Topographic Relationship between Senile Plaques and Cerebrovascular Amyloidosis in the Brain of Aged Dogs

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ABSTRACT. The distributions of senile plaques (SP) and cerebrovascular amyloidosis (CA) were studied by employing thioflavin S and modified Bielschowsky stains, and β-protein immunohistochemistry on serial sections of the brains of aged dogs older than 10 years. Mature and perivascular plaques, both of which contained compact amyloid deposits, always showed a close topographic relationship to CA. In contrast, the majority of diffuse plaques showed no topographic relationship to CA. Cell bodies of neurons and/or glia were almost always involved in the diffuse plaques. In addition, β-protein immunohistochemistry demonstrated amyloid deposits on the periphery of occasional neurons. These findings suggest that different mechanisms may be involved in the development of the different subtypes of SP in the brains of aged dogs.—KEY WORDS: cerebrovascular amyloidosis, dog, immunohistochemistry, senile plaque, β-protein.

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Senile plaques (SP) and cerebrovascular amyloidosis (CA) are well-known forms of cerebral amyloid deposits in the brain of human beings with Alzheimer's disease (AD) [20, 39]. Amyloid deposits are composed primarily of a 42–43 amino acid fragment (β/A4 amyloid protein or β-protein) of a larger amyloid precursor protein (APP) [16, 29, 36]. Changes in the transcription, translation or post-translational processing of APP are likely to play a role in the excessive β-protein formation in AD [6, 9, 12]. Amyloid deposits may also occur in non-demented aged human beings and other mammals, including nonhuman primates, polar bears and dogs [5, 31, 33, 40, 41]. Using β-protein immunohistochemistry, we have previously showed that CA and SP occurring in aged dogs were morphologically very similar to those of human beings [33].

As to the source of APP, there are two main hypotheses, neuronal and vascular. The primary evidence supporting the neuronal origin is the high expression of APP and/or APP mRNA in neurons [3, 4, 19]. The vascular hypothesis rests mainly on the deposition of amyloid in the wall of blood vessels in AD and on the intimate association of capillaries with SP [8, 23, 24]. However, the observation that CA is not always associated with AD suggests that the deposition of amyloid in the neuroparenchyma may differ in some ways from that in and around the vascular wall [38]. The resolution of the relationship of amyloid deposits to local elements of brain tissue, such as neurites, glia and capillaries has important implications in the pathogenesis of them [14].

In the present study, we investigated the cellular association of SP and the topographic relationship between SP and CA in the brain of aged dogs using sensitive techniques for the detection of amyloid deposits, such as β-protein immunohistochemistry, modified Bielschowsky and thioflavin S stains. The pathomorphogenesis of SP was discussed.

MATERIALS AND METHODS

The brains were obtained from 33 aged dogs which were killed by euthanasia or died of a variety of disorders including heart worm disease, renal failure, and tumor in the visceral or genital organs. They consisted of 15 males and 18 females, with an average age of 13.6 years (ranging from 10 to 17) (Table 1). Clinical and pathological data of some of the dogs have been reported elsewhere [33].

The brains were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. Serial sections (6 μm thick) systematically cut at four levels (frontal lobe, striatum, diencephalon and occipital lobe) (Fig. 3) of the left cerebral hemisphere and at various levels of the brain stem and cerebellum were stained with hematoxylin and eosin, thioflavin S and modified Bielschowsky stains [46]. Selected sections were also stained with periodic acid-methenamine silver [11],

Table 1. Clinical and pathological data of the dogs examined

Dog No.	Age (years)	Sex	SP[a] subtype			CA[b]
			Diffuse	Mature	Perivascular	
1	10	M	−	−	−	−
2	10	M	−	−	−	+
3	10	F	−	−	−	−
4	11	M	−	−	−	−
5	11	F	++	−	−	+
6	11	M	−	−	−	−
7	11	M	++	+	−	++
8	12	F	−	−	−	−
9	12	F	−	−	−	+
10	12	F	+++	+	+	++
11	12	M	++	−	−	−
12	12	F	+	−	−	++
13	12	F	−	−	−	−
14	13	M	−	−	−	−
15	13	F	−	−	−	−
16	13	M	+	−	−	++
17	13	F	−	−	−	+
18	13	F	−	−	−	+
19	13	M	−	−	−	++
20	13	F	+	−	−	++
21	14	F	−	−	−	−
22	14	M	−	−	−	+++
23	14	F	+++	−	−	+
24	14	F	++	−	−	+++
25	15	M	+	−	−	−
26	15	F	+++	+	+	+++
27	15	M	−	−	−	+
28	16	M	+	−	+	+++
29	16	F	+++	−	−	+
30	17	F	−	−	−	+
31	17	M	+++	−	−	+
32	17	M	−	−	−	++
33	17	F	+++	+	+	+++

a) SP: Senile plaque.
b) CA: Cerebrovascular amyloidosis.
SP (number/four coronal sections of the cerebral hemisphere): (−) absent, (+) 1–499, (++) 500–999, (+++)≧1000. CA: (−) not observed, (+) some lesions confined to meningeal vessels, (++) some lesions in meningeal and neuroparenchymal vessels, (+++) many lesions in both meninges and neuroparenchyma.

Luxol Fast Blue, Bodian and Congo red stains or processed for immunohistochemistry using antibody directed against β-protein (1–24 residues; rabbit polyclonal, kindly provided by Dr. N. Kitaguchi, Life Science Research Laboratories, Asahi Chemical Industry Co., Ltd.) [43]. The antibody was used at a dilution of 1:1000. Sections for immunohistochemistry were stained using an avidin-biotin peroxidase complex (ABC) method with diaminobenzidine as chromogen. Before the immunostaining, deparaffinized sections were immersed in formic acid (99%) for 5 min at room temperature to enhance the immunostaining of β-protein [18]. Control sections were stained with nonimmune rabbit serum IgG at a protein concentration equivalent to that of the primary antibody.

The number of SP was counted at a magnification of × 100; higher magnification (200 x) was used to differentiate between the plaque types. SP were classified into the following three types according to their morphology [33]: diffuse plaques (diffuse amyloid deposits lacking amyloid cores and apparent neuritic elements), mature plaques (amyloid cores with or tithout apparent neuritic elements) and perivascular plaques (dense amyloid deposits closely associated with blood vessels). Incidence of SP was graded from (−) to (+++) by the number of SP on four coronal sections of the cerebral hemisphere: (+++)≧ 1000, (++) 500–999, (+) 1–499 and (−) absent. Using the sections stained with thioflavin S and immunohistochemistry, CA was assessed semiquantitatively by division into four grades of severity: (−) not observed, (+) some lesions confined to meningeal vessels, (++) some lesions in meningeal and neuroparenchymal vessels, and (+++) many lesions were visible in both meninges and neuroparenchyma. The number and disribution of SP and CA were determined on the coronal sections by plotting these lesions with the aid of the camera lucida.

Histological examination, using thioflavin S, modified Bielschowsky stains and β-protein immunostain

for the presence of amyloid deposits, was also made on the spinal cord and non-neural tissues including the liver, spleen, kidney, heart, lung, gut, pancreas and skin of three dogs with prominent CA and SP formation (Dog Nos. 10, 26 and 33).

RESULTS

Gross and microscopic examinations of the central nervous system (CNS) of the aged dogs revealed no signs of neurological disorders other than typical aged changes, such as thickening of the meninges, mild dilatation of the ventricles, lipofuscin accumulation in neurons, as well as CA and SP formation (Fig. 1, Table 1). Neither obvious atrophy nor neurofibrillary tangles were observed in the brains examined.

All of the SP and CA, both of which reacted with β-protein antiserum, corresponded to those shown by the modified Bielschowsky and/or thioflavin S stains (Fig. 2). The relationship between age and the morphological changes is given in Table 1. SP and CA were detected in 15 (45%) and 22 (67%) of 33 dogs examined, respectively. Of the 15 dogs with SP, 13 (87%) were accompanied by CA. The brains with advanced lesions contained all the types of SP, and only the diffuse type was observed in the brains which had a small number of SP (Table 1).

In the dogs with mild CA, the lesions were confined to the meningeal vessels of the dorsal area of the cerebrum and, to a lesser degree, those of the cerebellum. In the dogs with moderate to severe CA, the lesions were observed in the penetrating vessels in the cerebral cortex and all over the meninges except the ventral area of the brain (Fgi. 3 A-D). No CA was observed in the neuroparenchyma of the other areas of the CNS. SP, which were confined to the cerebrum, were predominant in the medial and lateral areas of the cerebral hemisphere, especially in the cingulate and temporal cortices (Fig. 3 E-H).

Diffuse plaques were the most prominent of the plaque types being distributed throughout the cortical layers, especially the cellular layers, with no topographic relationship to CA (Figs. 1 and 3). Mature and perivascular plaques, both of which contained compact amyloid deposits, had close topographic relationship to CA (Figs. 3 and 4). These plaques often occurred in the dogs with advanced CA (Table 1). Diffuse plaques of larger size, being as large as 200 μm in diameter, often contained capillaries in addition to a few neurons and glial cells (Fig. 5), whereas those of smaller size (≦ 50 μm) seldom involved capillaries (Fig. 6A-H). β-Protein immunohistochemistry demonstrated amyloid deposits on the periphery of neurons in the

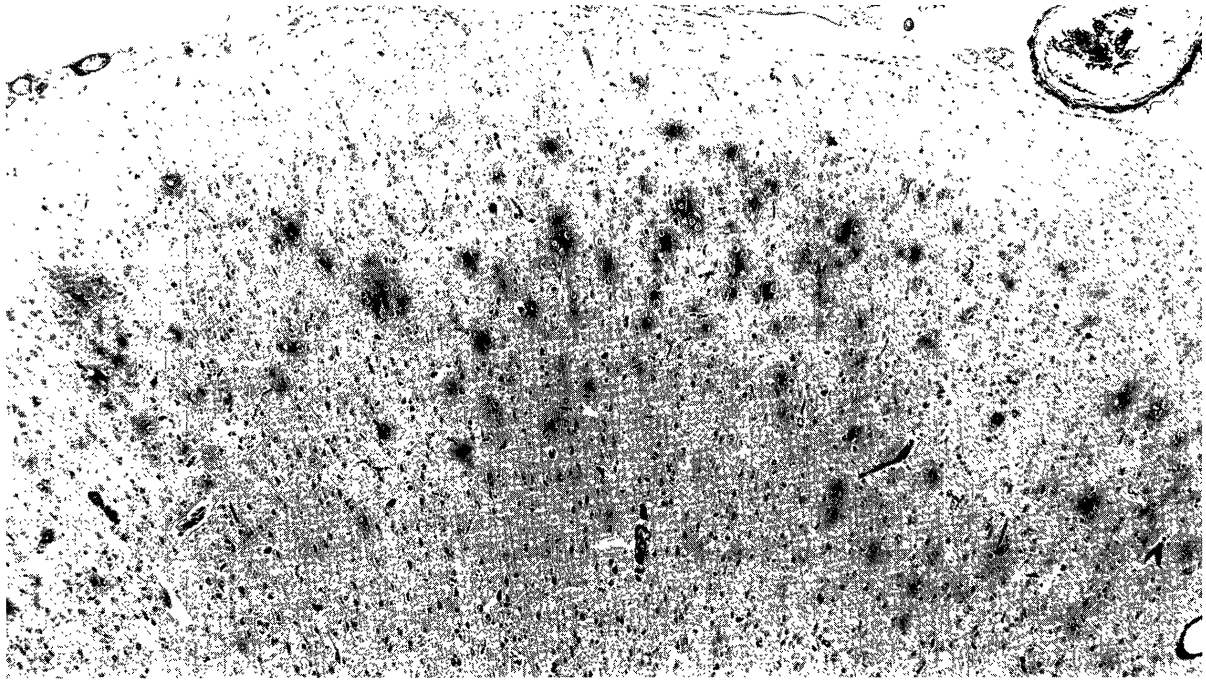

Fig. 1. Temporal cortex stained with modified Bielschowsky stain. Senile plaques (SP), most of which are diffuse plaques, are distributed throughout the cortical layers, especially in the cellular layer. Dog No. 33, × 60.

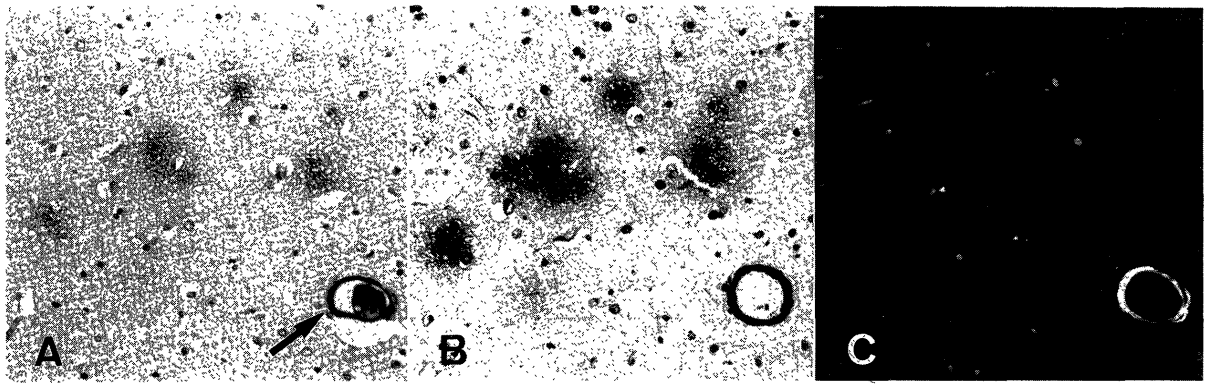

Fig. 2A-C. Serial sections of the temporal cortex stained with β-protein immunostain (A), modified Bielschowsky stain (B) and thioflavin S stain (C). Arrow indicates cerebrovascular amyloidosis (CA). Dog No. 16, × 180.

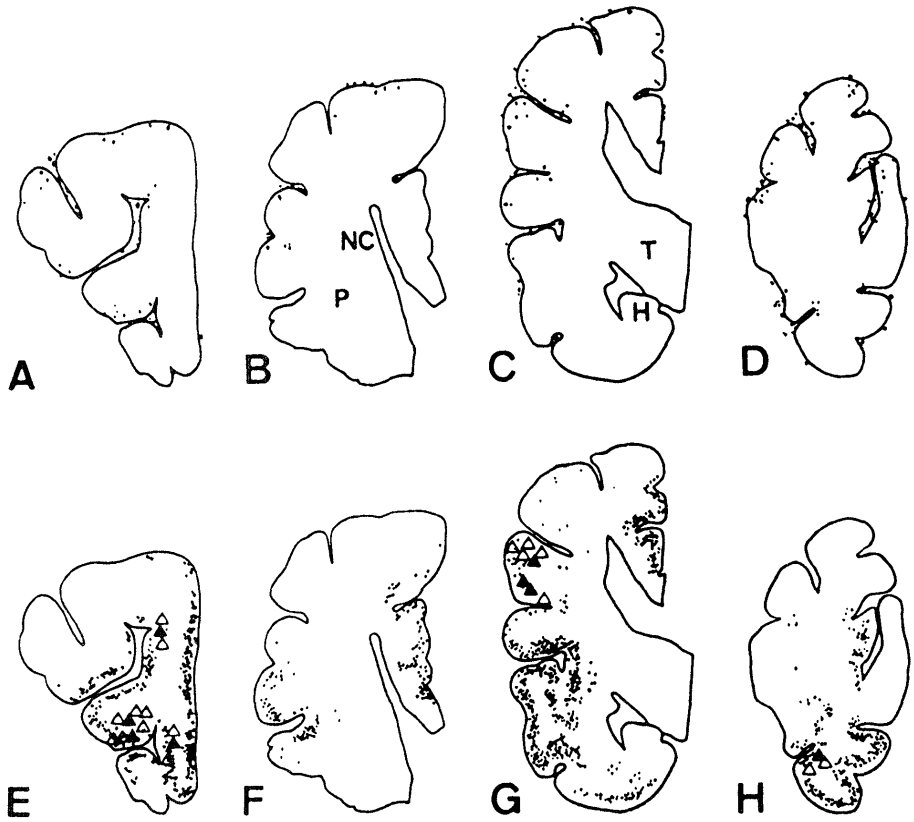

Fig. 3A-H. Schematic diagram of the distributions of CA and of three types of SP in four coronal sections at different levels through one cerebral hemisphere of a 17-year-old dog (Dog No. 33). Sections A-D: CA; E-H: SP. Dots represent CA in A-D and diffuse plaques in E-H, respectively. Mature plaques are represented by ▲ and perivascular plaques by △. H: hippocampus; NC: nucleus caudatus; P: putamen; T: thalamus.

cerebral cortex, although these neurons appeared histologically normal (Fig. 7). No amyloid deposits were detected in the non-neural tissues examined.

DISCUSSION

Using a thioflavin S stain, Selkoe *et al.* [31] demonstrated CA in all nine 10- to 12-year-old dogs examined. The incidence of SP and CA in the present study was comparable to that of non-demented aged human beings [26]. This implies that dogs may serve as a model for the study of brain amyloid deposits in the process of normal aging in human beings.

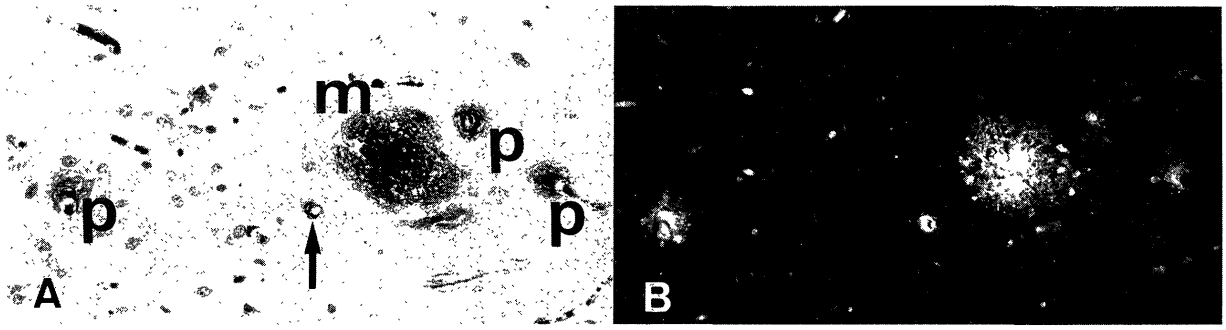

Fig. 4A, B. Serial sections of the frontal cortex stained with modified Bielschowsky stain (A) and thioflavin S stain (B). The mature plaque (m), perivascular plaques (p) and CA (arrow) are closely correlated. Dog No. 33, × 180.

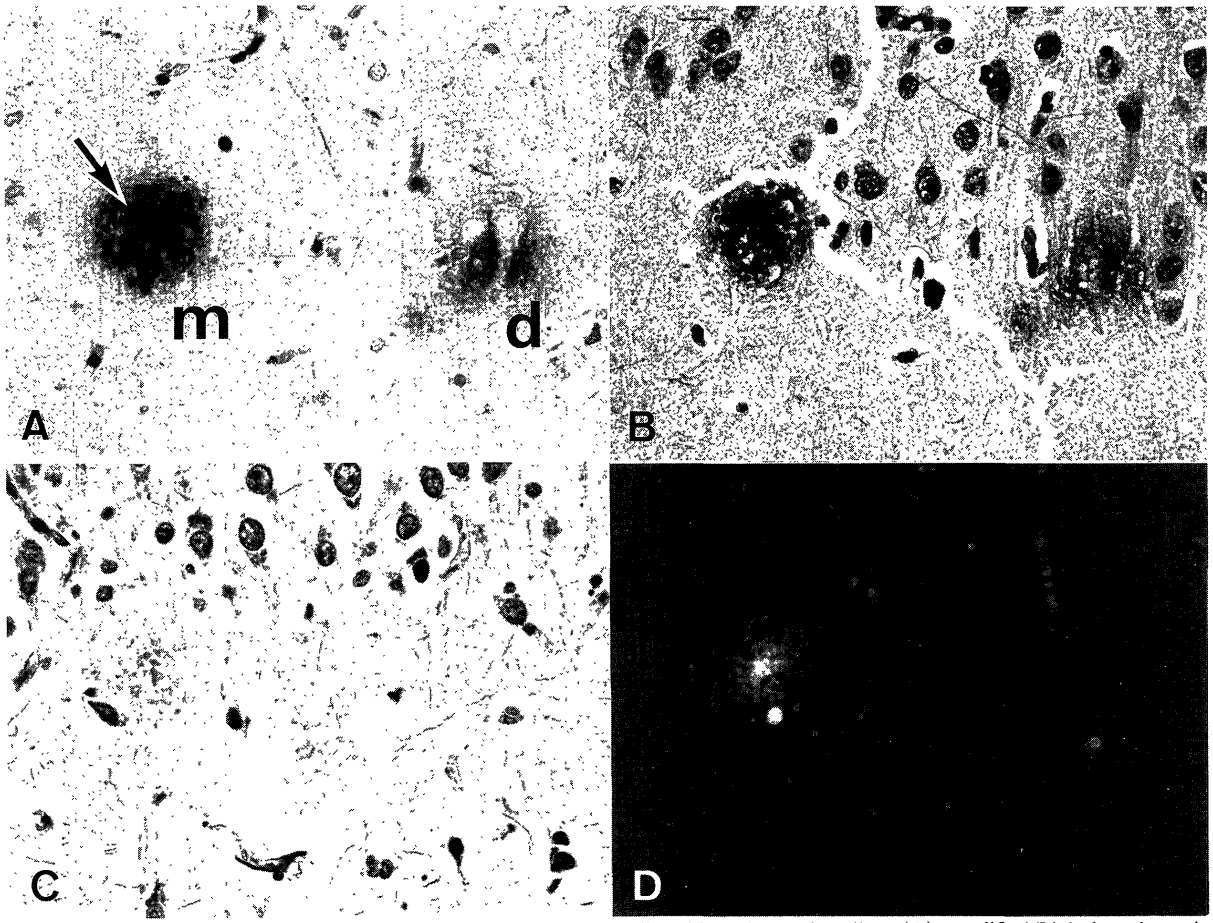

Fig. 5A-D. Serial sections of the frontal cortex stained with periodic acid-methenamine silver (A), modified Bielschowsky stain (B), Bodian stain (C), and thioflavin S stain (D). The mature plaque (m) contains a core of dense amyloid deposits (arrow). The diffuse plaque (d) is not demonstrated by Bodian and thioflavin S stains. Note the involved capillary, glial and nerve cell bodies in the diffuse plaque (d). Dog No. 10, × 360.

Previous studies on the brains of AD-affected and nondemented human beings demonstrated that diffuse plaques were predominant of the types of SP [35, 37, 45]. This finding coincides with our observation on the brain of the aged dogs. Diffuse plaques have been suggested to be very early SP based on the analysis of the Down's syndrome 'model' [2, 7, 13, 21]; this also appears to be the case in the aged dogs because no SP types other than diffuse plaques were observed in the dogs with a small number of SP.

In nondemented aged human beings and monk-

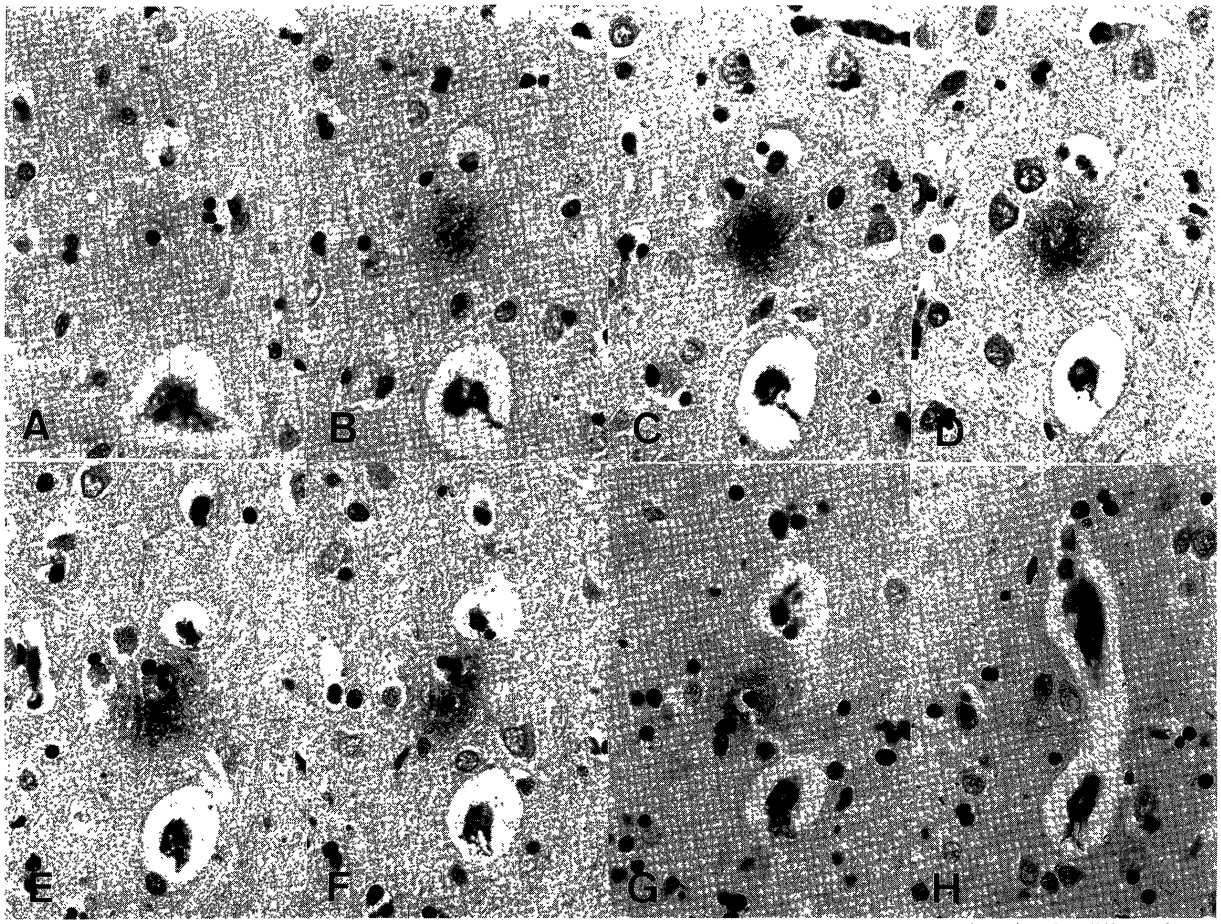

Fig. 6A-H. Serial sections through a small diffuse plaque stained with modified Bielschowsky stain (A-H). The diffuse plaque appears not to contain capillaries. Note the involved glial and nerve cell bodies in the diffuse plaque. Dog No. 5, × 360.

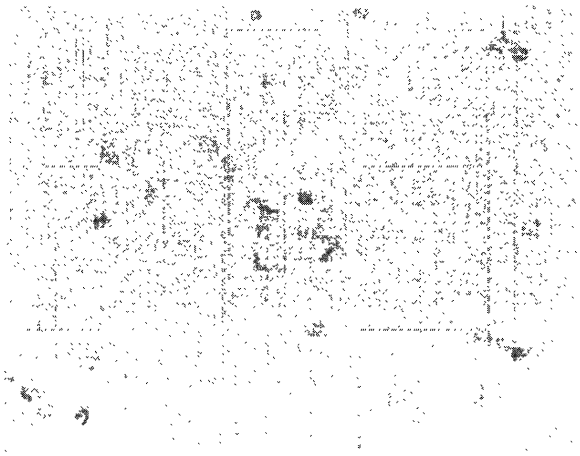

Fig. 7. β-Protein immunostain demonstrating amyloid deposits on the periphery of an apparently normal neuron in the temporal cortex. Dog No. 16, × 540.

eys, both SP and CA are mostly confined to the cerebrum [26, 38]. This distribution pattern is comparable to that of the aged dogs in this study. In addition, the severity of both lesions appeared to increase with advancing age, indicating that the lesions are age-related. Furthermore, of the dogs with SP, all but two were accompanied by various degrees of CA. Therefore, it is conceivable that SP and CA share some triggering causes and mechanisms which lead to amyloid deposition.

A close topographic relationship between capillaries with CA and all types of SP except diffuse plaques has been noted in human beings and monkeys [23, 38]. This finding agrees with the observations on the brains of the present dogs and supports the concept of a vascular origin, i.e. a circulating source for amyloid in certain types of SP [17, 23, 30].

Studies of the brains of human beings demonstrated the involvement of cell bodies of neurons

and/or glia in diffuse plaques [1, 2, 28, 45]. Diffuse plaques of smaller size, those being regarded as an initial stage of SP, often showed no association with capillaries [1]. Similar findings were also observed in this study. These findigns, combined with the observation that amyloid deposits were demonstrated on the periphery of neurons in the brains of the dogs, support the hypothesis of a neuronal or glial cell origin of plaque amyloid [3, 9, 10, 34].

Expression of APP and deposition of amloyid β-protein in non-neural tissues were demonstrated in AD affected and normal aged humans, which indicated a circulating source for amyloid [15, 32]. However, we failed to detect any sign of amyloid deposits in the non-neural tissues examined. This finding has two implications. First, the negative result may be attributable to insufficient stainability by the method used in this study, e.g. antiserum reactivity and immunohistochemical procedure. Second, the aged dogs examined had actually no amyloid deposits in the non-neural tissues examined. This is also likely because normal aged human beings showed only equivocal results in skin in β-protein immunohistochemistry, though definite and specific staining was observed in skin and intestine of AD patients [15].

Presence of two distinct modes of amyloid deposition in AD has been suggested based on the biochemical evidence that there are differences between CA and SP β-protein with respect to its solutibility, C-terminal sequence and N-terminal blockage [27]. Taken together, our results suggest that different mechanisms may also be involved in the pathogenesis of diffuse plaques, other types of SP and CA in the brain of aged dogs.

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