Bull. Yamaguchi Med. Sch., Vol. 28, Nos. 1-2, 1981

Pathological Study on Amyloidosis

—Localized Deposition of Amyloid; Dystrophic or Age-dependent Amyloid—

Hidenori Nakamura

First Department of Pathology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan.

(Received January 19, 1981)

Abstract. Interstitial microdeposits of congophilic substances in cases without systemic amyloidosis were described. They were found in the sternoclavicular joint, intervertebral disc, heart valve, pleura, thrombotic vessel, and gouty nodule. All congophilic substances exhibited green birefringence under polarized light and showed reactions similar to those of amyloid in secondary systemic amyloidosis with conventional histochemical methods for demonstrating amyloid. Ultrastructurally, congophilic foci in the sternoclavicular joint and heart valve showed fibrillar structure and granular material. Interstitial microdeposits of congophilic substances were classified as "localized deposition of amyloid" and were designated as dystrophic or age-dependent amyloid. The unlabeled antibody peroxidase-antiperoxidase method showed that dystrophic or age-dependent amyloid reacted neither to anti-AL antiserum nor to anti-AA antiserum. Some precursor proteins were considered to be transformed into amyloid fibril under certain local conditions.

Key Words: amyloidosis, dystrophic amyloid, aging, β-fibrilloses

Introduction

A close relationship between aging and small amounts of amyloid deposits in various sites has been well documented[1,2]. However, on careful examination of tissues obtained at autopsy or biopsy, microdeposits of Congo red positive substances are often noted in unexpected sites, irrespective of age[3], in cases in which systemic amyloidosis was ruled out.

In the present paper, localized interstitial microdeposits of congophilic substances (CPS) in the sternoclavicular joint, intervertebral disc, heart valve, pleura, thrombotic vessel, and gouty nodule were investigated whether they were amyloid or not. They were compared with AA amyloid (AA: amyloid protein A) using histochemical and ultrastructural techniques.

Materials and Methods

Specimens

All specimens were taken at autopsy or biopsy from cases in which systemic amyloidosis were ruled out.

1. Sternoclavicular joint: Sternoclavicular joints were taken from 174 patients ranged from birth to 88 years old as previously reported in part[4].
2. Intervertebral disc: Intervertebral discs at lum-

bar region were taken from 39 patients from birth to 85 years old.

3. Heart valve: Heart valves were surgically removed from 104 cases with chronic valvular diseases from 8 months to 64 years old as recently reported[5].

4. Pleura: Pleural tissues were obtained from 2 patients (61-year-old male and 70-year-old male) with chronic adhering pleuritis without effusion.

5. Thrombotic vessel: Thrombotic portal vein with calcification was obtained from a 67-year-old female with portal hypertension.

6. Gouty nodule: A thumb-head sized subcutaneous tumor at elbow was taken from a 49-year-old male with gout.

7. Control: Amyloid-laden thyroid tissues (AA amyloid) from a patient with systemic amyloidosis secondary to rheumatoid arthritis were examined.

Light microscopy

All specimens were fixed in 10% neutral formalin and tissues from the joint, disc, valve, and thrombotic vessel were decalcified if necessary. Paraffin-embedded sections were stained with hematoxylin-eosin (HE) and alkaline Congo red. Tissue sections containing substances that showed Congo red positivity and green birefringence under polarized light were stained with a variety of tinctorial methods as shown in Table. To characterize the protein nature of CPS, the following methods were performed; the dimethylaminobenzaldehyde nitrate (DMAB) method for tryptophan[6], the potassium permanganate method of Wright et al.[7], and the unlabeled antibody peroxidase-antiperoxidase (PAP) method[8] using anti-AL antiserum and anti-AA antiserum obtained through the courtesy of Drs. Fujihara and Glenner[8] and Dr. Imada (personal communication), respectively. Tissues stained with Congo red or Wolman's toluidine blue (STB)[9] were examined with a polarizing microscope. Thioflavin T stained tissues were examined with a fluorescence microscope.

Electron microscopy

Formalin-fixed specimens of the sternoclavicular joint and heart valve were applied for electron microscopy by the methods described previously[4,5,10,11]. Ultrathin sections were doubly stained with uranyl acetate and lead citrate, and were examined with a Hitachi HS-8 or H-300 electron microscope.

Table: Histochemical reactions of interstitial microdeposits of CPS and AA amyloid in the thyroid

Technique	Joint	Disc	Valve	Pleura	Vessel	Gout	Thyroid
HE	pink	light pink	pink	pink	pink	pink	pink
Congo red	++	+	++	+	+	+	+
Congo red polarization	GB	GB	GB	GB	GB	GB	GB
Thioflavin T	YF	YF	YF	YF	YF	YF	YF
Crystal violet	M	M	M	M	M	M	M
STB	orange	−	orange	orange	orange	orange	orange
SAB	yellow, green	green	yellowish green	green	green	green	light green
van Gieson	kahki	kahki	kahki	kahki	kahki	kahki	kahki
PAS	+	+	−, ±	+	+	±	±, +
Alcian blue	−, ±	±	±	±	−	−, ±	±
Azan-Mallory	red	red	grayish red	red	grayish red	light blue	light violet
PTAH	brown, violet	tan	brown, violet	tan, violet	violet	tan	tan
DMAB	+	+	+	+	+	+	+
Permanganate	R	R	R	R	R	R	S
PAP: anti-AL	−	−	−	−	−	−	−
PAP: anti-AA	−	−	−	−	−	−	+

GB: green birefringence, YF: yellow fluorescence, M: metachromasia, R: resistant, S: sensitive
++: strongly positive, +: positive, ±: weakly positive, −: negative

Results

Identification of CPS

1. Sternoclavicular joint: CPS were noted in a high incidence with an increase with age (146/174, 83.9%). The youngest patient with CPS was 36 years old. CPS were deposited in the meniscus and along the surface and fibrillation clefts of articular cartilage in linear or lamellar fashion, and also occurred in some superficial and deep areas in patchy or massive fashion (Fig. 1). Some cases were associated with inflammation and/or calcification.

2. Intervertebral disc: CPS were recognized in 22 out of 39 cases (56.4%) and the incidence and severity increased with aging. The youngest patient with CPS was 46 years old. Microdeposits of CPS occurred at the peripheral part of anulus fibrosus and were never seen at the nucleus polposus. The manner of deposits was linear or lamellar along slit-like fissures (Fig. 2) and patchy in some areas. Other significant pathological changes were not observed.

3. Heart valve: The result in detail was reported recently[5]. CPS could be seen in sclerotic or ulcerated areas in patchy, linear, or dotted fashion in degenerated valves (Fig. 3). The incidence of occurrence of CPS was 46 out of 104 (44.2%) and increased in relation to aging or duration of illness. The youngest patient with CPS was 29 years old.

4. Pleura: CPS were noted in destructed areas with inflammation or hyalinized areas in linear, lamellar, or granular fashion (Fig. 4).

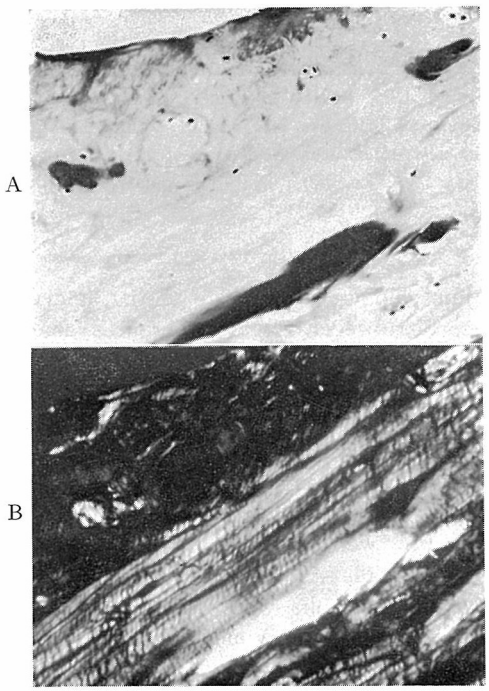

Fig. 1 CPS in the sternoclavicular joint are deposited in linear or lamellar fashion along the surface of articular cartilage and in patchy or massive fashion in deep area. (A) ordinary light, (B) polarized light. (Congo red, ×60)

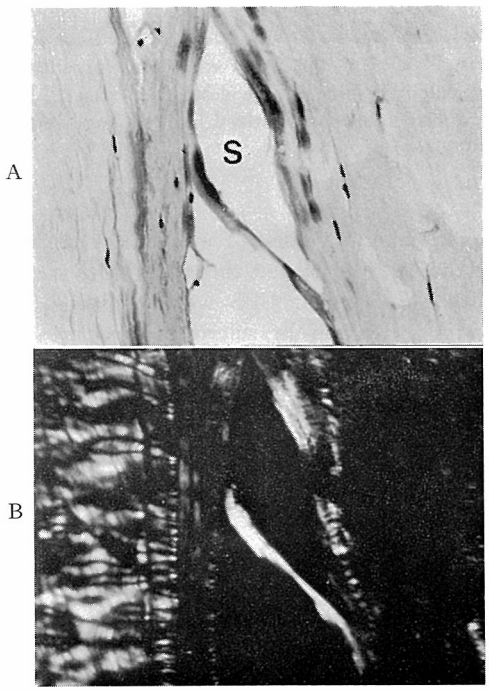

Fig. 2 CPS in the intervertebral disc are deposited in linear fashion. *S.*: slit-like fissure. (A) ordinary light, (B) polarized light. (Congo red, ×100)

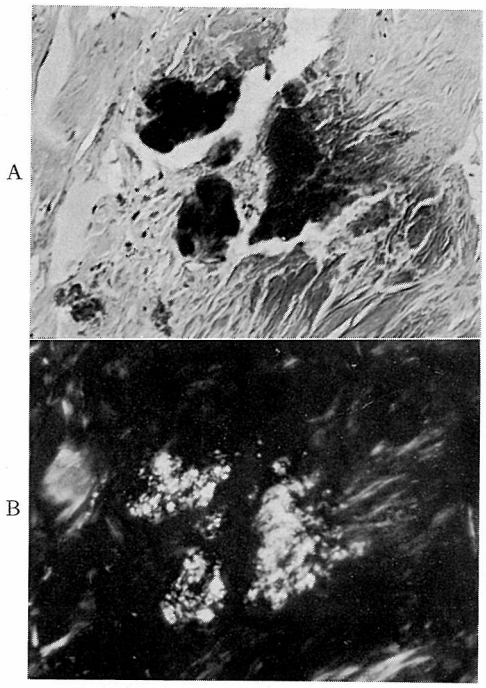

Fig. 3 CPS in the heart valve are deposited in patchy fashion in the destructed area. (A) ordinary light, (B) polarized light. (Congo red, ×100)

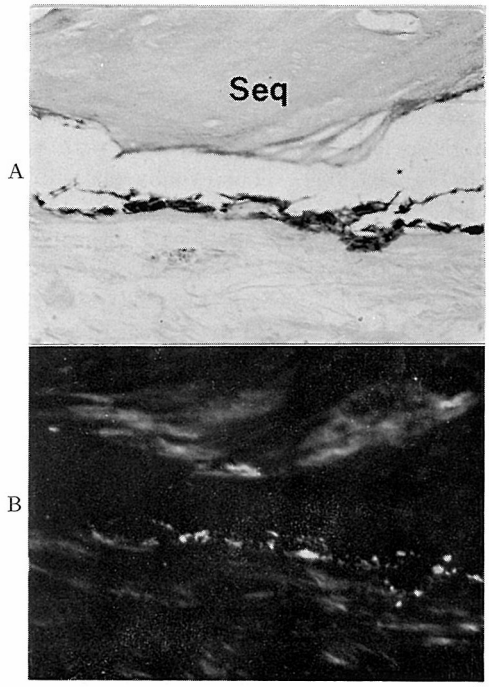

Fig. 4 CPS in the pleura. Dotted CPS are deposited in linear fashion along the cleft in the destructed area. *Seq*: sequestrating necrotic tissue. (A) ordinary light, (B) polarized light. (Congo red, ×100)

5. Thrombotic vessel: The portal vein was obstructed by calcified thrombus with recanalization. Granular CPS were deposited in band-like fashion in sclerotic areas around calcification (Fig. 5).

6. Gouty nodule: The subcutaneous tumor at elbow was composed of tophi. CPS were found in linear fashion within the tophus (Fig. 6).

Tinctorial and optical properties

The results are summarized in Table. All CPS described above exhibited green birefringence under polarized light. CPS in various sites showed reactions similar to those of AA amyloid in the thyroid with conventional histochemical methods generally accepted for the demonstration of amyloid, such as thioflavin T, crystal violet, STB, sodium sulphate alcian blue (SAB), and van Gieson. However, slight differences among CPS and from AA amyloid were recognized when stained with additional methods, such as periodic acid Schiff (PAS), alcian blue, Azan-Mallory, and phosphotungustic acid hematoxylin (PTAH). CPS in each site were positive for tryptophan by DMAB method. All CPS were potassium permanganate-resistant while AA amyloid was sensitive. No positive results were obtained by PAP method except for AA amyloid.

Electron microscopy

Congophilic foci in the sternoclavicular joint showed fibrillar structure with a mean width of 14nm surrounded by amorphous granular material (Fig. 7). The fibrils were

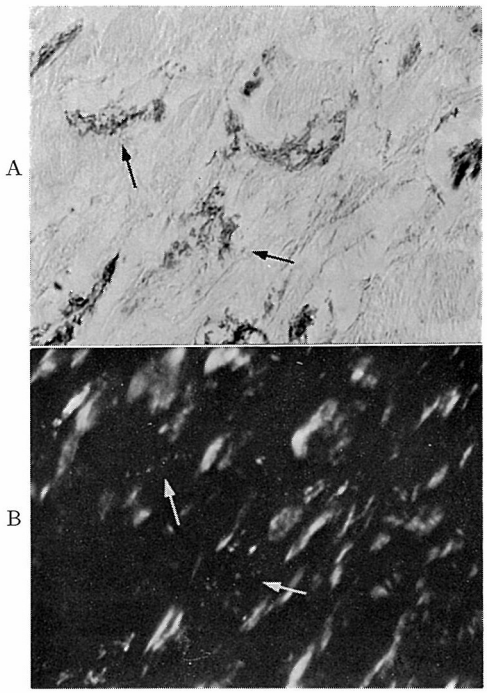

Fig. 5 CPS in the thrombotic vessel Dotted CPS (arrows) are deposited in band-like fashion. (A) ordinary light, (B) polarized light. (Congo red, ×200)

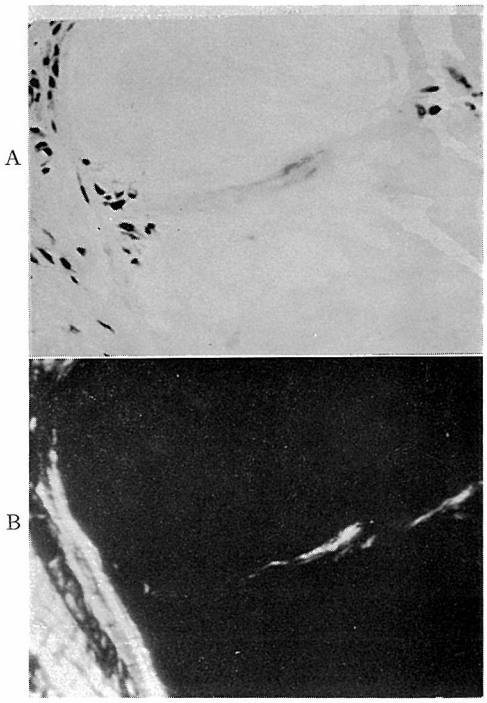

Fig. 6 CPS in the gouty nodule are deposited in fibrillar fashion within the tophus. (A) ordinary light, (B) polarized light. (Congo red, ×200)

straight and non-branching. Congophilic foci in the heart valve consisted of granular material and fibrils having a mean width of 10nm. The fibrils were short, non-branching and haphazard (Fig. 8). AA amyloid in the thyroid showed non-branching fibrillar structure in felt-like fashion. The mean width of fibrils was 8nm.

Discussion

Identification of CPS as amyloid

In general, when pathologists diagnose amyloid, the following criteria are used; 1) positivity for Congo red stain, 2) green birefringence under polarized light after Congo red staining, and 3) ultrastructural feature of accumulation of fibrils 8–10nm wide. Although a variety of tinctorial and optical methods have been applied for the demonstration of amyloid, they often provide false-positive or false-negative reactions[12,13]. Ultrastructural variations have been also sometimes noted[14]. Glenner[15] proposed the term "the β-fibrilloses" for amyloidosis and amyloid deposition on the basis of the unifying definition of amyloid as having a β-fibril structure in X-ray diffraction study[16]. Congo red birefringence (Congo red positivity and green birefringence under polarized light after Congo red staining) has been regarded as a histological marker for the β-fibril structure[15].

In a recent report[4], CPS in articular cartilage of the sternoclavicular joint were considered as amyloid-like substances because

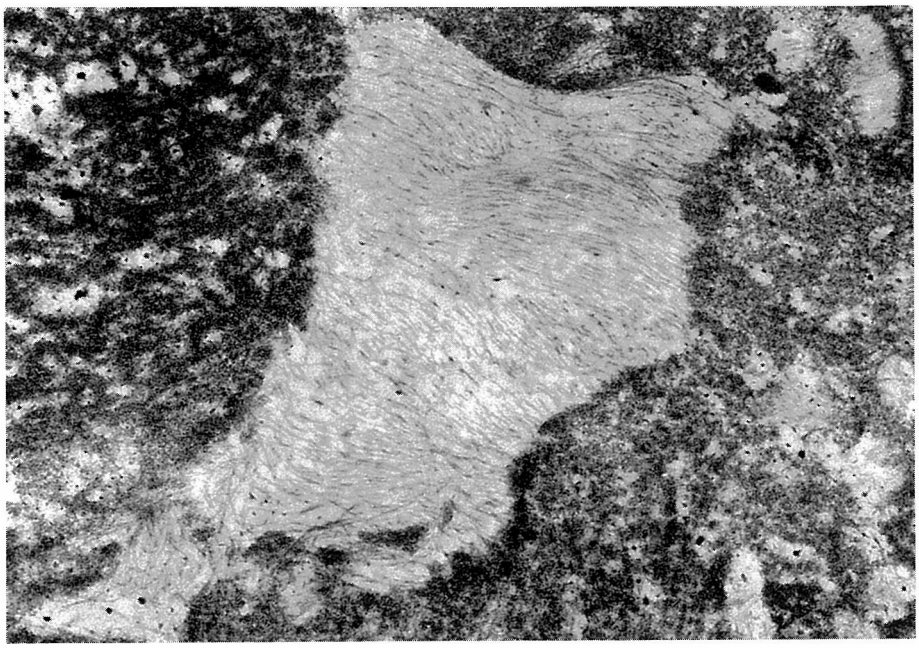

Fig. 7 CPS in the sternoclavicular joint show fibrillar structure surrounded by amorphous granular material. ×25, 000.

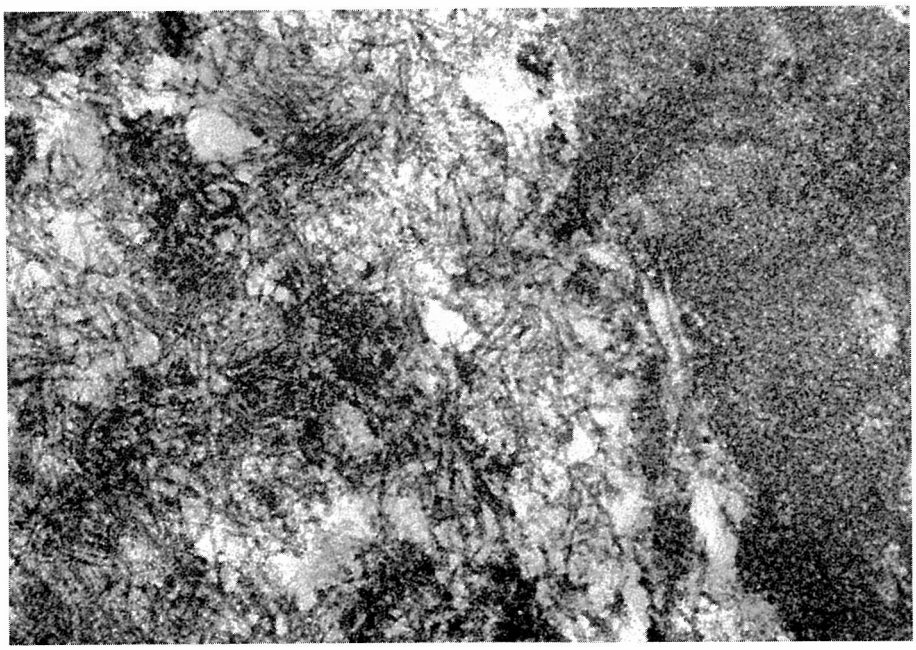

Fig. 8 CPS in the heart valve show short, haphazard fibrils and granular material. ×50, 000.

of the dissimilarities of their tinctorial properties with some staining methods and ultrastructural feature comparing with those of amyloid in systemic amyloidosis. However, according to Glenner, the CPS in the sternoclavicular joint are retrospectively defined as amyloid, not amyloid-like. Consequently, all CPS in this study could be identified as amyloid, and should be classified as localized deposition of amyloid[1] because they did not produce any clinical manifestations by themselves.

Microdeposits of amyloid in the joint, disc, and valve seem to be related aging. However, the youngest patient with CPS was 36, 46, and 29 years old, respectively. Therefore, it is inappropriate to regard them as "senile".

Sorensen and Christensen[17] and Teglbjærg et al.[18] noted amyloid deposits in the joint capsule, in osteoarthritis and pyrophosphate arthritis, respectively. They detected no relationship between age, duration, or severity of the diseases and incidence or quantity of amyloid deposits. They regarded amyloid in the joint as an end-product of the diseases. However, there were no joint diseases in the sternoclavicular joint in this study.

Reports on amyloid deposits in the intervertebral disc are rare. Ballou et al.[19] reported a single case in which systemic amyloidosis involving discs was associated with calcification. The discs examined showed no significant pathological changes.

Microdeposits of amyloid in surgically resected valves due to valvular dysfunction have been rarely reported. Goffin[20] described such type of amyloid deposits which appeared to be local scarring. Amyloid in the valve exhibited nearly identical results with those of Goffin.

Small amounts of amyloid deposits were also noted in destructed or hyalinized areas in the pleura with severe pleuritis and in the thrombotic vessel. In the gouty nodule, amyloid was deposited within the tophus.

Localized microdeposits of amyloid described above were commonly found in association with aging or at sites of pathological lesions. Goffin[20] designated the unique type of amyloid "dystrophic" comparing with the analogous situation in pathological calcification. However, all interstitial microdeposits of amyloid are not always considered to be dystrophic. The term "age-dependent" might as well be suitable for some of them, especially for ones in the joint and disc.

Characterization of dystrophic or age-dependent amyloid

Dystrophic or age-dependent amyloid showed reactions similar to those of AA amyloid with the conventional histochemical methods for amyloid. However, different reactions with some additional methods were detected from case to case. The heterogenous stainability and discrepancies might be due to the different amounts of amyloid component, such as acid mucopolysaccharide[4,17], to the different environmental conditions, or to the duration of existence. Ultrastructural features of amyloid in the joint and valve were different from those hithero reported, in spite of showing fibrillar structure. Those features might be due to the reasons mentioned above or to the results of degeneration by decalcification procedure which was sometimes used.

Recent advances in biochemical and immunological analyses have disclosed some chemically different types of amyloid fibril proteins and their related proteins (precursor proteins)[15,21-23]. The characterization of amyloid fibril is important for the comprehention of amyloidogenesis. Several attempts have been made to differentiate amyloid fibrils using histochemical or immunohistochemical techniques[7,8,24,25].

Dystrophic or age-dependent amyloid were positive for tryptophan[24]. Potassium permanganate method by Wright et al.[7] for distin-

guishing protein AA from other amyloid proteins was performed. Dystrophic or age-dependent amyloid in each site was permanganate-resistant. PAP method[8] showed that dystrophic or age-dependent amyloid reacted neither to anti-AL antiserum nor to anti-AA antiserum. From these results, it is considered that dystrophic or age-dependent amyloid has the diverse type of amyloid fibril protein other than protein AL or AA.

Some amyloid precursors, possibly derived from serum or ground substance, might be transformed by proteolytic cleavage[15] into amyloid fibril protein having a β-fibril structure under certain conditions during localized pathological course or aging process.

Acknowledgment

The author is gratefully indebted to Prof. Uchino for his kind guidance and encouragement throughout this study. Thanks are also due to Dr. Iwata, Dr. Ishihara, and Dr. Kamei for their advices, and to Mr. Yamashita and Mr. Murakami for excellent technical assistance.

This work was presented in part at the 69th Annual Meeting of the Japanese Pathological Society in Sapporo, June, 1980, and was supported by a Grant-in-Aid for Amyloidotic Neuropathy from the Ministry and Welfare.

References

1) Wright, J.R., Calkins, E., Breen, W.J., Stolte, G., and Schutz, R.T.: Relationship of amyloid to aging. Review of the literature and systemic study of 83 patients derived from a general hospital population. *Medicine*, 48 : 39-60, 1969.

2) Iwata, T., Kamei, T., Uchino, F., Mimaya, H., Yanagaki, T., and Etoh, H.: Pathological study on amyloidosis. Relationship of amyloid deposits in aorta to aging. *Acta Pathol. Jpn.*, 28 : 193-203, 1978.

3) Ravid, M., Gafni, J., Sohar, E., and Missmahl, H.P.: Incidence and origin of non-systemic microdeposits of amyloid. *J. Clin. Pathol.*, 20 : 15-20, 1967.

4) Uchino, F., Nakamura, H., Kamei, T., and Nagasawa, T.: Amyloid-like substances in cartilage of the sterno-clavicular joint. *In* G.G. Glenner, P.P. Costa, and F. Freitas (Eds.), *Amyloid and Amyloidosis.* Excerpta Medica, Amsterdam, 1980. p. 55-59.

5) Iwata, T., Nakamura, H., Nagasawa, T., Kamei, T., Fujihara, S., Yokota, T., and Uchino, F.: Pathological study on amyloidosis. Small deposits of amyloid in surgically removed heart valves. *Acta Pathol. Jpn.*, in press.

6) Adams, C.W.M.: A *p*-dimethylaminobenzaldehyde-nitrate method for the histochemical demonstration of tryptophan and related compounds. *J. Clin. Pathol.*, 10 : 56-62, 1957.

7) Wright, J.R., Calkins, E., and Humphrey, R.L.: Potassium permanganate reaction in amyloidosis. A histologic method to assist in differentiating forms of this disease. *Lab. Invest.*, 36 : 274-281, 1977.

8) Fujihara, S. and Glenner, G.G.: Primary localized amyloidsis of the genitourinary tract. Immunohistochemical study on eleven cases. *Lab. Invest.*, 44 : 55-60, 1981.

9) Wolman, M.: Amyloid, its nature and molecular structure comparison of a new toluidine blue polarized light method with traditionary procedures. *Lab. Invest.*, 25 : 104-110, 1971.

10) Shirahama, T. and Cohen, A.S.: A Congo red staining method for epoxy-embedded amyloid. *J. Histochem. Cytochem.*, 14 : 725-729, 1966.

11) Rossi, G.L., Luginbühl, H., and Probst, D.: A method for ultrastructural study of lesions found in conventional histological sections. *Virchows Arch. Pathol. Anat.*, 350 : 216-224, 1970.

12) Sümegi, I. and Rajka, G.: Amyloid-like substance surrounding mammary cancer and basal cell carcinoma. *Acta Pathol. Microbiol. Scand.* (A), 80 : 185-192, 1972.

13) Klatzkin, G.: Nonspecific green birefringence in Congo red-stained tissue. *Am. J. Pathol.*, 56 : 1-13, 1969.

14) Westermark, P.: Amyloid of human islets of Langerhans. Ⅱ. Electron microscopic analysis of isolated amyloid. *Virchows Arch. Pathol. Anat.*, 373 : 161-166, 1977.

15) Glenner, G.G.: Amyloid deposits and amyloidosis. The β-fibrilloses. *N. Engl. J. Med.*, 302: 1283-1292, 1333-1343, 1980.

16) Eanes, E.D. and Glenner, G.G.: X-ray diffraction studies on amyloid filaments. *J. Histochem.*

Cytochem., 16 : 673-677, 1968.
17) Sorensen, K.H. and Christensen, H.E.: Local amyloid formation in the hip joint capsule in osteoarthritis. *Acta Orthop. Scand.*, 44 : 460-466, 1973.
18) Teglbjærg, P.S., Ladefoged, C., Sorensen, K. H., and Christensen, H.E.: Local articular amyloid deposition in pyrophosphate arthritis. Histological aspects. *Acta Pathol. Microbiol. Scand.* (A), 87 : 307-311, 1979.
19) Ballou, S.P., Khan, M.A., and Kushner, I.: Diffuse intervertebral calcification in primary systemic amyloidosis. *Ann. Intern. Med.*, 85 : 616-617, 1977.
20) Goffin, Y.: Microscopic amyloid deposits in the heart valves. A common local combination of chronic damage and scarring. *J. Clin. Pathol.*, 33 : 262-268, 1980.
21) Glenner, G.G., Harbaugh, J., Ohms, J.I., Harada, M., and Cuatrecasas, P.: An amyloid protein. The amino-terminal variable fragment of an immunoglobulin light chain. *Biochem. Biophys. Res. Commun.*, 41 : 1287-1289, 1970.
22) Benditt, E.P. and Eriksen, N.: Chemical classes of amyloid substance. *Am. J. Pathol.*, 65 : 231-249, 1971.
23) Rosenthal, C.J., Franklin, E.C., Frangione, B., and Greenspan, J.: Isolation and partial characterization of SAA. An amyloid-related protein from human serum. *J. Immunol.* 116 : 1415-1418, 1975.
24) Pearse, A.G.E., Ewen, S.W.B., and polak, J.M.: The genesis of apudamyloid in endocrine polypeptide tumours. Histochemical distinction from immunamyloid. *Virchows Arch. Zellpathol.*, 10 : 93-107, 1972.
25) Romhányi, V.G.: Selektive Darstellung sowie methodologische Möglichkeiten der Analyse ultrastruktureller Unterschiede von Amyloidablagerungen. *Zentralbl. Allg. Pathol.*, 123 : 9-16, 1979.