Virchows Arch. A Path. Anat. and Histol. 376, 107–115 (1977)

*Virchows Archiv A*


# Histochemical Similarity of Senile Plaque Amyloid to Apudamyloid*

James M. Powers and Samuel S. Spicer

Department of Pathology, Veterans Administration Hospital and Medical University of South Carolina, Charleston, South Carolina 29401, USA

**Summary.** Senile plaque amyloid of both isolated and perivascular (dyshoric) types was compared histochemically to other major types of amyloid. In contrast to most amyloids tested, senile plaque amyloid contained less tryptophan and tyrosine reactivity and more alcianophilia. These histochemical features indicate that senile plaque amyloid is similar to apudamyloid, and suggest that local factors are more important than systemic circulating factors in its genesis. The implications of these histochemical observations are discussed.

**Key words:** Amyloid – Apudamyloid – Senile plaque – Tryptophan staining – Alzheimer's disease.

## Introduction

The senile plaque represents one of the fundamental brain lesions in Alzheimer's senile or pre-senile dementia (McMenemey, 1963). The presence of amyloid within the senile plaque is universally agreed upon, but its chemical nature, mode of formation, and exact relationship to the degenerating neuritic elements are unknown. Cerebral amyloid is commonly associated with cardiac and insular amyloid in the senium (Schwartz, 1970). Insular amyloid has been studied recently by Westermark (1974, 1975), who found that it resembled the amyloid associated with cells of Pearse's APUD series (Pearse, 1974). Apudamyloid differs from other amyloids because of its decreased aromatic amino acid content and autofluorescence, and its greater alcianophilia (Pearse et al., 1972). Previous attempts to histochemically define the aromatic amino acid content and alcianophilic properties of senile plaque amyloid have been limited.

* Supported in part by Veterans Administration General Research Fund and NIH Grant AM 11028

*For offprints contact:* James M. Powers, M.D., Department of Pathology, Medical University of South Carolina, 80 Barre Street, Charleston, South Carolina 29401, USA

We have studied three brains with senile plaques by carefully controlled histochemical methods and compared plaque amyloid to other major amyloids. We find that senile plaque amyloid is histochemically similar to apudamyloid. This data indicates that local factors may be more important than systemic circulating factors in the formation of senile plaque amyloid.

## Materials and Methods

Several types of amyloid (Table 1) were studied histochemically for tryptophan and tyrosine content. The rosindole reaction for tryptophan and the Millon reaction for tyrosine (Lillie, 1965) were utilized. Neutral buffered formalin fixation was employed. Three serial paraffin sections were cut at both 5 μ and 10 μ thickness on an American Optical microtome by one technician. The first and third sections of each set were stained for tryptophan and tyrosine, while the second was stained with alkaline Congo red and viewed with polarized light for positive identification of amyloid. All stains were performed by another technician. Positive controls (3 +, 4 +) for tryptophan and tyrosine consisted of mouse submandibular gland or pancreas. The 5 μ sections were generally 25 to 50% paler than the 10 μ sections. Internal positive controls, such as erythrocytes, fibrin, muscle, and elastica were also used. To identify senile plaque amyloid accurately, only the cores or perivascular plaques were evaluated. Tryptophan and tyrosine content was estimated semiquantitatively using a 0–4+ grading system by one of us (JMP) 6 to 8 h following completion of the stain. The serial sections were photographed between 18 and 24 h after stain completion. Composite lantern slides were then prepared and the reactions were again quantitated independently by the authors. The results of the two separate quantitations were comparable. The black and white photographs, which accompany this text, were similarly controlled. All specimens were photopraphed on a Zeiss Axiomat with automatic exposure, automatically developed in the same developer, and printed at the same exposures on the same grade of photographic paper.

It was necessary to perform some time studies on the effects of formalin fixation, since formalin had been reported to affect tryptophan staining adversely (Adams, 1957; Glenner and Lillie, 1957). Murine submandibular gland, pancreas, and amyloid-containing spleen were fixed in neutral buffered formalin for 3 h, 24 h, 3 days, 7 days or 14 days. There was no appreciable decrease in tryptophan or tyrosine reactivity between 3 h and 24 h, a mild decrease between 24 and 72 h, a substantial decrease (50%) by 7 days, and an almost total loss of reactivity at 14 days. Tyrosine was more

**Table 1.**

| Case | Age (yrs.) | Sex | Type | Organ | Pre-fixation interval | Formalin fixation |
|---|---|---|---|---|---|---|
| 1 | 78 | F | Primary | Heart | 3 h | 6 d |
| 2 | 50 | M | Primary | Heart | 1 h | 3 d |
| 3 | 58 | M | Myeloma | Tongue | 21 h | 5 d |
| 4 | 56 | M | Secondary | Heart, liver | 15 h | 5 d |
| 5 | 53 | F | Medullary carcinoma | Thyroid | 1 h | 8 h |
| 6 | 67 | M | Insular | Pancreas | 1 h | 3 d |
| 7 | 76 | M | Senile plaque | Brain | 18 h | 3 h |
| 8 | 78 | M | Senile plaque | Brain | 4 h | 3 h |
| 9a | 89 | M | Senile plaque | Brain | 6 h | 15 h |
| 9b | 89 | M | Senile cardiac | Heart | 6 h | 10 d |
| 10 | 3 mo. | M | Murine, casein induced | Spleen, liver | 0[a] | 3 h |
| 11 | 6 mo. | M | Murine, spontaneous (K.K) | Spleen, liver | 0[a] | 3 h |

[a] In situ perfusion fixation

resistant than tryptophan. In order to ascertain the effect of autolysis on tryptophan or tyrosine staining, unfixed samples of splenic amyloid were also placed in normal saline at room temperature for 24 h prior to 15 h of formalin fixation. No detectable alteration in staining was observed.

In an attempt to characterize the secondary contributions to amyloid formation, 5 μ sections from each major type of amyloid were stained with periodic acid-Schiff (PAS), alcian blue at pH 2.5 (AB), alcian blue at pH 2.5 in 0.2 molar magnesium chloride, Halmi's aldehyde fuchsin, and azure A at pH 4.0 for wet and post-dehydration metachromasia (Spicer et al., 1967). These stains were quantitated using the same grading system. To investigate further the nature of the proteoglycans or glycoproteins present, sections were pre-treated with bovine testicular hyaluronidase for 5 h or Vibrio cholera sialidase for 18 h, and then stained with alcian blue at pH 2.5.

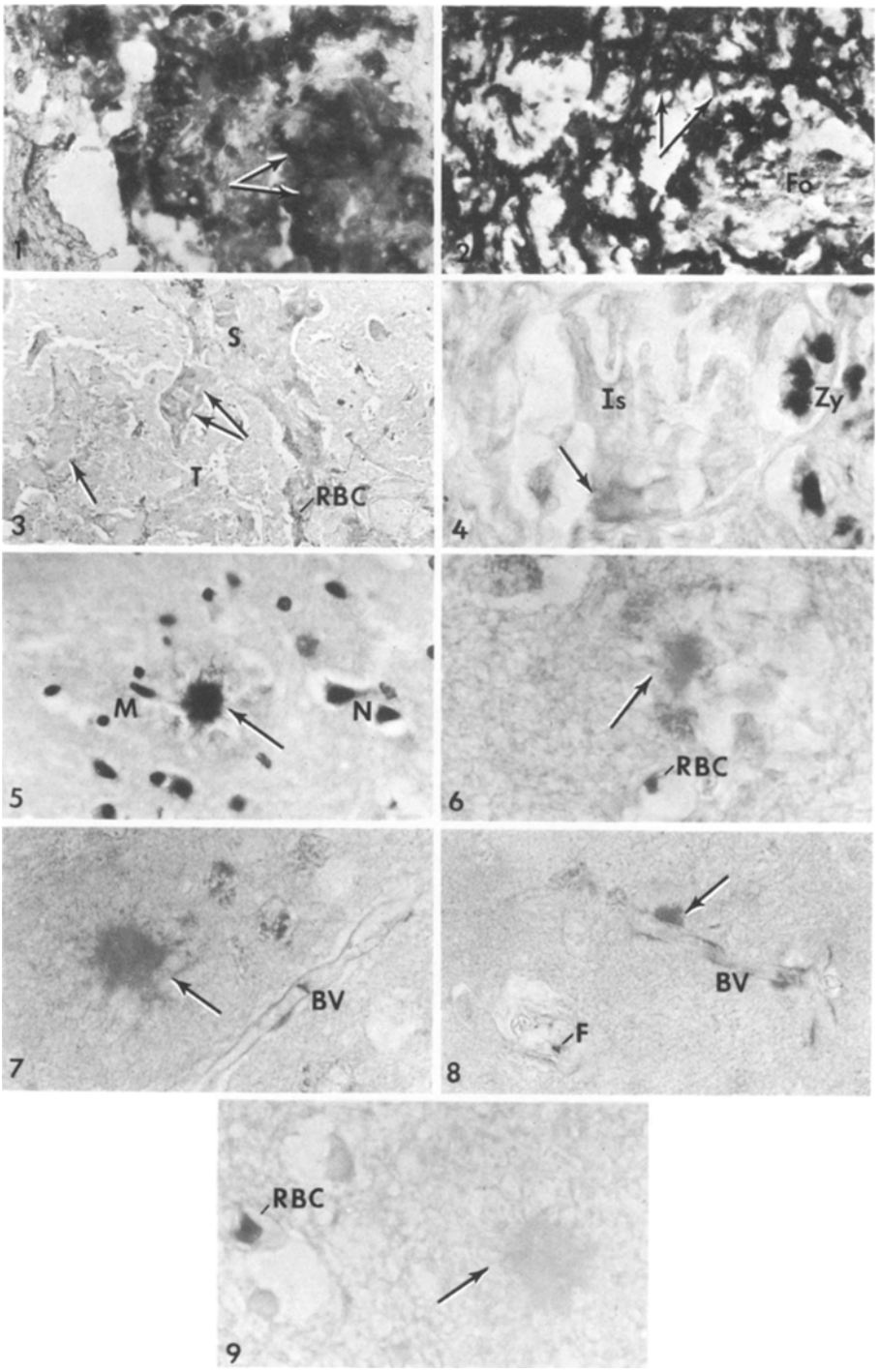
## Results

The results of the histochemical staining are presented in Table 2 and Figures 1–9. Senile plaque amyloid (Figs. 6–9) demonstrated intermediate tryptophan and tyrosine reactivity when compared to generalized amyloids (Figs. 1 and 2) and islet amyloid (Fig. 4), and closely approximated the amyloid of thyroid medullary carcinoma (Fig. 3). The plaque amyloid was often weaker than erythrocyte staining (Figs. 6 and 9) which usually is weakly (+) positive (Glenner and Lillie, 1957). Although neither tryptophan nor tyrosine could be demonstrated in the insular amyloid at 5 μ, trace staining was found in the 10 μ sections. The amyloid in medullary carcinoma of the thyroid was unexpectedly variable in its staining throughout the single specimen studied, but was generally weak. Interestingly, senile cardiac amyloid exhibited greater tryptophan staining than senile plaque amyloid of the same case, despite prolonged formalin fixation. The few cerebral vessels which exhibited congophilic angiopathy demonstrated tryptophan and tyrosine staining which was consistently greater than that of senile plaque amyloid and almost as strong as the staining of generalized amyloids. Isolated plaque and perivascular plaque amyloid was essentially identical. The few neurons undergoing Alzheimer's neurofibrillary degeneration displayed

**Table 2.** Comparative staining of amyloids

| Case | Congo red | PAS | Tryptophan (5 μ) | Tryptophan (10 μ) | Tyrosine (5 μ) | Tyrosine (10 μ) | Alcian blue pH 2.5 |
|---|---|---|---|---|---|---|---|
| 1 | 3+ | 4+ | 3+ | 4+ | 3+ | 3+ | 2+ |
| 2 | 3+ | 3+ | 2+ | 4+ | 2+ | 3+ | ··· |
| 3 | 3+ | 3+ | 3+ | ··· | 2+ | ··· | ··· |
| 4 | 3+ | 2+ | ··· | 2+ | ··· | 2+ | ··· |
| 5[a] | 3+ | 2+ | +/− | +/− to + | +/− | +/− | 2+ |
| 6 | 3+ | 2+ | 0 | +/− | 0 | +/− | 4+ |
| 7 | 3+ | 3+ | 0 to +/− | + | 0 | +/− | ··· |
| 8 | 3+ | 2+ | +/− | ··· | 0 | ··· | ··· |
| 9a | 3+ | 3+ | +/− to + | + | +/− to + | + | 4+ |
| 9b | 3+ | 2+ | 2+ | 3+ | +/− | 2+ | 2+ |
| 10 | 2+ | 2+ | 3+ | 4+ | +/− | +/− | 2+ |
| 11 | 2+ | 2+ | 3+ | 4+ | +/− | +/− | 2+ |

[a] Some variability with all stains; ··· not determined


1
2
Fo
S
T
RBC
3
Is
Zy
4
M
N
5
RBC
6
BV
7
BV
F
8
RBC
9

weak tyrosine staining, but only a trace of tryptophan reactivity.

All amyloids were alcianophilic, but the insular and senile plaque amyloids stained most intensely. Senile cardiac and plaque amyloids retained their strong alcianophilia in 0.2 M magnesium chloride. Only senile cardiac and plaque amyloids demonstrated wet metachromasia with azure A, which was lost after dehydration. Insular amyloid alone demonstrated aldehyde fuchsin positivity. Hyaluronidase digestion did not affect the alcianophilia of the amyloids tested, except for the senile type. Both the senile cardiac and plaque amyloids exhibited a slightly reduced alcianophilia after hyaluronidase. Sialidase pre-treatment resulted in a minimal increase in alcianophilia for all amyloids tested.

## Discussion

Amyloid is an extracellular pathological substance with hyaline, fibrillar and congophilic properties. For a recent review of its morphological features, the reader is referred to Stiller and Katenkamp's monograph (1975). Chemically, amyloid is a heterogeneous mixture consisting predominantly of a major fibrillar glycoprotein, serum globulins and mucosubstances (Glenner and Page, 1976). Most investigators agree that the fundamental nature of amyloid is glycoproteinaceous, with the serum globulins and local mucosubstances being secondary constituents. These secondary constituents, however, may play a decisive role in some properties of amyloid, such as its insolubility (Pras et al., 1971). Although some difference of opinion still exists, it appears that the major fibrillar

**Fig. 1.** Case 1. Subendocardial amyloid deposits (*arrows*). Ten micra section, tryptophan (×200)

**Fig. 2.** Case 10. Perifollicular (*Fo*) splenic amyloid (*arrows*). Ten micra section, tryptophan (×200)

**Fig. 3.** Case 5. Stromal (*S*) amyloid (*arrows*) separates masses of medullary carcinoma (*T*). For comparison, several clusters of weakly positive erythrocytes (*RBC*) are identified. Ten micra section, tryptophan (×50)

**Fig. 4.** Case 6. Pancreatic islet (*Is*) containing single nodule of amyloid (*arrow*). For comparison, several acinar cells with strongly positive zymogen granules (*Zy*) are included. Ten micra section, tryptophan (×500)

**Fig. 5.** Case 9. Senile plaque with amyloid core (*arrow*) and microglial cell (*M*) lies adjacent to some neurons (*N*). Ten micra section, Congo red (×350)

**Fig. 6.** Case 9. This serial section of the same senile plaque demonstrates that the amyloid core (*arrow*) stains less intensely than the erythrocyte (*RBC*). Ten micra section, tryptophan (×600)

**Fig. 7.** Case 9. This amyloid plaque (*arrow*) is pale and distinct from the blood vessel (*BV*). Ten micra section, tryptophan (×480)

**Fig. 8.** Case 9. A perivascular or dyshoric amyloid plaque (*arrow*) is paler than some intravascular material, presumably fibrin (*F*). Ten micra section, tryptophan (×400)

**Fig. 9.** Case 9. The amyloid core (*arrow*) is unreactive while the erythrocyte (*RBC*) stains quite well. Five micra section, tryptophan (×600)

glycoprotein found in "primary" or myeloma amyloid differs from the fibrillar glycoprotein in secondary amyloid. The "primary"-myeloma type is a fragment of the variable region of an immunoglobulin molecule (usually $V_L$), and has been designated B protein, protein type B, and amyloid of immunoglobulin origin (AIO). The secondary type is a different glycoprotein of unknown origin, which has been designated A protein, protein type A, amyloid of unknown origin (AUO) (Benditt and Eriksen, 1972; Glenner and Page, 1976). There is abundant evidence that both major glycoproteins, or their precursors, originate in the circulating blood (Husby and Natvig, 1974; Rosenthal and Franklin, 1975; Glenner and Page, 1976). Tryptophan has been a consistently reliable chemical or histochemical marker in these types of amyloidosis, as well as in hereditary forms and animal models (see Glenner and Page, 1976). The amyloid associated with APUD cells, such as that in medullary carcinoma of the thyroid (Pearse, 1972) and in diabetic or senile pancreatic islets (Westermark, 1974), contains little or no histochemically demonstrable tryptophan or tyrosine. This has been confirmed by autofluorescent studies and by spectrophotometric determination. Its hyaline, congophilic, and fibrillary properties, however, are indistinguishable from those of other amyloids. This third type of amyloid [apudamyloid, amyloid of endocrine origin (AEO)] has thus been separated from the primary and secondary types because of its low aromatic amino acid content (tryptophan and tyrosine). There is also both in vivo and in vitro biochemical and morphological evidence (Pearse, 1972; Westermark, 1973, 1974; Ibanez, 1974) which indicates that apudamyloid is mainly produced by the APUD cells themselves, and not primarily derived from the plasma.

APUD cells, purportedly originating in neural crest, contain fluorogenic amines. They have the capacity to take up amine precursors, decarboxylate amino acids, and secrete bioactive polypeptides (Pearse, 1974). They represent a diffuse system of modified nerve cells which have the same ultimate origin as the cells which populate cerebral cortex. One type of apudamyloid, senile or diabetic insular, is commonly associated with both senile cardiac and senile cerebral amyloid (Schwartz, 1970). Katenkamp and Stiller (1973), utilizing polarization optics, concluded that senile plaque amyloid differed from both primary and secondary amyloids. Therefore, one may expect a similarity between senile plaque amyloid and apudamyloid.

Biochemical studies on the composition of senile plaque amyloid have been limited. Nikaido et al. (1971) isolated a protein from plaque-enriched cortex in Alzheimer's disease. Differences in their methodology make it difficult to put their data into perspective. They did find, however, that the senile plaque protein was different from a control secondary amyloid in that it had a much higher carbohydrate content and different predominant amino acids. Their data revealed that the amyloid cores had more than trace amounts of chondroitin sulfate. Suzuki et al. (1965) found increased amounts of hyaluronic acid and chondroitin sulfate in the gray matter of Alzheimer's disease, and assumed that it had originated from the amyloid of senile plaques. Our histochemical data indicates the presence of hyaluronic acid and/or chondroitin sulfate in senile plaque amyloid. The bulk of the alcianophilia of plaque amyloid, however, appears related to a hyaluronidase-resistant mucosubstance, and could reflect the presence of dermatan, heparan, or keratan sulfate, or a sialidase-resistant sialoglycoprotein. Dermatan and heparan sulfate have been isolated from normal human and sheep brain (Singh and Bachhawat, 1968), and therefore appear to be likely condidates.

There have been many histochemical investigations of cerebral amyloid, but we wish to consider only the tryptophan and/or tyrosine histochemistry. Most investigators have not been interested specifically in senile plaque amyloid, except for Ishii (1958) and Stiller and Katenkamp (1974). Ishii, utilizing the coupled tetrazonium reaction, concluded that senile plaque amyloid contained tryptophan. The coupled tetrazonium reaction, however, is now considered a general protein stain which is not specific for tryptophan or tyrosine (Pearse, 1968). Stiller and Katenkamp (1974) demonstrated low tryptophan content in the amyloid of senile plaques when compared to secondary amyloid, and commented upon its similarity to the amyloid of thyroid medullary carcinoma (1975). They utilized the dimethylaminobenzaldehyde (DMAB) reaction of Adams and the rosindole reaction of Glenner and Lillie. Cooper (1969), using the DMAB reaction of Adams, found that cerebral amyloid and insular amyloid were always tryptophan positive. His identification of tryptophan in insular amyloid contradicts both Pearse's (1972) and Westermark's (1974) findings. Cooper did not specify which type of cerebral amyloid he evaluated, e.g. vascular or plaque. Most recently, Antonutto (1975), using the DMAB reaction, found that senile plaque amyloid was consistently negative, but cerebrovascular amyloid was positive. The section thickness was 5 μ and the formalin fixation time was not specified. Although some of these results may be at variance with each other, it appears that most of the variability may be explained by differences in methodology, including formalin fixation time and section thickness.

We have attempted to rectify this apparently contradictory data by controlling section thickness, formalin fixation time, and staining. We have utilized specific histochemical reactions for tryptophan and tyrosine. We have evaluated only the amyloid core of the senile or perivascular plaque, and have compared this to the other major types of amyloid. Our observations confirm those of Antonutto (1975) and Stiller and Katenkamp (1975) on the paucity of tryptophan in senile plaque amyloid. More importantly, we have demonstrated that senile plaque amyloid is almost histochemically identical to apudamyloid. These histochemical results are necessarily limited by the sensitivity of the methods and their semiquantitative nature. It should be emphasized, however, that histochemical staining is currently the most feasible technique available to study small deposits of amyloid.

The implication of the present histochemical observations relates directly to the significance of tryptophan in amyloid. Tryptophan is highly characteristic of most amyloids, constituting 2–3% of the amyloid fibril's amino acids. The tryptophan containing-fibril is currently believed to be the major glycoprotein in amyloid, and consequently it is of primary pathogenetic significance. Although the tryptophan containing-fibril probably originates in blood, the tryptophan deficient-fibril of apudamyloid appears to be produced locally by modified neural crest cells (see Glenner and Page, 1976). Our data showing similarity of plaque amyloid to apudamyloid indicates that the major fibrillar glycoprotein of senile plaque amyloid is also primarily derived from local neural sources, rather than from the circulating blood. Possible local candidates for a major fibrillar protein with low tryptophan and high acidic amino acid content could include intact or degenerated neurofilaments (Davison and Winslow, 1974) neurotubules (Davison and Huneeus, 1970), or glial filaments (Eng et al., 1971), the S 100 protein (Uozumi and Ryan, 1973), and perhaps the bifilar helix of Alzheimer's neurofibrillary degeneration. This is not a novel idea (see McMenemey, 1963), but it does contradict recent theories on the pathogenesis of senile plaque amyloid in which a vascular origin is invoked (Schwartz, 1970; Wiśniewski et al., 1975). Furthermore, the identification of a neuronal glyco-

protein as the major component of senile plaque amyloid would furnish convincing proof that plaque amyloid is secondary to neuritic degeneration, rather than the initial cause of it.

*Acknowledgements.* The authors wish to express their gratitude to Ms. Judy Bates and Ms. Betty Hall for their technical assistance, to Ms. Carol Moskos and Mr. James Nicholson for their photographic assistance, and to Ms. Wanda Ward and Ms. Geri Lanier for their preparation of the manuscript.

## References

Adams, C.W.M.: A p-dimethylaminobenzaldehyde-nitrite method for the histochemical demonstration of tryptophan and related compounds. J. clin. Path. **10**, 56–62 (1957)

Antonutto, G., Bartoli, M., Melato, M.: Histochemical study on systemic amyloid microdeposits with special reference to parathyroid intrafollicular deposits. Virchows Arch. A Path. Anat. and Histol. **368**, 23–34 (1975)

Benditt, E.P., Eriksen, N.: Chemical similarity among amyloid substances associated with long standing inflammation. Lab. Invest. **26**, 615–625 (1972)

Cooper, J.H.: An evaluation of current methods for the diagnostic histochemistry of amyloid. J. clin. Path. **22**, 410–413 (1969)

Davison, P.F., Huneeus, F.C.: Fibrillar proteins from squid axons. II. Microtubule protein. J. molec. Biol. **52**, 429–439 (1970)

Davison, P.F., Winslow, B.: The protein subunit of calf brain neurofilament. J. Neurobiol. **5**, 119–133 (1974)

Eng, L.F., Vanderhaeghen, J.J., Bignami, A., Gerstl, B.: An acidic protein isolated from fibrous astrocytes. Brain Res. **28**, 351–354 (1971)

Glenner, G.G., Lillie, R.D.: The histochemical demonstration of indole derivatives by the post-coupled p-dimethylaminobenzylidene reaction. J. Histochem. Cytochem. **5**, 279–296 (1957)

Glenner, G.G., Page, D.L.: Amyloid, amyloidosis and amyloidogenesis. In: International review of experimental pathology, 15, p. 1. New York: Academic Press 1976

Husby, G., Natvig, J.B.: A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. J. clin. Invest. **53**, 1054–1061 (1974)

Ibanez, M.L.: Medullary carcinoma of the thyroid gland. In: Pathology Annual, 9, p. 263. New York: Appleton-Century-Crofts 1974

Ishii, T.: Histochemistry of the senile changes of the brain of senile dementia. Psychiatria Neurol. jap. **60**, 768–781 (1958)

Katenkamp, D., Stiller, D.: Comparisons of the texture of amyloid, collagen, and Alzheimer cells. Virchows Arch. A Path. Anat. and Histol. **359**, 213–222 (1973)

Lillie, R.D.: Histopathologic technic and practical histochemistry, third edition. New York: McGraw-Hill 1965

McMenemey, W.H.: The dementias and progressive diseases of the basal ganglia. In: Greenfield's Neuropathology, second edition, p. 525. London: Arnold Ltd. 1963

Nikaido, T., Austin, J., Rinehart, R., Trueb, L., Hutchinson, J., Stukenbrok, H., Miles, B.: Studies in ageing of the brain. I. Isolation and preliminary characterization of Alzheimer plaques and cores. Arch. Neurol. **25**, 198–211 (1971)

Pearse, A.G.E.: Histochemistry, theoretical and applied, second edition. Boston: Little Brown and Company 1961

Pearse, A.G.E., Ewen, S.W.B., Polak, J.M.: The genesis of apudamyloid in endocrine polypeptide tumors: histochemical distinction from immunamyloid. Virchows Arch. B Zellpath. **10**, 93–107 (1972)

Pearse, A.G.E.: The APUD cell concept and its implications in pathology. In: Pathology Annual, volume 9, p. 27. New York: Appleton-Century-Crofts 1974

Pras, M., Nevo, Z., Schubert, M., Rotman, J., Matalon, R.: The significance of mucopolysaccharides in amyloid. J. Histochem. Cytochem. **19**, 443–447 (1971)

Rosenthal, C.J., Franklin, E.C.: Variation with age and disease of an amyloid A protein-related serum component. J. clin. Invest. **55**, 746–753 (1975)

Schwartz, P.: Amyloidosis—cause and manifestation of senile deterioration. Springfield: Charles C. Thomas 1970

Singh, M., Bachhawat, B.K.: Isolation and characterization of glycosaminoglycans of human brain of different age groups. J. Neurochem. **15**, 249–258 (1968)

Spicer, S.S., Horn, R.G., Leppi, T.J.: Histochemistry of connective tissue mucopolysaccharides. In: The connective tissue, International Academy of Pathology monograph number 7, p. 251. Baltimore: Williams and Wilkins 1967

Stiller, D., Katenkamp, D.: Histochemische Untersuchungen am Alzheimerschen Symptomen-Komplex. Sammlg. Psychiatrie **45**, 133–145 (1974)

Stiller, D., Katenkamp, D.: Histochemistry of amyloid. General considerations, light microscopical and ultrastructural examinations. Exp. Path. Suppl. **1**, 1–116 (1975)

Suzuki, K., Katzman, R., Korey, S.R.: Chemical studies on Alzheimer's disease. J. Neuropath. exp. Neurol. **24**, 211–223 (1965)

Uozumi, T., Ryan, R.J.: Isolation, amino acid composition, and radioimmunassay of human brain S 100 protein. Mayo Clinic Proc. **48**, 50–56 (1973)

Westermark, P.: On the nature of the amyloid in human islets of Langerhans. Histochem. **38**, 27–33 (1974)

Westermark, P.: Amyloid of human islets of Langerhans. I. Isolation and some characteristics. Acta Path. microbiol. Scand., Sect. C, **83**, 439–446 (1975)

Wiśniewski, H.M., Bruce, M.E., Fraser, H.: Infectious etiology of neuritic (senile) plaques in mice. Science **190**, 1108–1110 (1975)

*Received June 25, 1977*