circulating amyloid precursor. These and other pathologists recognized that while amyloid often accompanied chronic suppuration, it also was found in the absence of predisposing infection or inflammation. The chemistry of amyloid was initially investigated by Friedreich and Kekulé in 1859; these workers concluded that the substance was neither starch nor cellulose but largely protein. An experimental model of the disease was described in 1922 by Kuczynski who showed that many mice developed amyloid deposits following injections of casein. Divry and Florkin observed that amyloid was birefringent when viewed in the polarizing microscope. The introduction of the Congo red test and stain as well as the development of techniques for direct biopsy of suspected involved tissues in the 1920's made clinicians more aware of the natural history and variable manifestations of the disorder. Waldenström clearly demonstrated the disappearance of hepatic amyloid in patients with tuberculosis and chronic draining fistulae once the infections were arrested. Although it had been previously recognized that amyloidosis occasionally "complicated" multiple myeloma, Magnus-Levy emphasized the association between amyloid and Bence Jones proteinuria. Apitz called attention to the presence of marrow plasmacytosis in a large proportion of cases of idiopathic or "primary" amyloidosis and raised the possibility that many (?all) cases of this type represented occult or "pre-myeloma". Reimann et al proposed the popular classification of amyloidosis into primary, secondary, localized (tumor-forming), and myeloma-associated types. The situation was further complicated by the description of a hereditary amyloid syndrome in Portugal by Andrade in 1952.

Two principal theories of the pathogenesis of amyloid recur in the literature: 1) precipitation of a serum component (?antibody-antigen complexes, Letterer 1934); 2) local production by cells; amyloid formation dependent on a 2-phase aberration of the protein-synthesizing function of reticuloendothelial cells (Teilum 1956, 1964).

1. Cohen AS: Amyloidosis. New Eng J Med 277:522, 574, 628, 1967.

2. Letterer E: History and development of amyloid research. Symposium on Amyloidosis (Mandema E, Ruinen L, Scholten JH, Cohen AS, eds), Amsterdam, Excerpta Medica Foundation, 1968, p. 3.

3. Azar HA: Amyloidosis and plasma cell disorders, chap 7, Vol I. Multiple Myeloma and Related Disorders (Azar HA, Potter J, eds), Hagerstown, Harper & Row, Publishers, Inc, 1973.

4. Divry P, Florkin M: Sur les proprietes optiques l'amyloide. C R Soc Biol (Paris) 97:1808, 1927.

5. Waldenström H: On the formation and disappearance of amyloid in man. Acta Chir Scand 63:479, 1928.

6. Magnus-Levy A: Bence-Jones-Eiweiss und Amyloid. Ztschr Klin Med 116:510, 1931.

7. Magnus-Levy A: Amyloidosis in multiple myeloma. Progress noted in 50 years of personal observation. J Mt Sinai Hosp 19: 8, 1952.

8. Apitz K: Die Paraproteinosin. Über die Störung des Eiweiss-stoffwechsels bei Plasmocytom. Virchows Arch path Anat 306:631, 1940.

9. Reimann HA, Koucky RF, Eklund CM: Primary amyloidosis limited to tissue of mesenchymal origin. Am J Pathol 11:977, 1935.

10. Andrade C: A peculiar form of peripheral neuropathy. Brain 75: 408, 1952.

11. Teilum G: Pathogenesis of amyloidosis. The two-phase cellular theory of local secretion. Acta Pathol Microbiol Scand 61:21, 1964.

II. Spontaneous and Induced Amyloidosis in Animals

Amyloid occurs spontaneously in at least 16 species of animals including dogs, cats, mice and birds. As in man, its occurrence is often associated with aging. Amyloidosis can be induced experimentally in many species - mice, guinea pigs, rabbits, hamsters, etc. but susceptibility to the inducing regimen varies among species and strains. A variety of inducing agents have been utilized: bacterial toxins, antitoxins, whole serum, plasma globulins, dietary manipulation, RNA, methylcholanthrene, colloidal sulfur, experimental tumors and gamma irradiation. However, the most popular agent remains casein. The administration of nitrogen mustard or other cytotoxic agents has been alleged by some to accelerate amyloidogenesis. Deposits usually appear first in the perifollicular areas of the spleen, then in Disse's space in the liver and the mesangial region of the kidney. Later, widespread deposition occurs in many sites including the blood vessels. The histologic and EM appearance of experimentally induced amyloidosis in animals is fully comparable to the human counterpart.

It has been recognized for decades that horses given repeated toxoid injections to produce antiserum often develop amyloidosis. This fact together with the association of amyloidosis with chronic infection or inflammation suggested to many workers that hyperactivity or "exhaustion" of the immunologic apparatus led to the disorder. Some investigators have proposed that impairment of cellular immunity may be important in pathogenesis. Thus rabbits thymectomized as adults sometimes developed amyloidosis. If these animals were also irradiated, the frequency of amyloidosis increased; positive Coombs tests and a wasting

syndrome were also observed. Cohen and co-workers have postulated that tolerance to a specific immunogen may be crucial to the development of amyloidosis. Alterations in tolerance have also been invoked to explain the finding that parabiosis between animals differing at weak histocompatibility loci results in amyloidosis whereas parabiosis between syngeneic animals does not (Walford).

It should be noted that amyloidosis is not seen in mice with immunoglobulin-secreting plasmacytomas although it has been reported in some animals with atypical plasma cell leukemias. Amyloid has also been observed in the lupus-like disease of NZB mice.

12. Sorenson GD, Heefner WA, Kirkpatrick JB: Experimental amyloidosis. Methods and Achievements in Experimental Pathology, Vol I (Bajusz E, Jasmin G, eds), Basel, Karger, 1966, p. 514.

13. Walford RL: The Immunologic Theory of Aging. Baltimore, Williams & Wilkins, 1969, p. 84.

14. Cohen AS, Shirahama T: Animal model: Spontaneous and induced amyloidosis. Am J Pathol 68:441, 1972.

15. Willerson JT, Gordon JK, Talal N, Barth WF: Murine amyloid. II. Transfer of an amyloid-accelerating substance. Arth Rheum 12:232, 1969.

16. Barth WF, Willerson JT, Asofsky R, Sheagren JN, Wolff SM: Experimental murine amyloid. III. Amyloidosis induced with endotoxins. Arth Rheum 12:615, 1969.

17. Hardt F: Acceleration of casein-induced amyloidosis in mice by immunosuppressive agents. Acta path microbiol scand, Section A, 79:61, 1971.

18. Hardt F, Ranlov P: Amyloidogenesis. Israel J Med Sci 9:857, 1973.

19. Kellum MJ, Sutherland DER, Eckert E, Peterson RDA, Good RA: Wasting disease, Coombs-positivity, and amyloidosis in rabbits subjected to central lymphoid tissue extirpation and irradiation. Int Arch Allergy 27:6, 1965.

20. Cathcart ES, Mullarkey M, Cohen AS: Amyloidosis: An expression of immunological tolerance? Lancet 2:639, 1970.

21. Potter M: Immunoglobulin-producing tumors and myeloma proteins of mice. Physiol Rev 52:631, 1972.

Also see refs 1 and 3.

III. Structure

A. Morphologic Properties

Amyloid appears as an amorphous, eosinophilic, hyaline, extracellular substance in routine (H & E) tissue sections under the light microscope. It displays metachromatic properties when stained with crystal violet, methyl violet or Toluidine Blue and fluoresces after staining with thioflavine T or S. When tissue sections stained with Congo red are viewed under the polarizing microscope, a unique green birefringence is seen (see Table I).

TABLE I. Summary of Histological Techniques in the Study of Amyloid (from ref 22)

	Appearance of Amyloid	Advantages of Technique	Limitations of Technique
A. Stains			
1. Hematoxylin and eosin	Amorphous, pink, refractile	Good definition of general histology; presumptive evidence of amyloid in large deposits	Not specific; not useful in locating minimal deposits
2. Congo red	Pink	Few other substances stain with this dye	Poor contrast; not completely specific
3. Crystal or methyl violet	Metachromatic	Distinctive appearance of amyloid; valuable in locating small deposits; distinguishes amyloid from most other extracellular deposits	Variability of different batches of the dye; rapid deterioration of slides
4. van Gieson	Yellow-orange or khaki	Clearly differentiates amyloid from collagen	Only of suggestive value in screening; not specific
5. Periodic acid–Schiff	Pale violet	Of possible theoretical interest	Unhelpful differential diagnosis
B. Optical Techniques			
1. Fluorescence in u.v. light			
(*a*) Unstained	Weakly autofluorescent	None	Nonspecific; technically not suitable for routine use
(*b*) Stained with Congo red	Pink fluorescence	Very sensitive for small deposits of amyloid	Get false positives unless Congo red staining perfect; unsuitable for routine use
(*c*) Thioflavine T	Yellow fluorescence	Very sensitive	Need for fluorescent microscope; probably not specific
2. Polarization microscopy			
(*a*) Unstained	Weakly birefringent (weak pale green hue)	None	Difficult to read and interpret
(*b*) Congo red stain	Birefringent (green)	Possibly specific; very sensitive	Need for polarizing microscope

22. Cohen AS: The diagnosis of amyloidosis, chap II. Laboratory Diagnostic Procedures in the Rheumatic Diseases (Cohen AS, ed), Boston, Little, Brown & Co, 1967.

Ultrastructural studies have disclosed that all amyloid tissue deposits consist of discrete fibrils. These fibrils are 70-100 Å wide, rigid, non-branching and of indeterminate length. High-resolution electron microscopy has demonstrated that each 70 to 100 Å filament is made up of subunits (protofibrils) which are approximately 35 Å in diameter. The characteristic fibrillar component accounts for more than 95% of amyloid and is apparently unique to this material. Isolated fibrils possess all the classic tinctorial properties of tissue amyloid including staining with Congo red, green birefringence on polarization microscopy after such staining, and crystal violet metachromasia. X-ray diffraction studies have revealed that the fibrils consist of polypeptide chains arranged in an antiparallel, β-pleated sheet conformation. These physical properties are common to all types of amyloid.

In 1965, another structure, the pentagonal unit or plasma component (P component) was recognized in partially purified preparations though it has not been identified in tissues. The P component is ≅ 90 Å in diameter and appears as rod-like or doughnut-shaped structures under the electron microscope. It does not stain with Congo red or display crystal violet metachromasia and it is antigenically distinct from the fibril. This minor component apparently represents a normal serum α_1-globulin and is largely removed during most fractionation procedures. Its role in the pathogenesis of amyloidosis is unknown.

23. Cohen AS, Calkins E: Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature 183:1202, 1959.

24. Bladen HA, Nylen MU, Glenner GG: The ultrastructure of human amyloid as revealed by the negative staining technique. J Ultrastruct Res 14:449, 1966.

25. Shirahama T, Cohen AS: High-resolution electron microscopic analysis of the amyloid fibril. J Cell Biol 33:679, 1967.

26. Cathcart ES, Comerford FR, Cohen AS: Immunologic studies on a protein extracted from human secondary amyloid. New Eng J Med 273:143, 1965.

27. Glenner GG, Bladen HA: Purification and reconstitution of the periodic fibril and unit structure of human amyloid. Science 154:271, 1966.

28. Pras M, Schubert M, Zucker-Franklin D, Rimon A, Franklin EC: The characterization of soluble amyloid prepared in water. J Clin Invest 47:924, 1968.

29. Eanes ED, Glenner GG: X-ray diffraction studies on amyloid filaments. J Histochem Cytochem 16:673, 1968.

30. Cohen AS, Shirahama T: Electron microscopic analysis of isolated amyloid fibrils from patients with primary, secondary and myeloma-associated disease. Israel J Med Sci 9:849, 1973.

31. Franklin EC, Zucker-Franklin D: Current concepts of amyloid. Adv Immunol 15:249, 1972.

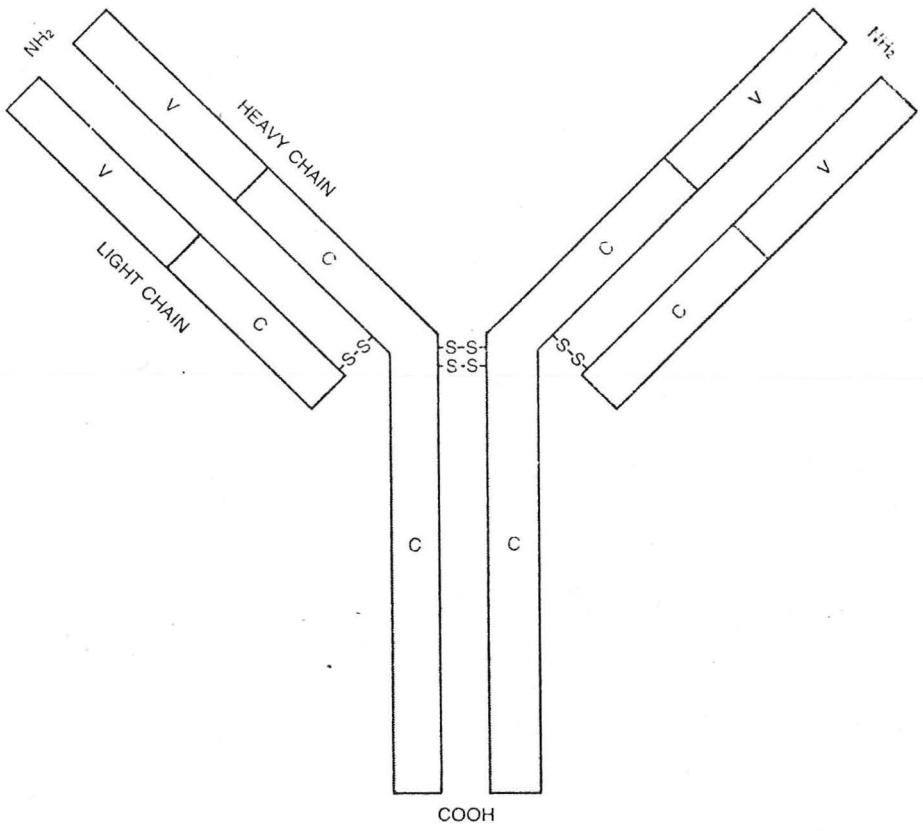
B. Chemical Characterization of Amyloid Fibrils

1. Immunoglobulin Derived

Early immunofluorescent studies of amyloid deposits disclosed the presence of γ-globulin (Vasquez & Dixon). Osserman et al (1964) studied 27 patients with amyloidosis and concluded that almost all had evidence of an underlying plasma cell dyscrasia. Moreover, immunofluorescence data supported the thesis that Bence Jones proteins (BJP)(monoclonal immunoglobulin light chains) might be directly responsible for the production of tissue amyloid. Others were unable to confirm these findings and the issue was hotly debated in the literature. Thus the Boston group found no immunochemical evidence of whole immunoglobulins, heavy chains or light chains in solubilized amyloid fibrils.

With the advent of methods for denaturing and degrading the fibrils, they became susceptible to analysis by the classic techniques of protein chemistry: peptide mapping, amino acid compositional data and primary sequence determination. The results have disclosed that certain amyloid proteins are derived from immunoglobulin light chains while others originate from non-immunoglobulin sources.

Immunoglobulin light (L) polypeptide chains have a molecular weight of $\cong$ 22,500 daltons and are composed of approximately 214 amino acid residues. The amino-terminal half, the first 107 amino acids, is characterized by variance in amino acid sequence; this half is referred to as the variable half (V_L). The remaining 107 amino acids, the carboxyl-terminal half, are characterized by constancy in amino acid sequence within a class (κ or λ); this portion is referred to as the common or constant half (C_L). (See Figure 1.)



LINEAR STRUCTURE of an antibody molecule is shown schematically. The two heavy chains and two light ones are connected by disulfide bridges. Each chain has an amino end (NH_2) and a carboxyl end (COOH). Chains are divided into variable (V) regions in which the amino acid sequence varies in different antibodies, and constant (C) regions.

Fig. 1
From: Jerne NK: The immune system. Sci Amer, July, 1973.

The strongest support for the immunoglobulin origin of some amyloid fibrils comes from determination of their amino acid sequence. In 1971, Glenner et al reported that the major component of two amyloid fibril proteins was homologous to the variable-region sequence of a kappa-type BJP (Table 2). The minor differences were no greater than those seen between any two BJP. These results demonstrated conclusively that the major protein constituent of these 2 amyloid fibril preparations was derived from the amino-terminal (variable) portions of homogeneous kappa L chains.

TABLE 2 **Sequence Analyses of Amyloid Protein X and VIII-b as Compared to the Sequence of the Prototype $V_{\kappa I}$, Ker. (Variant residues are in bold type; undetermined or equivocal residues are indicated by brackets.)**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ker	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
Amyloid X	Asp	Ile	Gln	Met	Thr	Gln	Ser	**Ala**	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
Amyloid VIII	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Ker	Val	Thr	Ile	Thr	Cys	Gln	Ala	Ser	Gln	Asp	Ile	Lys						
Amyloid X	Val	**Ile**	Ile	[ ]	Cys	Glx	Ala	[ ]	Glx	Asx	Ile	[ ]	Pro	Tyr	Leu	[ ]	[ ]	Tyr
Amyloid VIII	Val	Thr	Ile	Thr	Cys	Gln	Ala	Ser	Gln	Asx	Ile	**Gly**	[ ]	Tyr	Leu	[ ]	Trp	

From ref 35.

Further studies have confirmed the light chain origin of amyloid, particularly in patients with the "primary" type (Table 3).

Table 3. Amyloid Fibril Proteins of Immunoglobulin Origin (AIO)

Amyloid Protein	Subgroup Classification (Ref. 66)	Major Protein(s)*	Clinical Classification	Ref.
Cos (III)	$V_{\lambda I}$	V_L + Lc	Primary (65)	74
Spe (VI)	$V_{\lambda II}$ or IV	V_L	Primary (65)	74
Art (VIII)	$V_{\kappa I}$	V_L + Lc	Secondary (65)	62
Ale (X)	$V_{\kappa I}$	V_L	Primary (65)	62
Cra (XIV)	$V_{\lambda II}$ or IV	V_L	Primary (65)	74
Lua (L)	$V_{\lambda I}$	V_L	Isolated (43)	97
Tew	$V_{\kappa II}$	Lc	Plasma cell dyscrasia (93)	131

*Predominant amino-terminal variable fragment and whole light chain are designated V_L and Lc, respectively. From ref 45.

Of particular interest is the Tew protein which was isolated from the intestine of a patient with a history of chronic colitis who subsequently developed primary systemic amyloidosis and a plasma cell dyscrasia (κ Bence Jones proteinuria). Osserman et al had previously shown that fluorescein-labeled Tew BJP had an unusual binding affinity for small intestine which could be specifically blocked by pretreatment with the unlabeled BJP. It was therefore postulated that the Tew BJP might have been part of an anti-intestine antibody which was produced in great excess after long-term intestinal inflammatory disease. Terry et al have shown that the amyloid fibril protein from this patient was identical to his intact urinary BJP. Thus in some circumstances, complete monoclonal L chains may be directly incorporated into amyloid tissue deposits.

Glenner et al also were the first to show that some, but not all, BJP obtained from patients without amyloidosis can be made to form amyloid fibrils in vitro. BJP can be cleaved into constant and variable halves by

enzymatic methods. With some proteins a precipitate forms which has the typical Congo red birefringence, EM fibrillar appearance and X-ray diffraction pattern characteristic of all amyloid fibrils. Sequence analysis of one such protein showed that the fragment (mw 4600 daltons) was derived from the amino-terminal V region of the BJP. Cohen, Franklin and their co-workers have confirmed these findings. Thus V-region sequences of certain BJP ($\cong$ 15% of nearly 100 proteins) can become amyloid fibrils under near-physiologic conditions, i.e., they have V-region sequences which are "amyloidogenic". The preponderance of λ-type BJP in patients with amyloidosis (unlike the L-chain ratios in normal immunoglobulins and in myeloma without amyloidosis) suggests that such specific amyloidogenic V-regions occur more commonly in λ chains than in κ chains. In this regard it is noteworthy that λ BJP usually exist as dimers while κ BJP exist as monomers and dimers. In addition, λ chains have a β-conformation at body temperature whereas the κ-type acquire it before precipitation during the classic thermal reaction. These properties may account for the propensity of λ BJP to form amyloid.

The above studies also provide a reasonable explanation for the discrepancies noted in the earlier immunochemical studies on the nature of amyloid. Since most amyloid proteins of immunoglobulin origin are composed of V_L fragments, it is not surprising that many previous studies using anti-immunoglobulin or anti-L chain sera were negative. Most antisera to BJP react with the constant region (C_L) or to unique (idiotypic) determinants of the variable region thereby reducing markedly the possibility of an antiserum to L chains or whole immunoglobulins reacting with the amyloid fibrils of any one patient. Although native fibrils are only weakly immunogenic, antisera can be made to the denatured fibrils which cross-react with other structurally-related amyloid proteins and also with many BJP of the appropriate class.

To summarize, amyloid fibrils of immunoglobulin origin are demonstrable in patients with plasma cell dyscrasia and usually, but not exclusively, in those with the primary (idiopathic) type of the disorder. In these instances, the major protein of the amyloid fibril has been shown to be a monoclonal light polypeptide chain (BJP) and/or its amino-terminal (V_L) fragment. Amino acid sequence analyses and immunochemical studies have revealed that these amyloid fibril proteins may be classified as either κ or λ type based on their chemical homology and/or sharing of antigenic determinants with kappa or lambda Bence Jones proteins.

32. Vasquez JJ, Dixon FJ: Immunohistochemical analysis of amyloid by the fluorescent technique. J Exp Med 104:727, 1956.

33. Osserman EF, Takatsuki K, Talal N: The pathogenesis of "amyloidosis". Sem Hematol 1:3, 1964.

34. Cathcart ES, Cohen AS: Relation between isolated human amyloid fibrils and human γ-globulin and its subunits. J Immunol 96:239, 1966.

35. Glenner GG, Terry W, Haroda M, Isersky C, Page D: Amyloid fibril proteins: Proof of homology with immunoglobulin light chains by sequence analyses. Science 172:1150, 1971.

36. Terry WD, Page DL, Kimura S, Isobe T, Osserman EF, Glenner GG: Structural identity of Bence Jones and amyloid fibril proteins in a patient with plasma cell dyscrasia and amyloidosis. J Clin Invest 52:1276, 1973.

37. Glenner GG, Ein D, Eanes ED, Bladen HA, Terry W, Page DL: Creation of "amyloid" fibrils from Bence Jones proteins in vitro. Science 174:712, 1971.

38. Shirahama T, Benson MD, Cohen AS, Tanaka A: Fibrillar assemblage of variable segments of immunoglobulin light chains: An electron microscopic study. J Immunol 110:21, 1973.

39. Linke RP, Zucker-Franklin D, Franklin EC: Morphologic, chemical, and immunologic studies of amyloid-like fibrils formed from Bence Jones proteins by proteolysis. J Immunol 111:10, 1973.

40. Linke RP, Tischendorf FW, Zucker-Franklin, Franklin EC: The formation of amyloid-like fibrils in vitro from Bence Jones proteins of the Vλ1 subclass. J Immunol 111:24, 1973.

41. Pollet R, Rossi G, Edelhoch H: Fluorescence and circular dichroism of Bence Jones proteins at high temperature. J Biol Chem 247:5921, 1972.

42. Editorial: Immunoglobulin origin of amyloid. Lancet 1:1046, 1973.

43. Isersky C, Ein D, Page DL, Harada M, Glenner GG: Immunochemical cross-reactions of human amyloid proteins with human immunoglobulin light polypeptide chains. J Immunol 108: 486, 1972.

44. Glenner GG, Ein D, Terry WD: The immunoglobulin origin of amyloid. Am J Med 52:141, 1972.

45. Glenner GG, Terry WD, Isersky C: Amyloidosis: Its nature and pathogenesis. Sem Hematol 10:65, 1973.

46. Glenner GG: Immunoglobulin and amyloid fibril proteins. Brit J Hematol 24:533, 1973.

47. Cohen AS, Cathcart ES: Amyloidosis and immunoglobulins. Adv Int Med 19:41, 1974.

48. Glenner GG, Terry WD: Characterization of amyloid. Ann Rev Med 25:131, 1974.

2. Non-immunoglobulin Derived

Benditt and Ericksen carried out extensive studies on secondary amyloid deposits isolated from liver of a human and a monkey with tuberculosis. These investigators were able to isolate a component (amyloid A) with a molecular weight of 6000-8000 daltons. The proteins

had a characteristic amino acid composition and a unique sequence. Moreover, both displayed the ability to bind Congo red. Sequence data on the N-terminal 24 amino acids indicated that amyloid A was a completely new protein distinct from immunoglobulins and with a sequence unlike any other peptide previously reported. Other patients with secondary amyloid (associated with juvenile RA, ulcerative colitis, bronchiectasis or rheumatoid arthritis) also were found to have the unique protein A.

These findings have been confirmed in several other laboratories. Levin et al have characterized an amyloid fibril protein (ASF) obtained from patients with secondary amyloidosis (TB, bronchiectasis and Hodgkin's disease) and one type of familial amyloidosis, i.e., familial Mediterranean fever (FMF), which corresponds to protein A of Benditt and Ericksen. The protein from the FMF patient had a molecular weight of 8500 daltons. Husby and co-workers reported a nearly-identical protein (AS), mw 9145 daltons, obtained from the liver of a patient with secondary amyloidosis and rheumatoid arthritis. Glenner et al have also reported the same protein. The subtle differences in amino acid composition and size of the individual proteins reported implies that, as with most amyloid fibril proteins of immunoglobulin origin, these non-immunoglobulin amyloid proteins are derived from a larger precursor by proteolytic digestion. The nature of the protein of origin is, at present, unknown.

Table 4. Amyloid Fibril Protein of Unknown Origin (AUO)

Amyloid Protein	N-Terminal Sequence of Major Protein*	Clinical Classification	Ref.
Med (IV)	Arg-Ser-Phe-Phe-Ser-	Secondary (65)	40
Paa	Ser-Phe-Phe-Ser-	Primary (31)	52
Pab	Ser-Phe-Phe-Ser-	Secondary (31)	52
A-4	Arg-Ser-Phe-Phe-Ser-	Secondary (12)	14
Hodgkin's	Ser-Phe-Phe-Ser-	Secondary (48)	48
F.M.F.	Ser-Phe-Phe-Ser-	Heredo-familial (48)	48

*Sequences are aligned to afford maximal homology.

From ref 45. Protein AS is not included in the table but has an N-terminal sequence identical to proteins Med (IV) and A-4 (ref 51).

Levin et al have demonstrated that an antiserum to ASF cross-reacts with a human serum component (slightly larger than albumin) which has alpha-1 electrophoretic mobility. Seven per cent of normal sera and 50-60% of patients with certain chronic diseases, some of which are associated with amyloidosis, had elevated levels of this component.

Similarly, Husby and Natvig have described a serum component having mw $\cong$ 100,000 daltons and electrophoretic mobility in the α-β region which is antigenically related to amyloid protein AS. This protein (ASC) was detected in only 3% of normal sera but in 48 of 55 sera from patients with

various clinical types of amyloidosis. Protein ASC is structurally and antigenically distinct from the periodic rods (P component) seen in amyloid preparations. Protein ASC was also detected with increased frequency in sera of patients with diseases known to be complicated by amyloidosis. Moreover, it was detectable in 50% of aged but otherwise normal subjects and in a similar proportion of individuals with hypogammaglobulinemia. Interestingly in some instances, ASC was identified in sera of patients 2-3 years before the diagnosis of amyloidosis was established.

In summary, the major constituent of amyloid fibrils isolated from patients with secondary amyloidosis and FMF is a protein with mw ≅ 8000-9000 daltons which is unrelated to any known immunoglobulin chain or fragment. Its origin is obscure but it appears to be related to a minor component present in normal serum which rises in conditions known to predispose to amyloidosis and which may represent a circulating precursor of tissue amyloid.

49. Benditt EP, Ericksen N: Chemical classes of amyloid substance. Am J Pathol 65:231, 1971.

50. Levin M, Franklin EC, Frangione B, Pras M: The amino acid sequence of a major nonimmunoglobulin component of some amyloid fibrils. J Clin Invest 51:2773, 1972.

51. Sletten K, Husby G: The complete amino-acid sequence of non-immunoglobulin amyloid fibril protein AS in rheumatoid arthritis. Eur J Biochem 41:117, 1974.

52. Levin M, Pras M, Franklin EC: Immunologic studies of the major nonimmunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum component. J Exp Med 138:373, 1973.

53. Husby G, Natvig JB: A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. J Clin Invest 53:1054, 1974.

Also see refs 44-48 (reviews).

Although the correlation of L-chain derived amyloid with cases of primary and plasma cell dyscrasia-associated amyloidosis is striking, it is not invariable. Thus immunoglobulin-derived amyloid has been described in a case of apparent secondary disease and non-immunoglobulin-derived fibrils have been reported in a case of nonfamilial primary amyloidosis (see Tables 3 and 4). Small amounts of an immunoglobulin-like protein (? polyclonal) were detected by Franklin's group in their FMF and secondary cases. In addition, a single report of immunologically detectable amounts of the non-immunoglobulin protein AS in amyloid associated with plasma cell dyscrasia (IgG and IgM) has appeared.

Finally, a third class of amyloid protein has been reported recently. This component was isolated from the spleen of a patient with generalized primary amyloidosis who had no evidence of BJP or other M-component. Initial characterization of this protein suggests that it differs from any other known amyloid protein and also from immunoglobulin fragments. It seems likely that still other classes of amyloid will be identified in the future. Indeed, preliminary studies suggest that polymers of insulin and glucagon form β-pleated sheet fibrils having the morphologic characteristics of amyloid.

54. Husby G, Sletten K, Michaelsen TE, Natvig JB: Amyloid fibril protein subunit, "protein AS": Distribution in tissue and serum in different clinical types of amyloidosis including that associated with myelomatosis and Waldenstrom's macroglobulinemia. Scand J Immunol 2:395, 1973.

55. Husby G, Natvig JB, Sletten K: New, third class of amyloid fibril protein. J Exp Med 139:773, 1974.

56. Glenner GG, Eanes ED, Termine JD, Bladen HA, Linke RP: The structural characteristics of some proteins having the properties of Congo red-stained amyloid fibrils. J Histochem Cytochem 21: 406, 1973.

The principal features of human amyloid fibrils are listed in Table 5.

Table 5. CHARACTERIZATION OF HUMAN AMYLOID FIBRILS

I. PHYSICAL PROPERTIES: common to all forms of amyloid

A. Polarized light: green birefringence with Congo red
B. EM appearance: 70-100 Å nonbranching fibrils
C. X-ray diffraction pattern: β-pleated sheet conformation

II. CHEMICAL STUDIES: at least 2 distinct types of amyloid proteins

A. Intact monoclonal immunoglobulin light chains &/or fragments from N-terminal (variable) region

1) patients with nonfamilial primary systemic or plasma cell dyscrasia-associated amyloidosis

2) about 15% of Bence Jones proteins apparently "amyloidogenic" ($\lambda > \kappa$)

B. Protein A - also known as AS or ASF (not derived from L chains)

1) patients with secondary amyloidosis (rheumatoid arthritis, bronchiectasis, tuberculosis, Hodgkin's) and familial Mediterranean fever

2) mol wgt ≅ 9000 daltons

3) no relationship to any known immunoglobulin fragment

4) origin obscure but appears related to minor component in normal serum

C. Additional types of amyloid proteins likely

IV. Diagnosis

Dye extraction tests with Congo red or Evans blue have proved unsatisfactory. A high incidence of false negatives occurs with both agents and anaphylactic reactions have been reported.

Definitive diagnosis of amyloidosis requires adequate examination of involved tissue. A variety of biopsy sites have been utilized including skin, rectum, gingiva, tongue, liver, kidney, spleen, respiratory tract and small intestine. We have made the diagnosis from connective tissue removed at surgery for relief of the carpal tunnel syndrome and also from lymph node biopsy. The clinician should bear in mind the potential hazard of bleeding from friable amyloid tissue and the obvious fact that a negative biopsy does not rule out the diagnosis.

Rectal biopsy is the procedure of choice whenever amyloidosis is suspected. The diagnosis can be established in 75-85% of cases and morbidity is minimal. Amyloid deposits are most frequently observed in blood vessels, submucosa and muscularis mucosae. The key to correct diagnosis is appropriate staining and examination of tissue sections. All tissue should be stained with Congo red and examined for green birefringence under polarized light. If possible, electron microscopy should also be done.

Needle biopsy of subcutaneous abdominal fat has been recently advocated as an accurate method for diagnosis of secondary amyloidosis.

57. Blum A, Sohar E: The diagnosis of amyloidosis. Lancet 1:721, 1962.

58. Kyle RA, Spencer RJ, Dahlin DC: Value of rectal biopsy in the diagnosis of primary systemic amyloidosis. Am J Med Sci 251:501, 1966.

59. Westermark P, Stenkvist B: A new method for the diagnosis of systemic amyloidosis. Arch Int Med 132:522, 1973.

Also see ref 22.

V. Clinical Amyloidosis

A. Classification

There is no satisfactory classification of amyloidosis. Allocation of patients into primary, secondary, myeloma-associated, hereditary and localized categories has been largely on empirical grounds. Attempts at histologic distinction between primary and secondary types according to site of early amyloid deposition (perireticulin vs. pericollagenous) or tinctorial properties are not valid. Classification based on differences in organ distribution of amyloid has also been utilized. Thus secondary or "typical" amyloid usually involves liver, spleen, kidneys and adrenals. By contrast, the primary and myeloma-associated ("atypical") types tend to involve skin, tongue, G-I tract, heart, ligaments and peripheral nerves. However, considerable overlap occurs and such a scheme has limited clinical value. For example, renal disease is a major factor in most patients regardless of classification. Similarly, cardiac, G-I and skin involvement occurs frequently in subjects with the secondary type. It should be noted, however, that macroglossia and/or carpal tunnel syndrome generally indicate the primary or myeloma-associated variety.

Primary and secondary have also been employed to denote absence or presence of predisposing chronic disease (infection or inflammation), respectively. If this feature is used as the distinguishing criterion, most current cases will be labeled "primary". Clearly, the principal justification for retaining primary and secondary types according to chronic disease association is the recent chemical data on the nature of amyloid fibrils (see Section III). Moreover, there now seems little reason to

maintain a separate myeloma-associated group (Section V-B). Table 6 shows a tentative classification based on these considerations. A truly rational scheme awaits further elucidation of the chemical nature of the various kinds of amyloid.

60. Brandt K, Cathcart ES, Cohen AS: A clinical analysis of the course and prognosis of forty-two patients with amyloidosis. Am J Med 44:955, 1968.

Also see refs 1, 3 and 9.

Table 6. CLASSIFICATION OF AMYLOIDOSIS

1. Primary Systemic (nonhereditary)

   A. Immunoglobulin-derived
      i) M-component (usually Bence Jones protein) demonstrable in urine and/or serum
      ii) Overt myeloma may or may not be present

   B. Non-immunoglobulin-derived
      i) No demonstrable Bence Jones protein
      ii) Uncommon

2. Secondary Systemic

   A. Chronic disease association: TB, bronchiectasis, osteomyelitis, rheum. arthritis, leprosy, paraplegia, inflammatory bowel disease, Hodgkin's, renal cell carcinoma, etc.

   B. Most appear to be of non-immunoglobulin origin (no demonstrable Bence Jones protein)

3. Heredofamilial

4. Localized

   A. Amyloid "tumors" - esp. respiratory and urinary tracts

   B. Neoplasms with amyloid stroma

B. The Problem of Primary Systemic Amyloidosis

It has long been recognized that amyloidosis occurs in 10-15% of patients with typical multiple myeloma. As noted, Magnus-Levy and Apitz originally pointed out the association between primary amyloid, marrow plasmacytosis and Bence Jones proteinuria. Later, the relationship between primary amyloid and BJP was re-emphasized by Kyle and Bayrd and by Osserman whose data suggested that nearly all patients with primary amyloidosis had either frank myeloma or an underlying plasma cell dyscrasis (PCD) that would ultimately result in myeloma.

Many other investigators were unable to substantiate these findings (e.g. Barth et al). The magnitude of the conflict is perhaps best illustrated by Cohen's remarks in his extensive 1967 review (ref 1). After recommending that patients with primary systemic amyloidosis have electrophoretic and immunochemical studies on serum and urine performed to search for M-proteins, he stated, "Although these tests will occasionally uncover a patient with early multiple myeloma, it by no means indicates that all patients with amyloidosis have active or inapparent myeloma, as has been suggested" (by Osserman). In discussing amyloid associated with multiple myeloma, he wrote, "Although it has been somewhat attractive in the past to associate amyloidosis with the patients whose urine contained Bence Jones protein, and to speculate that the protein constituted part of the amyloid, this hypothesis has clearly become untenable in recent years".

61. Kyle RA, Bayrd ED: "Primary" systemic amyloidosis and myeloma. Discussion of relationship and review of 81 cases. Arch Int Med 107:344, 1961.

62. Barth WF, Willerson JT, Waldmann TA, Decker JL: Primary amyloidosis. Clinical, immunochemical and immunoglobulin metabolism studies in fifteen patients. Am J Med 47:259, 1969.

Also see refs 1, 6-8 and 33.

For the past few years, we have been interested in patients with "light chain disease" (LCD), i.e. patients whose only detectable monoclonal protein abnormality consists of excess production of either κ or λ light chains (Bence Jones protein). The diagnosis of myeloma in such individuals was based on the demonstration of the M-protein in urine and/or serum plus at least one of the following additional findings: marrow plasmacytosis in sheets or clusters $>$ 10 cells, lytic bone lesions, or Bence Jones proteinuria in excess of 300 mg/day. Our criteria for the diagnosis of myeloma are similar to those of others; all of 35 patients with LCD whom we studied met these criteria. Systemic amyloid was identified in 7 of the patients; their initial serum and urine protein findings are shown in Figure 2.

63. Stone MJ, Frenkel EP: The clinical spectrum of light chain myeloma. A study of thirty-five patients with special reference to the occurrence of amyloidosis. Am J Med, in press.

SERUM AND URINE PROTEIN STUDIES

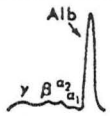
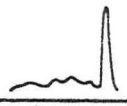
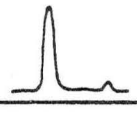
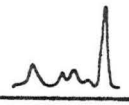
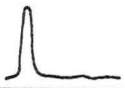
	SERUM							URINE			
	SPE	ALB gm/100ml	IgG ← mg/ml →	IgA	IgM	IgD	Bence Jones Proteinemia	UPE	Dipstix	TSA	mg/24hr
NORMAL		3.8-5.2	7.5-15.0	0.8-2.2	0.7-2.0	0-0.3	0		Neg	Neg	<200
CASE 2		2.5	3.2	0.5	<0.2	0	+	Not done	3+	---	oliguric
11		4.2	5.5	0.3	0.3	0	+		2+	Pos	985
20		3.7	5.2	0.2	0.3	0	+		Neg	Pos	4500
24		2.7	9.4	0.4	1.0	0	+		4+	Pos	2540
25		2.7	3.7	<0.2	<0.2	0	+		3+	Pos	8160
27		2.2	3.6	<0.2	<0.2	0	0		2+	Pos	5150
29		2.9	4.5	0.5	0.3	0	+		2+	Pos	1440

Fig. 2

Patients 2, 11 and 27 had type κ BJP; patients 20, 24, 25 and 29 had type λ BJP. (From ref 63.)

The following case protocols illustrate the clinical presentation and course of our patients with LCD and systemic amyloidosis.

Case 2. This 59 year old woman presented to another hospital with tarry stools. She was found to have anemia, proteinuria and diffuse infiltrates in both lung fields on chest roentgenograms. She was given transfusions and antibiotics. Blood urea nitrogen (BUN) had been 48 mg/100 ml initially but repeat determination on the twenty-first hospital day was 102 mg/100 ml. An intravenous pyelogram (IVP) did not visualize but retrograde pyelograms were normal. The development of oliguria led to her transfer to Parkland Memorial Hospital.

On admission, she was obtunded, edematous and in acute respiratory distress with diffuse rales and dullness at both lung bases. A crepitant 6 cm mass was noted in the region of the right 9th rib posteriorly. The liver was enlarged (overall span 18 cm) but splenomegaly and lymphadenopathy were absent.

Admission laboratory data disclosed hemoglobin 10.4 g/100 ml, Hct 32 per cent, white blood cell count 16,100/mm^3 with a left shift in the differential, and platelet count 260,000/mm^3. The BUN was 83, serum creatinine 13.5 and serum uric acid 12.2 mg/100 ml. Except for a plasma chloride of 83 meq/liter, electrolytes were normal. The arterial pH was 7.45, carbon dioxide tension (pCO_2) 33 mm Hg, oxygen tension (pO_2) 43 mm Hg and oxygen saturation 83 per cent. Serum protein electrophoresis (SPE) showed hypoalbuminemia, increased alpha-2 globulin and no evidence of a monoclonal spike (Figure 2). Urine showed 3+ protein by Dipstix with negative heat test for Bence Jones protein. The stool guaiac was 3+. Chest roentgenograms disclosed bilateral diffuse reticulonodular infiltrates and a lytic lesion of the right posterior 9th rib. Skull films were negative. The bone marrow was hypercellular with 83 per cent pleomorphic plasma cells in sheets. Serum IgG, IgA and IgM levels were markedly decreased (Figure 2); IgD and IgE were undetectable. IEP demonstrated Bence Jones proteinemia, type κ. The patient became anuric and, in spite of peritoneal dialysis, expired.

Autopsy confirmed the diagnosis of multiple myeloma; the 9th rib lesion was due to sheets of plasma cells. The patient also had amyloidosis involving the lungs, gastrointestinal tract, kidneys, liver and spleen. The pulmonary alveolar septa and renal glomeruli were diffusely involved with amyloid. Extensive casts filled the kidney tubules; atrophic tubular epithelium and giant cells were present (myeloma kidney).

Comment: This patient presented with unexplained renal failure and proteinuria. The initial evaluation failed to quantify and characterize this proteinuria. An IVP was performed after which the patient developed oliguric renal failure, an event extensively documented in the past. Myeloma was considered in the differential diagnosis of her renal failure but the absence of a monoclonal serum spike and the negative Bence Jones heat test misled her physicians.

This patient also had generalized amyloidosis. Osteolytic lesions are rare in patients with amyloidosis and may be due to amyloid infiltration of bone or, as in this case, focal plasma cell tumor.

64. Gardner H: Bone lesions in primary systemic amyloidosis. Brit J Radiol 34:778, 1961.

65. Grossman RE, Hensley GT: Bone lesions in primary amyloidosis. Am J Roentgen 101:872, 1967.

66. Gordon DA, Pruzanski W, Ogryzlo MA, Little HA: Amyloid arthritis simulating rheumatoid disease in five patients with multiple myeloma. Am J Med 55:142, 1973.

Case 20. This 59 year old man developed bilateral carpal-tunnel syndrome (surgically relieved), hoarseness, a thick tongue and bilateral submandibular swelling during the year prior to admission.

On physical exam his tongue was indurated and enlarged as were the submandibular glands. The jugular veins were distended and cardiomegaly was present. Scrotal and pretibial edema also was noted. The hemoglobin was 14.2 g/100 ml, hematocrit 42 per cent, white blood cell count 8,200/mm^3, platelets 265,000/mm^3 and ESR 15 mm/hour. Rouleau was absent. Blood chemistries (BUN, creat., calcium and uric acid) were normal. SPE showed hypoalbuminemia and hypogammaglobulinemia (Figure 2). Serum levels of IgG, IgA and IgM were low; IgD and IgE were undetectable. Urine protein was negative by Dipstix but 4+ by SSA; quantitative urine protein excretion was 4.5 g/24 hours. Bence Jones protein was present by both the heat and TSA tests. Urine protein electrophoresis demonstrated a typical M-spike in the fast γ region which accounted for $>$ 90 per cent of the protein present (see Figure 2); IEP showed a marked increase in λ light chains, a small amount of albumin and no evidence of κ determinants. Serum IEP revealed λ Bence Jones proteinemia. Voltage was normal on the electrocardiogram and a bone survey was negative.

Needle biopsy of the tongue showed marked infiltration with amyloid. Bone marrow aspiration demonstrated 75 per cent pleomorphic plasma cells in sheets and clusters.

The patient was begun on intermittent melphalan. His 24 hour urine protein excretion was reduced to 650 mg 10 months later and 260 mg 16 months after institution of therapy, although λ Bence Jones protein remained the major urine protein component. His macroglossia did not change. However, he developed claudication of thigh muscles and expired from refractory congestive heart failure 18 months after diagnosis.

Post mortem examination disclosed a 690 g heart with extensive amyloidosis. Amyloid also was present in the tongue, esophagus, liver, spleen, kidneys and small arterioles of the muscles. Only occasional renal glomeruli were involved with amyloid and there was no evidence of myeloma kidney.

Comment: This patient presented with the classic features of primary systemic amyloidosis: bilateral carpal-tunnel syndrome, macroglossia, and congestive heart failure in the absence of a coexisting chronic disease. His persistent muscle pain on exertion was ischemic in origin due to amyloid infiltration of arterioles with consequent limited capacity for vasodilatation during exercise.

Case 20 is a striking example of the relationship between primary systemic amyloidosis and multiple myeloma. As was true in our other amyloid patients, the initial clinical manifestations and subsequent course were dominated by the sequelae of amyloid deposition. It is noteworthy that this patient did not have anemia, lytic bone lesions or evidence of severe renal functional impairment despite diffuse infiltration of his marrow by plasma cells and profound Bence Jones proteinuria.

67. Zelis R, Mason DT, Barth W: Abnormal peripheral vascular dynamics in systemic amyloidosis. Ann Int Med 70:1167, 1969.

Also see refs 3, 33, 44 and 61.

Case 24. This 60 year old woman presented with a 2 year history of intermittent melena, right upper quadrant pain and a 20 pound weight loss. On examination, she appeared pale and chronically ill. The only other abnormal finding was liver enlargement to 7 cm below the left costal margin.

Laboratory examination revealed a microcytic hypochromic anemia (hemoglobin 7.1 g/100 ml) with anisocytosis, poikilocytosis, target cells and Howell-Jolly bodies. Stool guaiac was positive. Urinalysis showed 4+ protein by Dipstix but was otherwise negative. Liver function chemistries revealed hypoalbuminemia (2.7 g/100 ml) and an elevated alkaline phosphatase (28 King-Armstrong units). Other blood chemistries and coagulation studies were within normal limits. Roentgenograms of the chest, esophagus, stomach, colon and bones were negative; an oral cholecystogram demonstrated cholelithiasis. A ^{99m}Tc-sulfur colloid scan confirmed the presence of marked hepatomegaly; no splenic uptake was seen. Percutaneous needle biopsy of the liver demonstrated extensive infiltration by amyloid. SPE disclosed total protein of 5.8 g/100 ml and an abnormal component in the γ region (Figure 2). Serum levels of IgG and IgM were normal; IgA was reduced. Neither IgD nor IgE was detectable in the serum. IEP demonstrated λ Bence Jones proteinemia. Urine protein excretion was 2.54 g/24 hours. Electrophoresis (Figure 2) and IEP confirmed multiple-component proteinuria. Both light chain classes were present but with altered κ/λ ratio and the λ precipitin line was bowed anodally. Bone marrow examination showed 24 per cent pleomorphic plasma cells, demonstrable amyloid and absence of stainable iron. Amyloid was also seen in small bowel and rectal biopsies. A diagnosis of polymeric λ Bence Jones proteinemia with primary systemic amyloidosis was made. Therapy consisted of melphalan, allopurinol and oral iron. She did well except for the development of orthostatic hypotension which was felt to be due to amyloid dysautonomia. After 4 courses of melphalan, urinary protein excretion was $<$ 500 mg/24 hours and remained so throughout her course; however, Bence Jones proteinemia persisted. She died of massive gastrointestinal bleeding 17 months after diagnosis.

Post mortem examination disclosed extensive amyloid involvement of the liver (3900 g), spleen (320 g), adrenals, kidneys and entire gastrointestinal tract. Amyloid deposits also were present in the small arterioles, heart, lungs, peripheral nerves, pancreas, ovaries, uterus, neurohypophysis and dura mater. Multifocal mucosal hemorrhages were present throughout the gastrointestinal tract.

Comment: This patient presented with abdominal pain, hepatomegaly and anemia secondary to gastrointestinal blood loss. Biopsies of multiple organs demonstrated amyloidosis. There was no history of previous symptomatic chronic suppurative or inflammatory disease. The abnormal component on SPE (Figure 2) was identified as λ Bence Jones protein. Although the urine electrophoretic pattern did not suggest the presence of an M-protein, the intensity and contour of the precipitin arc developed with monospecific anti-λ serum demonstrated that a small amount of the monoclonal light chain was present in the urine. Since the patient was neither oliguric nor azotemic, the finding of marked Bence Jones proteinemia with only a small amount of this protein in the urine was distinctly unusual and suggested that high molecular weight polymers of λ light chains were present which could not be filtered through the glomeruli.

Similar instances of Bence Jones proteinemia with little or no Bence Jones proteinuria have been described. Most Bence Jones proteins exist naturally as dissociable or stable dimers of molecular weight 45,000 daltons; these are filtered by normal glomeruli. Our patient closely resembles the one described by Parr et al.; their patient also had generalized amyloidosis with λ Bence Jones proteinemia and, to a minor extent, proteinuria. In that case, the monoclonal light chain existed as a tetramer in both serum and urine. Presumably, the increased glomerular permeability resulting from renal amyloidosis allowed passage of some of the tetrameric Bence Jones protein into the urine. The size of the lambda polymer in our patient's serum has not been precisely established but preliminary gel filtration studies indicate that it has a molecular weight in excess of 100,000 daltons (i.e., larger than a tetramer) and that the λ light chains are covalently bound to each other.

In view of the relationship between monoclonal light chains and some amyloid fibril proteins, it is intriguing to consider that selective retention of an "amyloidogenic" Bence Jones protein in this patient's serum accelerated the incorporation of light chain fragments into amyloid fibrils. Such speculation suggests the potential therapeutic value of a vigorous plasmapheresis program. High polymeric Bence Jones proteinemia does not appear to be invariably associated with either type λ light chains or amyloidosis. Another of our patients had persistent κ Bence Jones proteinemia in the absence of urine Bence Jones protein and azotemia. Except for his early age of onset, this patient had typical multiple myeloma without clinical or autopsy evidence of amyloidosis. Similarly, the patients with tetrameric Bence Jones proteinemia reported by Grey and Kohler and Caggiano et al. had no evidence of amyloid deposition.

Several other unusual clinical features noted in patients with amyloidosis were present in this patient. Despite marked hepatomegaly due to amyloid infiltration, abnormalities of liver function chemistries were limited to an increased serum alkaline phosphatase and hypoalbuminemia. This

discrepancy between extent of hepatic amyloidosis and derangement of liver function tests has been well documented. Gastrointestinal bleeding, an initial finding in this patient, is common in amyloidosis and occurred in more than half the patients described by Brandt et al. Similarly, the development of severe orthostatic hypotension in our patient appeared due to autonomic impairment by amyloid. The acquired dysautonomia associated with amyloidosis may involve abnormalities in control of gastrointestinal and respiratory as well as circulatory function.

Finally, the triad of abnormal red cell forms with Howell-Jolly bodies on peripheral blood smear, absence of splenic uptake following injection of ^{99m}Tc-sulfur colloid, and splenomegaly established the presence of functional hyposplenism in this patient. Although the occurrence of functional hyposplenism in amyloidosis has not been reported, we have recently seen it in 3 other patients with amyloidosis and plasma cell dyscrasia.

68. Parr DM, Pruzanski W, Scott JG, Mills DM: Primary amyloidosis with plasmacytic dyscrasia and a tetramer of Bence Jones type lambda globulin in the serum and urine. Blood 37:473, 1971.

69. Grey HM, Kohler PF: A case of tetramer Bence Jones proteinemia. Clin Exp Immunol 3:277, 1968.

70. Caggiano V, Dominguez C, Opfell RW, Kochwa S, Wasserman LR: IgG myeloma with closed tetrameric Bence Jones proteinemia. Am J Med 47:978, 1969.

71. Levine RA: Amyloid disease of the liver. Correlation of clinical, functional and morphologic features in forty-seven patients. Am J Med 33:349, 1962.

72. Kyle RA, Kottke BA, Schirger: Orthostatic hypotension as a clue to primary systemic amyloidosis. Circulation 34:883, 1966.

73. Capone R, Amsterdam EA, Mason DT, Zelis R: Systemic amyloidosis, functional coronary insufficiency and autonomic impairment. Ann Int Med 76:599, 1972.

74. French JM, Hall G, Parish DJ, Smith WT: Peripheral and autonomic nerve involvement in primary amyloidosis associated with uncontrollable diarrhea and steatorrhoea. Am J Med 29:277, 1965.

75. Eisele JH, Cross CE, Rausch DC, Kurpershoek CJ, Zelis RF: Abnormal respiratory control in acquired dysautonomia. New Eng J Med 285:366, 1971.

76. Stone MJ: Functional hyposplenism in amyloidosis. Proc 17th Annual Meeting Am Soc Hematology, 1974.

Also see refs 60 and 62.

The 7 subjects with primary amyloidosis differed in several respects from the remaining 28 patients in our study. A presentation with carpal-tunnel syndrome (5 patients), acute renal failure (1 patient), or gastro-intestinal symptoms (1 patient) characterized the patients with amyloid deposition. In addition, macroglossia, congestive heart failure, arthralgias and peripheral neuropathy were seen only in the amyloid group. By contrast, patients without evidence of amyloidosis presented with the more typical symptoms of myeloma, viz., skeletal pain. Although we cannot prove that all patients in this latter group had no tissue amyloid, we failed to obtain evidence for it at autopsy or on various biopsies in 16 of these individuals.

Clinical and laboratory findings in the amyloid and "nonamyloid" patients are compared in Table 7. Age at diagnosis, κ/λ frequency and hemoglobin values were similar in both groups. Although the initial BUN was somewhat lower in amyloid subjects, serum creatinines were identical. Serum uric acid levels and degree of marrow plasmacytosis also were similar in both groups. Serum IgG levels tended to be lower and daily proteinuric rates higher in patients with amyloidosis. Albuminuria paralleled the severity of glomerular involvement with amyloid. In the absence of extensive glomerular amyloid deposition, the predominant urine protein component consisted of Bence Jones protein (Cases 20, 25, 27 and 29)(Fig. 2).

Three major differences between the amyloid patients and the other light chain myeloma subjects were apparent (Table 7). First, the serum albumin levels were significantly lower in the amyloid group. Second, lytic bone lesions were rare in amyloid patients but present in 75 per cent of the others. This difference in frequency of skeletal lesions appeared to explain the higher serum calcium values observed in the "non-amyloid" group. Finally, median survival after diagnosis was 7 months in amyloid patients and 19 months in the "non-amyloid" group.

Table 7. COMPARISON OF CLINICAL AND LABORATORY FEATURES IN AMYLOID AND "NON-AMYLOID" PATIENTS

Parameter	Amyloid	"Non-Amyloid"	
No. patients	7	28	
Light chain class	3 κ, 4 λ	13 κ, 15 λ	
Mean age (yr.)	56	60	
Mean hemoglobin (g/100 ml)	11.7	11.4	
Mean BUN (mg/100 ml)	28	40	
Mean serum creatinine (mg/100 ml)	3.3	3.3	
Mean serum calcium (mg/100 ml)	9.7	11.1	
Mean serum uric acid (mg/100 ml)	8.1	8.4	
Mean serum albumin (g/100 ml)	3.0	3.9	$p < 0.01$
Mean serum IgG (mg/ml)	5.0	6.8	
Bence Jones proteinemia	6/7	22/28	
Lytic bone lesions (%)	1/7	21/28	$p < 0.025$
Marrow plasma cells (%)	50	54	
Mean 24 hr. proteinuria (g)	3.80	3.05	
Median survival (mo.)	7	19	$p < 0.001$

(From Ref. 63)

The recent insights into the chemical nature of amyloid fibrils have clinical implications which are pertinent to our study. The amyloid patients presented with complaints directly relating to tissue deposition of amyloid, whereas the other LCD patients exhibited the usual symptoms and signs of myeloma. Yet the monoclonal light chain abnormality and degree of evident plasma cell proliferation were similar in both groups. These considerations emphasize that the distinction between "primary amyloidosis associated with plasmacytic dyscrasia" and "myeloma-associated amyloidosis" is vague, particularly when the M-protein disturbance involves overproduction of Bence Jones protein exclusively. The experimental studies relating monoclonal light chains and amyloid (see Section III-B) may explain the rather high incidence (20 per cent) of amyloidosis which we observed. Amyloid has been identified only rarely in the remainder of our myeloma population, a finding similar to that of Hobbs. Thus accurate appraisal of the incidence of amyloidosis in multiple myeloma is largely dependent on the LCD subgroup. The data are consistent with the hypothesis that LCD patients who synthesize and secrete Bence Jones proteins possessing "amyloidogenic" properties tend to have an illness dominated by the features of primary amyloidosis instead of the usual manifestations noted in other myeloma patients. The resulting clinical picture would be, therefore, more dependent on the molecular structure of the individual Bence Jones protein produced than on any intrinsic difference between primary amyloidosis and multiple myeloma. Such a hypothesis does not dictate that every patient producing "amyloidogenic" light chains need necessarily develop clinical amyloidosis; some clearly do not suggesting that additional factors play a role in the tissue deposition of amyloid fibrils.

Skeletal destruction was rare in the amyloid group. In fact only 1 of 7 patients had an osteolytic lesion (Case 2). This rarity may be due to an earlier presentation of patients because of symptoms referable to the amyloid deposition. Cell kinetic data in IgG myeloma support such a concept since patients with more than 2×10^{12} "myeloma" cells generally have osteolytic lesions whereas such lesions are usually absent when the tumor cell burden is less than 1×10^{12} cells. This hypothesis has not been tested by cell kinetic studies in LCD.

77. Hobbs JR: Immunochemical classes of myelomatosis. Brit J Haematol 16:599, 1969.

78. Salmon SE: Immunoglobulin synthesis and tumor kinetics of multiple myeloma. Sem Hematol 10:135, 1973.

Also see refs 31, 37, 44 and 46.

In all we have studied 20 patients with biopsy-proved amyloidosis during the past 5 years. Sixteen had the primary systemic type; the remaining 4 had only localized involvement. As shown in Table 8, 14 of the 16 subjects with generalized amyloidosis had identifiable M-components in serum and/or urine. Bence Jones protein alone accounted for the monoclonal protein abnormality in 10 patients (3 κ, 7 λ).

Table 8. AMYLOIDOSIS - 20 PATIENTS
11/69 - 9/74

	No.	
A. WITH associated plasma cell dyscrasia (all had systemic involvement)	14	
Light chains (Bence Jones protein) only	10	(3κ,7λ)
IgG, κ	1	
IgA, κ	1	
IgA, λ	1	
IgM, λ	1	
B. WITHOUT associated plasma cell dyscrasia	6	
Localized	4	
Systemic	2	

Much more extensive data has recently been published. Isobe and Osserman documented M-proteins in 88 of 100 patients with primary (Pattern I) and secondary (Pattern II) amyloidosis (see Table 9). These included 50/50 in primary cases, 9/17 in secondary cases and 26/30 in mixed cases. 36/50 primary cases had BJP only and 10 others had BJP associated with IgG or IgA serum spikes. Therefore 92% of the primary cases were characterized by the production of free monoclonal L chains. The relatively higher frequency of type λ proteins is evident from the table (κ/λ amyloidosis=0.75; κ/λ myeloma without amyloidosis=1.1). The possibility that amyloid-related Bence Jones proteins are fragments of auto-reactive antibodies which initially bind to tissues in a specific manner was proposed by Osserman in 1964 (see ref 33). Indeed, Glenner has suggested that Bence Jones dimers themselves might mimic Fab fragments and bind specifically to tissue antigens prior to becoming β-pleated sheet fibrils (see Fig. 3). It seems probable that L-chain polymers and V-region half molecules could arise in vivo via enzymatic degradation which might be related to normal light catabolism. Thus Tan and Epstein have found that a lysosomal enzyme preparation from normal human kidney can degrade BJP; during this process polymers are formed which are insoluble under physiologic conditions. These insoluble polymers have the EM appearance of amyloid fibrils and display green polarization birefringence after staining with Congo red.

79. Isobe T, Osserman EF: Patterns of amyloidosis and their association with plasma-cell dyscrasia, monoclonal immunoglobulins and Bence Jones proteins. New Eng J Med 290:473, 1974.

80. Tan M, Epstein W: Polymer formation during the degradation of human light chain and Bence Jones proteins by an extract of the lysosomal fraction of normal human kidney. Immunochemistry 9:9, 1972.

81. Epstein WV, Tan M, Wood IS: Formation of "amyloid" fibrils in vitro by action of human kidney lysosomal enzymes on Bence Jones proteins. J Lab Clin Med 84:107, 1974.

Table 9. Frequency of M-proteins in Different Patterns of Amyloidosis.

M-Protein	Amyloid Distribution				
	Pattern I*	Mixed	Pattern II†	Localized	Total
	pattern I & II no. of cases				
BJκ only	17	3	1		21
BJλ only	19	6			25
IgG only		4	5	1	10
IgG + BJκ	3				3
IgG + BJλ	5	4			9
IgA only	4	2	1		7
IgA + BJκ	1				1
IgA + BJλ	1				1
IgD only		1			1
IgD + BJλ		1			1
IgM only		4	2	1	7
IgM + BJκ		1		1	2
No monoclonal protein detected		4	8		12
Totals	50	30	17	3	100

*Heart, tongue, gastrointestinal, etc. †Liver, spleen & kidney.

From ref. 79

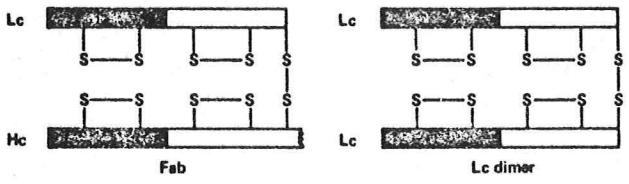


Fig. 3. Similarity between Fab fragment, having antigen binding activity, and interchain disulfide dimer of light chains. Variable regions are in black.

From ref. 45.

Two other recent studies are noteworthy. The Boston group has reported finding M-proteins in serum or urine of 7 of 14 cases of primary amyloidosis. No monoclonal proteins were identified in 8 patients with localized amyloid, 6 patients with hereditary amyloidosis or in 23 patients with secondary amyloidosis. These authors raise the possibility that the M-proteins in the primary group might simply represent coexisting and unrelated nonprogressive (benign) plasma cell dyscrasias which are known to occur in a variety of conditions as well as in healthy and aged persons. If so, one wonders why M-proteins were uniformly absent in their other groups of amyloid patients!

82. Cathcart ES, Ritchie RF, Cohen AS, Brandt K: Immunoglobulins and amyloidosis. An immunologic study of sixty-two patients with biopsy-proved disease. Am J Med 52:93, 1972.

In an autopsy study of 35 myeloma patients and 138 age-matched controls, Limas et al concluded that myeloma did not enhance the development of age-related amyloid. Their more interesting finding was that the overall frequency of amyloid infiltration in the myeloma patients did not differ significantly from that in the age-matched control group. The authors suggest that one possible explanation for the alleged frequency of amyloidosis in myeloma might be that previous workers did not give sufficient consideration to the incidence of amyloid accumulation in elderly persons without myeloma. Interestingly, the criteria for diagnosis of myeloma and the number of patients studied were essentially identical to those in our study (ref 63). However, most subjects had serum spikes and immunochemical typing of M-proteins was performed in only 13 of the 35 patients studied by Limas et al. Failure to focus on the light chain myeloma group leads to erroneous conclusions regarding the incidence of amyloidosis in myeloma.

83. Limas C, Wright JR, Matsuzaki M, Calkins E: Amyloidosis and multiple myeloma. A reevaluation using a control population. Am J Med 54:166, 1973.

Finally, all homogeneous components on electrophoresis of urine from patients with amyloidosis are not Bence Jones protein. We have recently studied a previously healthy 40 y/o man (AB) who developed the nephrotic syndrome. Renal biopsy demonstrated amyloid in all glomeruli present (Congo red and EM). Liver biopsy and bone marrow examination were negative. Urinary protein excretion was 8.5 g/day; the urine was positive for BJP by both the heat and TSA methods. Serum and urine electrophoretic patterns are shown in Fig. 4. Immunochemical analysis disclosed that the homogeneous β-migrating urine component was transferrin which was present in a concentration of 150 mg%. Transferrin purified from normal human serum exhibited thermal properties virtually identical to those of true Bence Jones proteins.

Thus another potential source of confusion in amyloidosis!

84. Guest WS, Stone MJ: Urine transferrin masquerading as Bence Jones protein in the nephrotic syndrome of primary amyloidosis. Clin Res 22:529A, 1974.

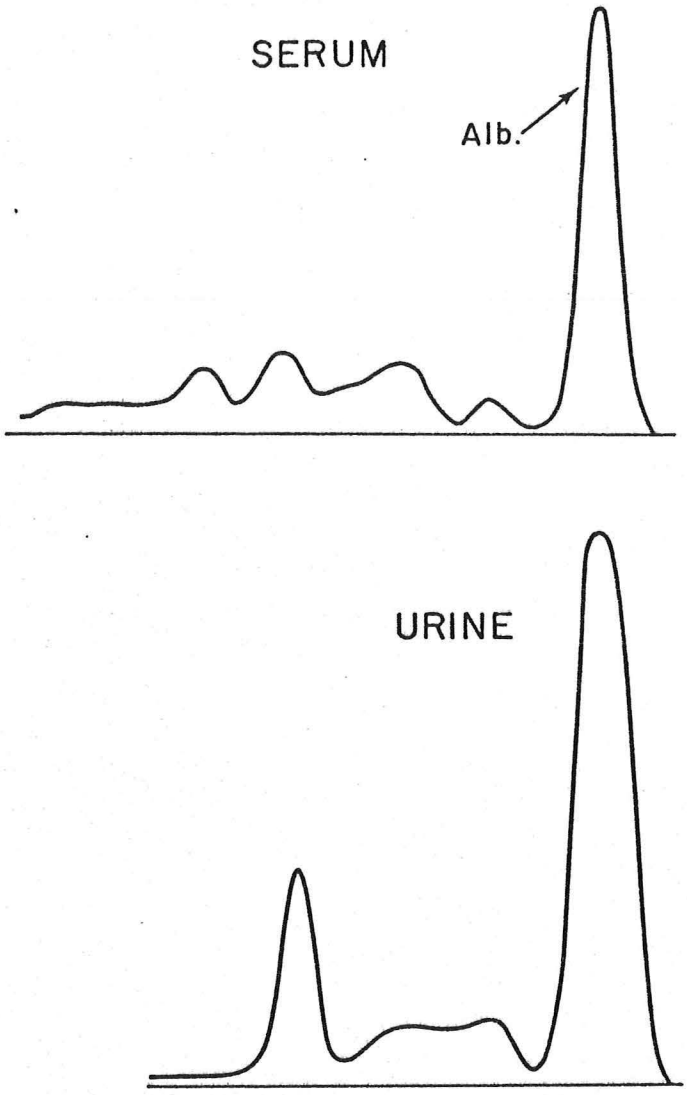


Fig. 4

C. Heredofamilial Amyloidoses

These syndromes are listed in Tables 10 and 11 according to their principal clinical patterns of involvement. Except for familial Mediterranean fever (FMF), the mode of inheritance is autosomal dominant. No characteristic biochemical, hematologic or immunologic abnormalities have been identified thus far. As noted in Section III, the amyloid fibril protein in FMF is of non-immunoglobulin origin.

Table 10. HEREDOFAMILIAL AMYLOIDOSES

Neuropathy
1. Lower limb (D)
   a. Portuguese
   b. Japanese
   c. Other (? U.S.A.—Greek variety)
2. Upper limb—carpal tunnel—vitreous opacities (D)
   a. Swiss (Indiana)
   b. German (Maryland)
3. Lower then upper limb plus nephropathy (D)
   a. English—Irish—Scottish (Iowa)

Nephropathy
1. With marked neuropathy [(3) above]
2. Familial Mediterranean fever (r)
   Non-Ashkenazi Jews; Armenians; Arabs; Turks
3. Fever and abdominal pain (D)
   Swedish
   Sicilian
4. Urticaria, deafness, and renal disease (D)
5. Renal failure and hypertension (D)

Cardiopathy
1. Progressive heart failure
   Danish (D)
2. Persistent atrial standstill

Miscellaneous
1. Medullary carcinoma of the thyroid
2. Lattice corneal dystrophy
3. Cutaneous

From ref 85.

Table II. Comparison of the Hereditary Amyloidoses

Phase	Portuguese-Japanese families	Indiana-Maryland families	Iowa family	Familial Mediterranean fever
Genetic mode of transmission	Autosomal, dominant	Autosomal, dominant	Autosomal, dominant	Autosomal, recessive
Ethnic Predilection	Portuguese and Japanese	Swiss and German	Scotch-English-Irish	Mediterranean Jews and Armenians
Age of onset (decades)	3rd-4th	4th-5th	3rd-5th	1st-2nd
Progression onset to death (yr)	10-12	16-35	1-26*	2-10
Neuropathy	++++ Lower extremities	++++ Upper extremities	++++ Lower extremities; ++ Upper extremities	—
Nephropathy	—	—	++++	++++
Vitreous opacities	—	++	—	—

* 12, average.

From ref. 86.

85. Cohen AS: Inherited Systemic Amyloidosis, chap 50. The Metabolic Basis of Inherited Disease (Stanbury JB, Wyngaarden JB, Fredrickson DS, eds), New York, McGraw-Hill, 1972.

86. Andrade C, Araki S, Block WD, Cohen AS, Jackson CE, Kuroiwa Y, McKusick VA, Nissim J, Sohar E, Van Allen MW: Hereditary Amyloidosis. Arth Rheum 13:902, 1970.

87. Blum A, Gafni J, Sohar E, Shibolet S, Heller H: Amyloidosis as the sole manifestation of familial Mediterranean fever (FMF). Further evidence of its genetic nature. Ann Int Med 57: 795, 1962.

88. Sohar E, Gafni J, Pras M, Heller H: Familial Mediterranean fever. A survey of 470 cases and review of the literature. Am J Med 43:227, 1967.

89. Rukavina JG, Block WD, Jackson CE, Falls HF, Carey JH, Curtis AC: Primary systemic amyloidosis: A review and an experimental, genetic and clinical study of 29 cases with particular emphasis on the familial form. Medicine 35:239, 1956.

90. Mahloudji M, Teasdall RD, Adamkiewicz JJ, Hartmann WH, Lambird PA, McKusick VA: The genetic amyloidoses: With particular reference to hereditary neuropathic amyloidosis, type II (Indiana or Rukavina type). Medicine 48:1, 1969.

91. Lambird PA, Hartmann WH: Hereditary amyloidosis, the flexor retinaculum, and the carpal tunnel syndrome. Am J Clin Pathol 52:714, 1969.

92. Frederiksen T, Gotzsche H, Harboe N, Kiaer W, Mellemgaard K: Familial primary amyloidosis with severe amyloid heart disease. Am J Med 33:328, 1962.

93. Allensworth DC, Rice GJ, Lowe GW: Persistent atrial standstill in a family with myocardial disease. Am J Med 47:775, 1969.

Also see ref 50.

D. Neoplasms with Amyloid Stroma

Localized amyloid deposits are characteristically found in certain types of tumors. These include medullary carcinoma of the thyroid, pancreatic β-cell adenoma or insulinoma, calcifying epithelial odontogenic tumor and basal cell carcinoma. Microdeposits of amyloid also may be found in non-neoplastic pituitary, thyroid and adrenal glands as well as in the islets of Langerhans. It seems likely that amyloid in these areas may be derived from fragments of polypeptide hormones.

94. Meyer JS, Hutton WE, Kenny AD: Medullary carcinoma of the thyroid gland. Subcellular distribution of calcitonin and relationship between granules and amyloid. Cancer 31:433, 1973.

95. Gordon PR, Huvos AG, Strong EW: Medullary carcinoma of the thyroid gland. A clinicopathologic study of 40 cases. Cancer 31: 915, 1973.

96. Hill CS, Ibanez ML, Samaan NA, Ahearn MJ, Clark RL: Medullary (solid) carcinoma of the thyroid gland: An analysis of the M. D. Anderson Hospital experience with patients with the tumor, its special features, and its histogenesis. Medicine 52:141, 1973.

97. DeLellis RA, Gleser RA: Amyloid in endocrine tumors (Letter). New Eng J Med 288:1024, 1973.

98. Wolfe HJ, Melvin KEW, Cervi-Skinner SJ, Al Saadi AA, Juliar JF, Jackson CJ, Tashjian AH: C-cell hyperplasia preceding medullary thyroid carcinoma. New Eng J Med 289:437, 1973.

99. Porta EA, Yerry R, Scott RF: Amyloidosis of functioning islet cell adenomas of the pancreas. Am J Pathol 41:623, 1962.

100. Gardner DG, Michaels L, Liepa E: Calcifying epithelial odontogenic tumor: An amyloid-producing neoplasm. Oral Surg 26:812, 1968.

101. Ravid M, Gafni J, Sohar E, Missmahl HP: Incidence and origin of non-systemic microdeposits of amyloid. J Clin Pathol 20: 15, 1967.

Also see refs 3 and 56.

E. Amyloidosis and Aging

Senile amyloidosis has been said to be the single most ubiquitous disease of vertebrates. The frequency and extent of amyloid deposition increases with age in both animals and humans. Amyloid-like material occurs in the brain as senile plaques, in Down's syndrome and Alzheimer's disease, and following head injury or X-irradiation. The incidence of cardiac amyloidosis apparently increases with each decade and is a well-recognized cause of noncoronary cardiomyopathy. Similarly, the incidence and severity of pancreatic islet amyloid is age-related. In an autopsy study of 83 general hospital patients, Wright et al determined the distribution and incidence of amyloid deposits. In those individuals over 70 years of age, cerebral amyloid was identified in 63%; cardiac amyloid in 31%; aortic amyloid in 50%; and pancreatic amyloid in 30%. The incidence of these deposits was significantly greater in the over 70 age group than in the group between 30 and 70 years of age. The same workers have reported that the incidence of amyloidosis in patients with rheumatoid arthritis is no greater than in age-matched controls.

Although the chemical nature of senile amyloid is unknown, the studies of Husby and Natvig (ref 53) suggest that it is of non-immunoglobulin origin.

102. Walford RL: Immunologic theory of aging: Current status. Fed Proc 33:2020, 1974.

103. Schwartz P: Senile cerebral, pancreatic insular and cardiac amyloidosis. Trans NY Acad Sci 27:393, 1965.

104. Terry RD, Gonatas NK, Weiss M: Ultrastructural studies in Alzheimer's presenile dementia. Am J Pathol 44:269, 1964.

105. Mandybur TI, Gore I: Amyloid in late postirradiation necrosis of brain. Neurology 19:983, 1969.

106. Editorial: Amyloidosis of Alzheimer's disease: A clue to ageing? Lancet 2:598, 1970.

107. Schwartz P: Amyloidosis. Cause and Manifestation of Senile Deterioration. Springfield, Charles C. Thomas, 1970.

108. Wright JR, Calkins E, Breen WJ, Stolte G, Schultz RT: Relationship of amyloid to aging: Review of the literature and systematic study of 83 patients derived from a general hospital population. Medicine 48:39, 1969.

109. Ozdemir AI, Wright JR, Calkins E: Influence of rheumatoid arthritis on amyloidosis of aging. Comparison of 47 rheumatoid patients with 47 controls matched for age and sex. New Eng J Med 285: 534, 1971.

Also see refs. 13, 53, 83, 101 and Section V-D.

F. Specific Organ Involvement and Clinical Features

Amyloid deposits may occur anywhere in the body and nearly always involve blood vessels to some extent. Consequently, the clinical manifestations are legion. An excellent discussion of amyloidosis of the kidney, heart, G-I tract, liver, respiratory tract, nervous system, eye and skin can be found in ref 1. In addition, the following are recommended:

Kidney

110. Heptinstall RH: Amyloidosis, multiple myeloma, and Waldenstrom's macroglobulinemia, chap 20. Pathology of the kidney. Boston, Little, Brown & Co., 1966.

111. Carone FA, Epstein FH: Nephrogenic diabetes insipidus caused by amyloid disease. Evidence in man of the role of the collecting ducts in concentrating urine. Am J Med 29:539, 1960.

112. Sebastian A, McSherry E, Ueki I, Morris RC: Renal amyloidosis, nephrotic syndrome, and impaired renal tubular reabsorption of bicarbonate. Ann Int Med 69:541, 1968.

113. Rosenmann E, Pollak VE, Pirani CL: Renal vein thrombosis in the adult: A clinical and pathologic study based on renal biopsies. Medicine 47:269, 1968.

114. Craciun EC, Nicolesco S, Rau C, Tomescu E: Amyloid in Balkan nephropathy. Lancet 1:676, 1969.

115. Littman E: Renal amyloidosis with nephrotic syndrome associated with retroperitoneal fibrosis. Ann Int Med 74:240, 1971.

116. Gardner KD, Castellino RA, Kempson R, Young BW, Stamey TA: Primary amyloidosis of the renal pelvis. New Eng J Med 284:1196, 1971.

117. Ooi BS, Pesce AJ, Pollak VE, Mandalenakis N: Multiple myeloma with massive proteinuria and terminal renal failure. Am J Med 52: 538, 1972.

Also see refs. 60, 62, 63, 84, 85, 87, 88

Heart

118. James TN: Pathology of the cardiac conduction system in amyloidosis. Ann Int Med 65:28, 1966.

119. Buja LM, Khoi NBA, Roberts WC: Clinically significant cardiac amyloidosis. Clinicopathologic findings in 15 patients. Am J Cardiol 26:394, 1970.

120. Barth RF, Willerson JT, Buja LM, Decker JL, Roberts WC: Amyloid coronary artery disease, primary systemic amyloidosis and paraproteinemia. Arch Int Med 126:627, 1970.

121. Case records of the MGH (Case 7-1972). New Eng J Med 286:364, 1972. (Excellent discussion by J. Willerson.)

122. Case records of the MGH (Case 25-1974). New Eng J Med 290:1474, 1974. (Sinus node involvement by amyloid. Interesting comment at end of discussion.)

Also see refs. 60, 62, 63, 67, 72, 73, 92, 93, 103, 108

Respiratory tract

123. Kamberg S, Loitman BS, Holtz S: Amyloidosis of the tracheobronchial tree. New Eng J Med 266:587, 1962.

124. Domm BM, Vassallo CL, Adams CL: Amyloid deposition localized to the lower respiratory tract. Am J Med 38:151, 1965.

125. Gonzalez-Cueto DM, Rigoli M, Gioseffi LM, Lancelle B, Martinez A: Diffuse pulmonary amyloidosis. Am J Med 48:668, 1970.

126. Gallego FG, Canelas JL: Hilar enlargement in amyloidosis (Letter). New Eng J Med 291:531, 1974.

Also see refs 63 and 75.

G-I tract, liver

127. Gregg JA, Herskovic T, Bartholomew LG: Ascites in systemic amyloidosis. Arch Int Med 116:605, 1965.

128. Katz J, Savin R, Spiro HM: The basal cell nevus syndrome and inflammatory disease of the bowel. Am J Med 44:483, 1968.

129. Gilat T, Spiro HM: Amyloidosis and the gut. Am J Digest Dis 13:619, 1968.

130. Mawas C, Sors C, Bernier JJ: Amyloidosis associated with primary agammaglobulinemia, severe diarrhea and familial hypogammaglobulinemia. Am J Med 46:624, 1969.

Also see refs 57, 58, 60, 62, 63, 71, 74, 133, 134.

Joints, connective tissue

131. Gelderman AH, Levine RA, Arndt KA: Dermatomyositis complicated by generalized amyloidosis. New Eng J Med 267:858, 1962.

132. Goldberg A, Brodsky I, McCarty D: Multiple myeloma with paramyloidosis presenting as rheumatoid disease. Am J Med 37:653, 1964.

133. Benedek TG, Zawadzki ZA: Ankylosing spondylitis with ulcerative colitis and amyloidosis. Am J Med 40:431, 1966.

134. Lake B, Andrews G: Rheumatoid arthritis with secondary amyloidosis and malabsorption syndrome. Am J Med 44:105, 1968.

135. Wiernik PH: Amyloid joint disease. Medicine 51:465, 1972.

136. Caughey DE, Wakem CJ: A fatal case of Reiter's disease complicated by amyloidosis. Arth Rheum 16:695, 1973.

137. Katz GA, Peter JB, Pearson CM, Adams WS: The shoulder-pad sign - a diagnostic feature of amyloid arthropathy. New Eng J Med 288: 354, 1973.

Also see refs 60,62, 63, 66, 88, 91, 109.

Misc.

138. Pechet L, Kastrul JJ: Amyloidosis associated with Factor X (Stuart) deficiency. Ann Int Med 61:315, 1964.

139. Daoud FS, Nieman RE, Vilter RW: Amyloid goiter in a case of generalized primary amyloidosis. Am J Med 43:604, 1967.

VI. Therapy and Prognosis

As noted in Section I, the reversibility of hepatic amyloid after treatment for chronic tuberculous osteomyelitis was demonstrated nearly a half century ago. Remission of the nephrotic syndrome in 2 patients with renal amyloidosis secondary to infective endocarditis and tuberculosis with bronchiectasis, respectively, has followed appropriate therapy for the infections. Renal transplantation has been performed in 2 patients (FMF and amyloid 2° osteomyelitis) with surprisingly good results.

The outlook for patients with nonhereditary primary systemic amyloidosis is poor, median survival after diagnosis ranging from 7 to 13 months. In those patients with demonstrable Bence Jones protein, it seems rational to suppress light chain production by plasma cells (or other B cells) with cytotoxic agents so that further accumulation in tissues is diminished. Although encouraging results have been reported in patients with primary renal amyloidosis, such myeloma-type therapy has not been extensively evaluated. Since amyloid is known to arise from non-immunoglobulin sources and its deposition in experimental animals may be enhanced by immunosuppressive agents, cytotoxic chemotherapy is not recommended for patients without Bence Jones proteins.

Native amyloid fibrils are insoluble in physiological media and relatively resistant to chemical, physical and proteolytic agents. Once deposited in the tissues, their extracellular location makes them inaccessible. Despite these discouraging facts, the observations in patients with secondary amyloidosis indicate that tissue deposits can be mobilized if the inciting stimulus leading to their production is removed. Hopefully, further elucidation of the mechanisms responsible for resorption in various sites will lead to more effective therapy for this fascinating group of disorders.

140. Lowenstein J, Gallo G: Remission of the nephrotic syndrome in renal amyloidosis. New Eng J Med 282:128, 1970.

141. Cohen AS, Bricetti AB, Harrington JT, Mannick JA: Renal transplantation in two cases of amyloidosis. Lancet 2:513, 1971.

142. Jones NF, Hilton PJ, Tighe JR, Hobbs JR: Treatment of "primary" renal amyloidosis with melphalan. Lancet 2:616, 1972.

143. Fields M, Polliack A, Laufer A: Resorption of amyloid and enzymatic studies in amyloidosis. Israel J Med Sci 9:875, 1973.

Also see refs 5, 17, 45, 60, 62, 63.

"Then you should say what you mean," the March Hare went on.
"I do," Alice hastily replied; "at least — at least I mean what I say — that's the same thing, you know."
"Not the same thing a bit!" said the Hatter. "Why, you might just as well say that 'I see what I eat' is the same thing as 'I eat what I see'!"

- Lewis Carroll