PATHOLOGICAL STUDIES ON SENILE PLAQUES, CEREBROVASCULAR AMYLOIDOSIS AND ASTROCYTIC GLIOSIS IN THE CENTRAL NERVOUS SYSTEM (CNS) OF AGED DOGS: AN ANIMAL MODEL SHARING FEATURES WITH CNS AGEING OF HUMAN BEINGS

AKINORI SHIMADA

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CONTENTS

PREFACE

Changes normally associated with aging in the central nervous system (CNS) of human beings include shrinkage of the brain and probable loss of neurons, gliosis, amyloid deposits such as senile plaques (SP) and cerebrovascular amyloidosis (CA), deposition of lipofuscin pigments in neurons and glial cells, and cytoskeletal abnormalities of neurons such as axonal dystrophy, neurofibrillary tangles (NFT), granulovacuolar degeneration, Hirano bodies and Lewy bodies. Corpora amylacea in the astrocytic processes are also a known form of aged changes.

The presence of numerous SP and nerve cells containing NFT in the cerebral cortex, hippocampus and amygdala is widely regarded as representing the histopathological hallmark of Alzheimer's disease (AD) [72]. It is also well recognized that these same pathological changes occur, but to a much lesser extent, in many non-demented individuals, particularly after the age of 65 years [18, 76, 80, 128, 131].

AD occurs throughout the world and accounts for one-half to two-thirds of all cases of late-life intellectual failure in many developed countries that have achieved high life expectancies. The application of biochemical and molecular approaches to AD has now led to the identification of several gene products whose alterations may underlie the progressive dysfunction and dystrophy of neurons and glia that occur in the limbic and association cortices and in certain subcortical nuclei that project to them. The greatest challenge to investigators studying AD, and the greatest source of ongoing controversy, derives from attempts to place the observed morphological and

biochemical changes into a temporal sequence of pathogenesis.

Animal models of human diseases usually bring closer the day when we can help affected people. The need for an animal model for AD is particularly pressing considering the number of people who are suffering or will suffer from this disease. Amyloid deposits similar to those observed in human brains have been described in aged non-human primates [19, 59, 60, 120, 135, 136, 139]. Numerous SP were also observed in the brains of aged bears [14]. SP and CA in the brains of dogs were observed by Wisniewski et al. [140] and Uchida et al. [130]. An immunohistochemical study revealed the presence of SP in five species of aged mammals including dogs, and showed that amyloid deposits that are a part of these SP consist of the amyloid β-protein (AβP) [112]. Recently, Kawabata et al. [51] produced transgenic mice carrying SP, NFT and neuronal degeneration similar to those in the AD brain.

In this thesis, data on the spectrum of morphological appearance of amyloid deposits in the CNS of aged dogs are presented. This study shows that the cerebral amyloid deposits in aged dogs are morphologically and immunohistochemically similar to those in human beings. Diffuse astrocytic gliosis, which is the change known to be associated with normal aging and AD, is also demonstrated in aged dogs. Aged dogs, therefore, may become the animal of choice for studying the mechanisms involved in CNS aging of human beings.

Current information on the pathology of cerebral amyloid deposition will be presented in this introduction, because this study attempts to get a insight into the pathogenesis of cerebral amyloid deposition by the systemic

examination of the CNS of aged dogs.

Review: the pathology of cerebral amyloid deposition

The classic SP of AD is a complex lesion of the cortical neuropil containing several abnormal elements: a central deposit of extracellular amyloid fibrils (the core) surrounded by dystrophic neurites (both dendrites and axon terminals), activated microglia, and hypertrophic astrocytes [142]. The sequence of involvement of these elements in plaque formation and the time required to generate such 'mature' plaques are poorly understood. Although plaques with these characteristics can occasionally be observed in other age-related degenerative brain diseases, they occur abundantly in three conditions: AD, trisomy 21, and, to a lesser extent, normal brain aging. The fibrils of the plaque core are ultrastructurally distinct from paired helical filaments of NFT (the core fibrils are extracellular, unpaired, and ~8 nm in diameter), but closely resemble the biochemically diverse amyloid filaments that accumulate extracellularly in nonneuronal tissues in a variety of unrelated systemic amyloidosis [33]. Amyloidosis is a general term in pathology that designates diseases in which deposits of 5–10 nm proteinaceous fibrils (amyloid) accumulate progressively in the extracellular spaces of tissues and their vasculature. The amyloid filaments are usually composed of proteolytic fragments of normal or mutant gene products; the particular polypeptide forming the filaments differs among the amyloidoses. Because the amyloid deposits in such diseases invariably lead to local tissue injury rather than accumulate as inert by-products of

the pathological process, it has long been assumed by some that this would also be the case in AD. In addition to plaque cores, amyloid deposits occur in the walls of some or many cerebral and leptomeningeal blood vessels in AD.

In 1984, Glenner and Wong [34] first reported the subunit composition of amyloid filaments isolated from meningeal vessels and solubilized in guanidine hydrochloride. The ~4 kd monomer was sequenced to residue 24 and found to be a novel peptide, designated the AβP (Fig. 1). The sequence of AβP derived from the meningovascular deposits was later shown to extend to 40 residues [46]. The purification of SP cores from AD cortex revealed an ~4 kd subunit protein of essentially identical amino acid composition [79, 111]. Antibodies to either the synthetic AβP [79, 145] or the native, purified AβP [46, 111] revealed the complete cross-reaction of vascular and plaque amyloid. Moreover, AβP immunohistochemistry of AD cortex has revealed innumerable noncompacted deposits of AβP ('diffuse' or 'preamyloid' plaques) that contain very few or no surrounding dystrophic neurites or glia [147]. Such amorphous deposits were not detected by the classic amyloid stains, Congo red and thioflavin S. These and many other recent studies indicate that there is a much greater amount of AβP in AD brain than previously believed, including many diffuse plaques in brain regions that appear to be largely unaffected clinically (for example, cerebellum, striatum, and thalamus) [49, 90].

The initial cloning of AβP cDNAs demonstrated that the isolated peptide was a proteolytic fragment of a 695 residue precursor protein (β-amyloid precursor protein: βAPP), whose sequence predicted a glycosylated polypeptide spanning the membrane once near its C-terminus [50]. The

39-42 residue AβP region comprises the 28 amino acids just outside of the membrane plus the first 11-14 amino acids of the hydrophobic transmembrane domain (Fig. 2). Subsequent cloning has identified several alternatively spliced transcripts (563, 751, or 770 amino acids long), which contain an insert with ~50% homology to the Kunitz family of serine protease inhibitors (KPI) [21, 57, 99, 124]. The βAPP polypeptides themselves have been identified in brain, nonneural tissues, and cultured cells as a complex group of 135 kd membrane-associated proteins [114]. In cultured cells, βAPP has been shown to undergo N- and O-glycosylation and tyrosine sulfation [93, 137], as well as constitutive proteolytic cleavage that releases the large, amino-terminal soluble protein into the medium [137] and retains an ~10 kd hydrophobic carboxy-terminal fragment in the membrane [114]. Recently, Esch and colleagues [25] have purified and sequenced both of these fragments from cDNA-transfected cells and established that constitutive cleavage occurs at residue 16 of the AβP region (residue 687 of βAPP$_{770}$) (Fig. 2), thereby precluding β-amyloid formation. Two (or more) alternative cleavages must occur in at least a subset of βAPP molecules during aging and in AD to liberate the intact AβP fragment. The secreted amino-terminal fragment containing the KPI insert has been shown to be identical to the previously described serine protease inhibitor, protease nexin II (PN-II) [92, 132].

The biological functions of βAPP are not yet well understood. The high degree of evolutionary conservation of βAPP molecules, their expression in virtually all cells and tissues examined to date, and the characteristics of the βAPP promoter region [105] all suggest that there are important,

multiple functions of this family of alternatively spliced proteins. Forms of βAPP containing the KPI motif have been shown to inhibit trypsin and other serine proteases in vitro [57, 117]. Based on studies of the secreted serine protease inhibitor, protease nexin-I, it has been hypothesized that the PN-II portion of βAPP may be secreted from cells in order to regulate extracellular serine proteases, following which the protease inhibitor complex may bind back to a cell surface receptor and be internalized. Another putative function for βAPP based on in vitro experiments is as an autocrine or growth-promoting molecule. This hypothesis is based on the finding of a reduced rate of fibroblast proliferation following treatment of the cells with βAPP antisense oligonucleotides; addition of exogenous PN-II restored normal growth [104]. PN-II has also recently been shown to be an inhibitor of coagulation factor XIa in vitro [118]. Its storage in the α granules of platelets and release following stimulation with platelet agonists such as thrombin or collagen have suggested a role in scar formation and wound repair [132]. Other vitro evidence suggests that βAPP is a component of the extracellular matrix and could participate in cell adhesion [61, 109]. The primary structure of βAPP based on its original cDNA sequence led to the hypothesis that it serves as a cell surface receptor [50], although no direct evidence of such a function has yet been published.

Additional studies establishing the normal biological functions of βAPP will be needed to ascertain whether one or more of these functions is perturbed in AD. It may be that only a small minority of βAPP molecules are alternatively cleaved to release the amyloidogenic AβP fragment and that most precursor molecules continue to be normally processed and remain

functional throughout the disease. Indeed, the lack of phenotypic abnormalities in nonneural tissues that express βAPP as well as in numerous brain and spinal cord regions that contain abundant β APP-producing cells suggests that widespread loss of normal βAPP function is not a fundamental event that underlies the pathogenesis of AD.

Although compositional analysis and fibril reconstitution studies suggest that AβP is the sole subunit protein of the amyloid fibrils in AD, there is growing evidence that several other distinct proteins are intimately and specifically associated with the β-amyloid deposits. These other constituents of the deposits, designated as β-amyloid-associated proteins, include α_1-antichymotrypsin (ACT) [1], complement factors C1q, C3c, and C3d [103], the serum amyloid P protein [13], and heparan sulfate proteoglycans [119]. The latter two constituents are now unique to the β-amyloid of AD, but are found in all types of amyloid deposits occurring in a variety of human diseases. ACT has been found to be present in β-amyloid deposits in the several disorders in which these occur (brain aging in humans and monkeys, AD, and Down's syndrome) [1], but not in other types of amyloid. Moreover, ACT remains associated with the AβP fibrils following heating in sodium dodecyl sulfate [1]. Such a tight association has also recently been reported for heparin sulfate proteoglycan [119].

The presence of complement components, acute phase protein such as ACT and serum amyloid P, and activated microglia [45, 143] in β-amyloid deposits suggests that an inflammatory process is occurring within the SP. The nature of this inflammatory process, the possible role of cytokines [39], and the question of whether the process may represent an immune-mediated

reaction [82] remain issues for further study.

The discovery that amorphous, largely nonfilamentous deposits of AβP that have few or no surrounding dystrophic neurites, designated as 'diffuse plaques', are much more common in both AD and normal aged brains than are 'classic plaques' has heightened interest in the long-standing and unresolved question of the origin of β-amyloid deposits. The juxtaposition of the amyloid cores of classic plaques to degenerating neurites (including axonal terminals) and activated glial cells has led to suggestions that the extracellular amyloid may arise from βAPP in altered neurons [78], astrocytes[116], or microglia [143]. The high levels of βAPP expression in neurons demonstrated by in situ hybridization [5] and the finding that βAPP undergoes fast axonal transport [63] have been used to support a neuronal origin for AβP deposits. However, virtually all cells in the nervous system, including astrocytes, microglia, and endothelial cells, as well as innumerable nonneural cells, express βAPP. Furthermore, many diffuse AβP deposits in AD brain are now known to contain very few or no morphologically altered neurites [47, 49, 147]. Electron microscopy of these deposits reveals occasional altered cell processes, but much of the AβP-immunoreactive neuropil is ultrastructurally indistinguishable from the surrounding normal neuropil [149].

Support for the hypothesis that such amorphous AβP deposits precede rather than follow neuritic and glial alteration comes from immunocytochemical studies of Down's syndrome. Humans with trisomy 21 develop typical amyloid-bearing neuritic plaques and neurofibrillary tangles indistinguishable from those in AD if they survive into their fifth

and sixth decades. It is generally assumed that the invariant occurrence of β-amyloid lesions in middle-aged subjects with trisomy 21 is related to the increased dosage of the βAPP gene, which has been localized to the mid-portion of the long arm of chromosome 21. Examining the brains of trisomic patients dying at various ages should reveal the temporal sequence of AD-type neuropathological changes. Such studies have now shown that diffuse AβP deposits can be detected in Down's subjects as early as the mid-teens and occur commonly in the twenties and thirties [30, 73]. At these ages, few or no mature neuritic plaques, dystrophic glia, or neurofibrillary tangles are observed. Since the brain lesions of older trisomic 21 subjects are indistinguishable from those of AD, it is likely that similar deposition of AβP in diffuse form precedes neuritic and glial alteration in AD as well. Indeed, both the cerebral cortex and some clinically uninvolved regions of AD brain show myriad diffuse plaques with little or no neuritic/neuronal or glial changes, even at the end stage of the disease.

SP and many cerebral microvessels in aged monkeys and dogs were shown to contain AβP immunochemically identical to that in AD and Down's syndrome [112]. Recently, cloning of the homologs of all three major human βAPP isoforms in cynomolgus monkey has revealed that βAPP_{695} is completely homologous to the human polypeptide, and βAPP_{751} and βAPP_{770} contain only 1 and 4 amino acid substitutions, respectively [97]. The complete homology of βAPP_{695} between the monkey and human contrasts to the 18 and 22 amino acid substitutions found in the rat and mouse, respectively [115, 146]. Such study on βAPP of dogs is not yet performed.

Studies of diffuse plaques in Down's and AD brains indicate that

degenerating neurites and glia are not a prerequisite for AβP deposition. Therefore, the βAPP molecules that give rise to AβP originate either from structurally normal neurons or glia or from another source. The frequently abundant AβP deposits found in the wall of meningeal arteries outside of brain tissue suggest that cells intrinsic to the vessel wall (for example, endothelial or smooth muscle cells) or cellular elements of the blood could serve as a source of the βAPP molecules that yield vascular AβP. A hematogenous origin for these deposits would be in concert with certain systemic amyloidoses known to derive from circulating precursors, for example, immunoglobulin amyloid in multiple myeloma and primary systemic amyloidosis, AA amyloid in the secondary amyloidosis of chronic inflammatory diseases, and transthyretin amyloid in familial amyloidotic polyneuropathy and senile systemic amyloidosis. Circumstantial evidence that the β-amyloid of SP might also be vessel- or blood-derived comes from the ultrastructural findings of Miyakawa et al. [86], which demonstrated a capillary bearing amyloid at its basement membrane in every SP subjected to serial ultrathin sectioning. In support of this hypothesis, occasional deposits of AβP immunoreactivity have been observed in and around microvessels of the dermis, subcutaneous tissue, small and large intestine, and adrenal gland in subjects with AD, familial AD, and Down's syndrome [48]. The chemical nature of the AβP deposits in nonneuronal tissues and their relationship to the much more abundant cerebral deposits remain unclear. However, the detection of extracerebral AβP deposits in and around selected microvessels supports the notion that AβP can be deposited without preceding neuronal/glial degeneration, indeed, in the

absence of local neurons and glia. Furthermore, the discovery of nonneural AβP-immunoreactive deposits strengthens the parallels of β-amyloidosis with the systemic amyloidoses and provides circumstantial support for a vascular or hematogenous origin. The resolution of the origin of cerebral β-amyloid has potentially important therapeutic implications, since a hematogenous origin should be easier to interrupt than an origin from neurons or other intrinsic brain cells. Much further study is needed before the question of whether AβP originates from βAPP in neurons, glia, vascular cells, extracellular fluid (blood and cerebrospinal fluid), or from multiple sources can be settled.

CHAPTER I

MODIFIED BIELSCHOWSKY AND IMMUNOHISTOCHEMICAL STUDIES ON SENILE PLAQUES AND CEREBROVASCULAR AMYLOIDOSIS IN THE CENTRAL NERVOUS SYSTEM OF AGED DOGS

I. INTRODUCTION

Senile plaques (SP) and cerebrovascular amyloidosis (CA) are well-known forms of cerebral amyloid deposits in the brains of human beings with Alzheimer's disease (AD) [72, 138]. Amyloid deposits are composed primarily of a 42-43 amino acid fragment, designated amyloid β-protein (AβP) of a larger amyloid precursor protein (βAPP) [36, 50, 99, 102, 115, 123, 137]. Changes in the transcription, translation or post-translational processing of βAPP are likely to play a role in the excessive AβP formation in AD [12, 24, 35, 43, 94, 115]. Amyloid deposits may also occur in nondemented aged human beings and other mammals, including nonhuman primates, polar bears and dogs [18, 112, 113, 120, 128, 130, 136, 139, 140].

There are two main hypotheses, the neuronal and vascular, as to the source of βAPP. The primary evidence supporting the neuronal origin is the high expression of βAPP and/or βAPP mRNA in neurons [5, 7, 62]. 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 senile plaques [32, 85, 86]. 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 [136]. The resolution of the relationship of amyloid deposits to local elements of the brain tissue, such as neurites, glia and capillaries has important implications for the pathogenesis of them [47].

In the present study, the morphology of SP and the topographic relationship between SP and CA in the brains of aged dogs were

investigated using sensitive techniques for the detection of amyloid deposits. And then, histopathogenesis of SP was discussed.

II. 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 of visceral or genital organs. They consisted of 15 males and 18 females, with an average age of 13.6 years (range 10 to 17 years) (Table 1). Most of the aged dogs showed symptoms known to be characteristic of aging: inactivity, sleepiness and poor mobility. No neurological signs were observed in the dogs examined.

The brains were fixed in 10% neutral buffered formalin, and processed routinely into paraffin wax embedding. Serial sections (6 μm thick) systematically cut at four levels (frontal lobe, striatum, diencephalon and occipital lobe) (refer Fig. 6) of the left cerebral hemisphere and various levels of the brain stem and cerebellum were stained with hematoxylin and eosin, thioflavin S and modified Bielschowsky stains [152]. Selected sections were also stained with periodic acid-methenamine silver [41], Luxol Fast Blue, Bodian and Congo red stains or processed for immunohistochemistry using antibody directed against AβP (1-24 residues; rabbit polyclonal, kindly provided by Dr. N. Kitaguchi of Life Science Research Laboratories, Asahi Chemical Industry Co., Ltd.) [145]. 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 AβP [58]. 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 x100. Observations at the higher magnification (x200) were used to better differentiate plaque types. The 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. On thioflavin S-stained and immunostained sections, CA was assessed semiquantitatively by division into four grades of severity: - ; not observed, +; some lesions confined to meningeal vessels, ++; some lesions in both meningeal and neuroparenchymal vessels, and +++; many lesions in both meninges and neuroparenchyma. The number and distribution of SP and CA were determined on the coronal sections by making plots for these lesions with the aid of the camera lucida method.

Histological examination using thioflavin S and modified Bielschowsky stains, and AβP immunostain for the presence of amyloid deposits was also made to the spinal cord and non-neural tissues including the liver, spleen, kidney, heart, lung, gut, pancreas and skin of the three dogs with the prominent CA and SP formation (Dog Nos. 10, 26 and 33).

III. RESULTS

The brains of the aged dogs showed normal gross-appearance except moderate to severe thickening of the meninges (Fig. 3) and mild dilatation of the ventricles. There appeared to be no difference in the age-related symptoms and gross findings of the central nervous system (CNS) between the dogs with SP and ones without SP. Histological examination of the CNS revealed typical aged changes, such as nerve cell loss, lipofuscin accumulation in neurons, occasional dystrophic axons and presence of corpora amylacea. AβP immunohistochemistry demonstrated SP and CA, these lesions being identically shown by the modified Bielschowsky and/or thioflavin S stains (Fig. 5). No neurofibrillary tangles were identified in neurons.

Most SP were observed in the neocortex of the cerebrum. Their densities were highest in the cingulate and temporal cortices. The lesion spared dorsal aspect of the coronal sections cut at various levels of the cerebral hemispheres. There were occasional SP in the subcortical nuclei, including the caudate nucleus and putamen, and hippocampus. No SP were detected in the cerebellum, brain stem and spinal cord.

Based on the morphological arrangement and relative proportions of amyloid and neuritic components, SP have been classified to several types [101, 121, 142, 148]. In the present study, three types of SP were identified on the basis of the modified Bielschowsky stain: diffuse plaques (diffuse amyloid deposits lacking amyloid cores and apparent neuritic elements), mature plaques (amyloid cores with or without apparent neuritic elements),

and perivascular plaques (dense amyloid deposits closely associated with blood vessels). Thioflavin S stain confirmed the presence of discrete deposits of amyloid that corresponded to the locations of CA and SP with dense amyloid deposits. The relationship between the age and 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. No SP subtypes other than diffuse plaques were observed in the brains which had a small number of SP; the brains with advanced CA contained all subtypes 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 (Fig. 6). No CA was observed in 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. 6).

Diffuse plaques, which predominated in the plaque subtypes, were distributed throughout the cortical layers, especially in cellular layers with no topographic relationship to CA (Figs. 4 and 6). Mature and perivascular plaques, both of which contained compact amyloid deposits, showed close topographic relationship to CA (Figs. 6 and 7). 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

Table I. Pathological data of the dogs examined

Dog No.	Age (years)	Sex	SP[a] subtype			CA[b]
			Diffuse	Mature	Perivascular	
1	10	M	−[c]	−	−	−[d]
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.
c) Number/four coronal sections of the cerebral hemisphere:
−; absent, +; 1-499, ++; 500-999, +++; ≧1000.
d) −; not observed, +; some lesions confined to meningeal vessels, ++; some lesions in both meningeal and neuroparenchymal vessels, +++; many lesions in both meninges and neuroparenchyma.

few neurons and glial cells (Fig. 8). Whereas, those of smaller size ($\leqq 50\,\mu m$) seldom involved capillaries (Fig. 9). AβP immunohistochemistry demonstrated amyloid deposits on the periphery of neurons with a normal appearance in the cerebral cortex (Fig. 10). No amyloid deposits were detected in the non-neural tissues examined.

IV. DISCUSSION

This study demonstrated that all of the SP and CA, both of which were reacted with AβP antiserum, corresponded to those shown by the modified Bielschowsky and/or thioflavin S stains. Studies of aged human brains have also shown this correspondence [44, 78]. Because of restrictions such as limited availability of the antibody and required skills, few AβP immunohistochemistry has been employed on brain tissues of domestic animals. Therefore, the findings shown in this study may be helpful to the comparative study of aged animals.

Amyloid deposits have been demonstrated in SP and cerebral vessels in aged human beings, nonhuman primates and several other mammals [53, 112, 136, 148]. In the present study, using the sensitive methods for the detection of amyloid deposits, such as AβP immunohistochemistry, modified Bielschowsky and thioflavin S stains [112, 144, 147], SP and CA were demonstrated in 45% and 67% of the aged dogs, respectively. The incidence of amyloid deposits in the aged dogs is comparable to that of nondemented aged human beings [90]. Using a thioflavin S stain, Selkoe et al. [112] demonstrated CA in all nine 10- to 12-year-old dogs examined. In contrast, no sign of any form of amyloid deposits, SP or CA, was demonstrated in the brains of cats, including three animals over 18 years of age (unpublished data by the present author, A. Shimada). Studies on this possible species difference may be useful in elucidating the mechanism of AβP deosition in the brain.

The immunohistochemical characteristics of brain amyloid deposits in the

present aged dogs, combined with their morphological appearance, suggest a strong similarity to the human type of lesions and make it likely that, as in the human beings, the AβP [34] is the main proteinaceous constituent of the SP and CA. Large numbers of SP have been proposed as a diagnostic criterion for the neuropathological diagnosis of AD [55]. Application of this criterion to the dog brains examined in this study would yield a positive diagnosis in some cases. One point, however, has to be kept in mind: no neurofibrillary tangles were detected in the brains investigated.

Diffuse plaques show AβP-immunoreactivity with little evidence of Congophilic amyloid deposition, whereas other subtypes of SP contain dense amyloid (AβP) deposits which are birefringent and fluorescent [122]. Electron microscopy demonstrated that diffuse plaques contained only a sparse accumulation of amyloid fibrils, which might be below the threshold level that could be detected by thioflavin or Congo red staining at the light microscope level [149, 150]. Of the subtypes of SP found in this study, diffuse plaques were predominant. This finding coincides with previous observations on the brains of AD-affected and nondemented human beings [121, 131, 148]. Diffuse plaques have been suggested to be very early SP based on the analysis of the Down's syndrome 'model' [3, 30, 44, 74]; this also appears to be the case in the present aged dogs because no SP subtypes other than diffuse plaques were observed in the dogs with a small number of SP. The detailed mechanism of formation of amyloid fibrils in SP and cerebrovascular walls is still unknown.

There have been controversies as to the relationship between SP and CA because the two kinds of lesions do not invariably coexist [75, 87, 136]. In

the aged dogs, both SP and CA were mostly confined to the cerebrum. This distribution pattern is comparable to that of nondemented aged human beings and monkeys [90, 136]. 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.

As to the topographic relationship between SP and CA, a close association between capillaries with CA and all subtypes of SP except diffuse plaques were demonstrated in the present study. This finding agrees with the observations on human beings and monkeys [85, 136] and supports the concept of the vasculature origin, namely circulating source for amyloid in these subtypes of SP [52, 85, 110]. Expression of βAPP and deposition of AβP in non-neural tissues further support this hypothesis [48, 114]. However, no sign of amyloid deposits was detected in the non-neural tissues examined in the present dogs. This finding has two implications. First, the negative staining is attributable to the method used in this study, such as antiserum and process of immunohistochemistry. 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 the skin in AβP immunohistochemistry, though definite and specific staining was observed in the skin and intestine of AD patients [48].

Cell bodies of neurons and/or glia were almost always involved in diffuse plaques. In addition, diffuse plaques of smaller size, those being regarded as an initial stage of SP, often showed no association with capillaries.

Similar finding was also observed in human beings [4, 101, 148]. These findings, 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 [5, 16, 35, 40, 78, 81, 116].

Presence of two distinct modes of amyloid deposition in AD has been suggested based on the biochemical evidences, including differences in the C-terminal sequence, N-terminal blockage, and solubility of the AβP between CA and SP [83, 100]. Taken together, results of the present study suggest that different mechanisms may also be involved in the pathogenesis of diffuse plaques, other subtypes of SP and CA in the brain of aged dogs.

V. SUMMARY

The morphology of senile plaques (SP) and the distributions of SP and cerebrovascular amyloidosis (CA) were studied by employing thioflavin S and modified Bielschowsky stains, and AβP immunohistochemistry on serial sections of the brains of aged dogs older than 10 years of age. The aged dogs developed SP which were morphologically and immunohistochemically similar to those occurring in aged human beings. Based on the morphological characteristics demonstrated by the modified Bielschowsky stain, SP in the brains of the dogs were grouped into three types; diffuse, mature and perivascular. Out of them diffuse plaques were predominant. Most SP were observed in the cerebral cortex. Their densities were highest in the cingulate and temporal cortices. There were occasional SP in the subcortical nuclei, including the caudate nucleus and putamen, and hippocampus. Mature and perivascular plaques, both of which contained compact amyloid deposits, always showed close topographic relationship to blood vessels with CA. Whereas, most of diffuse plaques showed no topographic relationship to CA. Cell bodies of neurons and/or glia were almost always involved in diffuse plaques. In addition, AβP immunohistochemistry demonstrated amyloid deposits on the periphery of occasional neurons. These findings suggested that different mechanisms may be involved in the development and evolution of the different subtypes of SP in the brain of aged dogs.

CHAPTER II

IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON AGE-RELATED ASTROCYTIC GLIOSIS IN THE CENTRAL NERVOUS SYSTEM OF DOGS

I. INTRODUCTION

Astrocytic gliosis (AG) in the brain has been reported in aged normal human beings and in patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer type [6, 23, 42, 69, 106, 127]. The phenomenon is believed to be a reaction to the loss of neurons and dendrites [6, 108]. AG as an aged change was also reported in the brains of animals, including rats, macaque monkeys and mice [2, 20, 29, 65, 68, 91]. No consistent results were shown in these studies of animals and this discrepancy was attributed to differing methods used, such as the number of animals, staining methods and counting techniques [42]. In addition, few studies on age-related ultrastructural changes in glial cells have been performed in both human beings and animals [20].

In this study, electron microscopy and immunohistochemistry for glial fibrillary acidic protein (GFAP), which is the primary constituent of glial intermediate filaments and is considered to be exclusively localized in astrocytes [8], were employed. Thus, as Mandybur et al. [71] stated, the term 'astrocytic gliosis' in this study refers to 'increase in GFAP-positive astrocytes (GFAP-PA)' or 'increased GFAP immunoreactivity' because not all astrocytic glia is positively stained by the GFAP immunohistochemistry. The aim of the present study is to report the pattern of age-related AG in the central nervous system (CNS) of dogs.

II. MATERIALS AND METHODS

Twenty-six dogs, 16 females and 10 males, were used in the present study (Table II). Seventeen were old dogs, ranging in age from 11 to 18 years (mean 14.5 years), and nine were young controls, 2 months to 4 years of age (Dog Nos. 10-15, 17-20, 22-25 of this Chapter correspond to Dog Nos. 7, 10, 8, 11, 16, 20, 24, 23, 27, 26, 29, 30, 32 and 33 of Chapter I, respectively). No signs of neurological disorders were observed in the dogs. The dogs were killed by euthanasia or died of a variety of disorders, including heart worm disease, renal failure, and tumor of visceral or genital organs. Four aged dogs (Dog Nos. 10, 13, 17 and 26) and four young controls (Dog Nos. 2, 3, 5 and 6) were anaesthetized with pentobarbital and perfused through the heart with 2 liters of 4% paraformaldehide in phosphate buffer (pH 7.2). During the perfusion, the descending aorta was clamped with a hemostat to save fixative. The dogs were decapitated and the exposed brain was re-immersed in perfusate for overnight at 4°C.

Complete autopsies were performed on these dogs. For light microscopy, the CNS, including the cerebrum, cerebellum, brain stem and spinal cord was fixed in 10% neutral buffered formalin at autopsy. A 5-mm-thick coronal slice cut at various levels of the CNS was dissected. Both left and right cerebral hemispheres of three aged dogs and left cerebral hemispheres of the others were used in this study. The tissue block was then routinely processed and embedded in paraffin. Serial sections (6 μm) were stained by hematoxylin and eosin (HE), Luxol Fast Blue, Nissl and Bodian. Serial sections were also processed for immunohistochemistry using the

peroxidase-antiperoxidase (PAP) method with diaminobenzidine as chromogen. The primary antibody was anti-human GFAP (rabbit polyclonal, Medac GmbH, West Germany). The antibody was used at a dilution of 1 : 200. The sections were counterstained with hematoxylin. Method specificity was evaluated by substituting normal rabbit serum with the primary antibody.

For electron microscopy small tissue blocks (1 mm^3) were taken from the cerebral cortex of the perfused dogs. These tissue blocks were post-fixed in phosphate-buffered 1% OsO_4 for 1 hr, dehydrated in ascending concentrations of ethanol and embedded in Epon. Semithin sections (1 μm) were stained with toluidine blue and were examined light microscopically. Thin sections were stained with lead citrate and uranyl acetate, and were examined with a JEM-100CXII.

At autopsy, tissue samples were also taken from a variety of organs for routine light microscopic studies and fixed in 10% neutral buffered formalin. Paraffin sections (4 μm) were made and stained with HE.

III. RESULTS

Gross and microscopic examinations of the CNS of the aged dogs revealed neither changes indicating ischemia nor signs of neurological disorders other than typical aged changes, such as thickening of the meninges, mild dilatation of the ventricles, occasional dystrophic axons, lipofuscin accumulation in neurons, and nerve cell loss (Fig. 11). No obvious gross atrophy was observed in the brains examined.

An increase in GFAP-PA in the aged dogs, as compared to the young controls, was the most constant histological feature observed in the GFAP immunohistochemistry (Table II). No correlation was noted between the intensity of AG and clinical findings, such as breed, sex and diseases which affected the dogs. AG consisted of hypertrophic astrocytes being characterized by either intense GFAP immunostaining in light microscopy (Fig. 12) or by thick bundles of intermediate filaments in electron microscopy (Fig. 13). These GFAP-PA were observed both interstitially and perivascularly (Fig. 14). There were occasional GFAP-PA surrounding nerve cell bodies (Fig. 15). In all 17 aged dogs, moderate to severe AG was observed with a consistent distribution pattern in the CNS. The change was symmetrical in the dogs whose CNS, including left and right cerebral hemispheres were examined.

A marked increase in GFAP-PA was seen in the subcortical and deep white matters in the cerebrum (Fig. 16a), especially at the cortico-medullary junction (Fig. 16b), corpus callosum, internal capsule, subcortical nuclei in the cerebrum, central nuclei in the cerebellum, various nuclei in the brain

Table Ⅱ. Pathological data of the dogs examined

Dog No.	Age	Sex	Breed	AG[a]
1	2 m[b]	F	Mongrel	±[f]
2	2 m	M	Mongrel	±
3	3 m	M	Mongrel	±
4	6 m	F	Mongrel	±
5	8 m	F	Mongrel	±
6	12 m	F	Mongrel	±
7	12 m	M	Mongrel	±
8	12 m	F	Mongrel	±
9	4 y[c]	M	Mongrel	±
10	11 y	M	Y[d].terrier	++
11	12 y	F	Poodle	+
12	12 y	F	Mongrel	+
13	12 y	M	Mongrel	++
14	13 y	M	Mongrel	++
15	13 y	F	Mongrel	++
16	13 y	F	Maltese	+++
17	14 y	F	Maltese	++
18	14 y	F	Mongrel	+++
19	15 y	M	Mongrel	+++
20	15 y	F	Shiba	+++
21	16 y	F	Shiba	+++
22	17 y	F	Mongrel	++
23	17 y	F	Mongrel	+++
24	17 y	M	M[e].schnauzer	+++
25	17 y	F	Mongrel	+++
26	18 y	M	Mongrel	++

a) AG: Astrocytic gliosis.
b) m: months.
c) y: years.
d) Y: Yorkshire.
e) M: Miniature.
f) The neuropathological lesion is graded: −; absent, ±; slight, +; mild, ++; moderate, +++; marked.

stem, and gray matter of the spinal cord (Fig. 16c). A prominent increase of them was also seen in the cerebral and cerebellar cortices (Figs. 12 and 16d) and thalamus (Fig. 16e).

A moderate increase was observed in the hippocampus (Fig. 16f), white matter of the cerebellum and spinal cord, reticular formation, optic tract and subependymal areas (Fig. 16g) and periaqueductal zone throughout the CNS.

Young control dogs showed minimum numbers of GFAP-PA in the CNS; the molecular layer of the cerebrum and white matter throughout the CNS consistently showed a slight increase in GFAP-PA (Fig. 16h).

Light microscopy failed to demonstrate no obvious degenerative changes of the neural architecture in the area with prominent AG (Fig. 17). Electron microscopy revealed a variety of degenerative profiles of neural components, such as dark axon terminals, swollen synapse-like vesicles with dense contents, and heterogeneous dense bodies in dendrites in the cerebral cortex of the aged dogs (Fig. 18). These changes were often located in the vicinity of hypertrophic astrocytic processes (Fig. 19). Few such profiles were observed in the young controls examined (Fig. 20).

IV. DISCUSSION

In contrast to slight staining of GFAP in the young dogs, prominent GFAP-immunoreactivity was shown in astrocytes of the CNS of the aged dogs, indicating that age-related AG also occurs in dogs. There are several reports which demonstrated astrocytic hypertrophy in the aging CNS [2, 9, 29, 68]. It was not certain whether there was an increase in the volume of GFAP per astrocyte or just an increase in astrocyte size [9]. In the present study, hypertrophic astrocytes with either intense GFAP immunostaining in light microscopy or thick bundles of intermediate filaments in electron microscopy were frequently observed. These findings may suggest that the observed age-related hypertrophy of GFAP-PA be related to an increase in the volume of GFAP per astrocyte. This morphological finding appears to correspond to the results from the biochemical studies of the brains of the experimental animals, in which age-related increase in the concentration of either GFAP or GFAP mRNA was demonstrated [38, 89].

The brains of the aged dogs showed normal gross-appearance except mild dilatation of the ventricles and thickening of the meninges. There appeared to be no difference in the thickness of the cerebral cortex between the aged dogs and the young controls. Similarly, no statistical difference as to the cortical thickness was noted between the AD group and the controls in the morphometric studies of human brains [69, 106, 126]. Therefore, the increase of GFAP-PA could not be related to the shrinkage or condensation of the tissue [69, 106].

Studies of the brains of aged rats demonstrated synaptic deterioration

and functional changes in synaptic transmission mechanisms [64, 133]. Age-related alterations in neural components, including dendrites and synapses have been considered as the stimulus for AG in aging [42, 66]. In this ultrastructural study, degenerating profiles of presynaptic axon terminals and dendrites, some of which were located in the vicinity of reactive astrocytic processes, were frequently observed. These findings are in accord with those of aged human beings [108].

The neocortical neuropil of AD patients is characterized by extensive synaptic pathology, such as synaptic loss [17, 107], dilatation of presynaptic terminals [17, 107] and abnormal synapses [37]. These changes are accompanied by focal amyloid deposition [78, 122], dystrophic neurite formation [31, 125], glial cell hypertrophy in plaques [22], and massive accumulation of abnormal cytoskeletal filaments in dendrites [11]. Recent studies in aged monkeys [15] and Down's syndrome cases [30] have suggested that abnormal neurites and synapse alterations scattered in neuropil precede amyloid deposition. In this regard, it is important to establish the origin and nature of the dystrophic neurites and their relation to synaptic pathology in the cerebral cortex. Electron microscopic studies in AD and aged animals [140, 141] have supported various theories about the origin of the abnormal neurites, including (1) Wallerian degeneration, (2) 'dying back' axonal degeneration, (3) 'dying back' of dendrites [31], and (4) a primary pathological process in the presynaptic terminal. Using synaptophysin-immunoelectron microscopy, Masliah et al. [77] suggested that the dystrophic neurites in the AD neocortex are distorted presynaptic terminal. Changes of the neural components in the present study may, at

least in part, originate from the presynaptic terminal, because of the similarity of these changes in the conventional electron microscopy to those in the AD neocortex.

Recent studies suggested that astrocytes have a phagocytic function, which may contribute to remove degenerative debris in the CNS [2, 88]. However, no dense bodies suggestive of ingestion of degenerative neural components were observed even in the cytoplasm of reactive astrocytes occurring among the profiles of degenerating axon terminals. Recent studies demonstrated evidence of new roles for astrocytes, including neurotransmitter uptake [10, 56], synthesis and secretion of trophic factors [27, 28, 54, 98], targets for hormones and immune system cytokines [26, 67, 134], and regulation of cerebral blood flow [96]. Thus, astrocytes may play important roles in the regulation of the microenvironment in the CNS. Therefore, the possibility exists that astrocytic hypertrophy represents a primary effect of aging rather than a mere secondary response to degenerative events in the CNS [89].

As to the patterns of age-related AG, increased numbers of GFAP-PA have been reported in the cerebral cortex and hippocampus in AD and normal aging brains of human beings [6, 23, 42, 69, 106, 127]. The pattern of AG observed in the present study is similar to that demonstrated in the recent study of the AD and aging cerebrums, in which extensive AG was observed in the deep cerebral white matter, cortico-medullary junction and subcortical gray matter in addition to the cerebral cortex [6]. Using GFAP immunohistochemistry, Mandybur et al. [71] systematically examined the brains of old nude mice, in which an increase in the number of GFAP-PA and

their hypertrophy were also observed in a pattern similar to that found in the dogs except no evidence of AG in the cerebral and cerebellar cortices in the nude mice. This discrepancy may be attributable to the species difference in neuroanatomical and functional specificity of the CNS between the two species.

In animals, occurrence of age-related specific changes, such as senile plaques and cerebrovascular amyloidosis is restricted to some species and in small quantity [71]. In Chapter I, both two changes, which were morphologically and immunohistochemically similar to those of human beings, were demonstrated to occur in the brains of aged dogs with a high incidence. In addition, the present study demonstrated the occurrence of age-related AG with a consistent pattern similar to that of human beings. It thus appears that aged dogs may be suitable as an animal model for the study of the mechanisms involved in CNS aging of human beings.

V. SUMMARY

The pattern of astrocytic gliosis (AG) was examined in 26 2-month-old to 18-year-old dogs using glial fibrillary acidic protein (GFAP) immunohistochemistry and electron microscopy. Coronal sections from various levels of the central nervous system (CNS) were stained with hematoxylin and eosin, Luxol Fast Blue, Nissl, and Bodian in addition to GFAP immunohistochemistry. A consistent pattern of age-related AG was observed in the dogs; the pattern was similar to that of aged human beings. The white matter, cortico-medullary junction, and subcortical nuclei in the cerebrum, central nuclei in the cerebellum, various nuclei in the brain stem, and grey matter of the spinal cord showed even and intense GFAP staining. AG was also prominent in the cerebral and cerebellar cortices and thalamus. Moderate AG was observed in the hippocampus and white matter of the cerebellum and spinal cord. Electron microscopy demonstrated increased numbers of profiles of degenerative neural components in the vicinity of hypertrophic astrocytes in the cerebral cortex of the aged dogs. Moderate to severe AG was consistently shown in the CNS of the aged dogs. In contrast, young normal dogs showed minimum numbers of GFAP-positive astrocytes in the CNS. These findings suggest that the observed AG in the CNS of the dogs may be a morphological expression of aging.

CHAPTER III

THE TOPOGRAPHIC RELATIONSHIPS BETWEEN ASTROCYTIC GLIOSIS AND SENILE PLAQUES, AND ASTROCYTIC GLIOSIS AND CEREBROVASCULAR AMYLOIDOSIS IN THE CEREBRAL CORTEX OF AGED DOGS

I. INTRODUCTION

Astrocytic gliosis (AG) as an aged change was demonstrated in the brains of dogs in Chapter II. AG has been believed to be a reaction to the loss of neural components [6, 106]. However, recent studies of the brains from patients affected with Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT) suggested the possible association of astrocytes in the process of senile plaque (SP) formation [6, 95, 129, 150, 151]. Furthermore, following the observation of β-amyloid precursor protein (β APP) in reactive astrocytes in the experimentally induced lesions of the brains of rats, Siman et al. [116] suggested that astrocytes may be a source of amyloid β-protein (AβP) for SP and cerebrovascular amyloidosis (CA). In Chapter I, SP and CA were demonstrated in the brains of aged dogs, both of which were morphologically and immunohistochemically similar to those occurring in aged humans. In the present study, the topographic relationships between AG and SP, and AG and CA in the cerebral cortex of aged dogs were examined and the possibility of the astrocytic involvement in the evolution of SP was discussed.

II. MATERIALS AND METHODS

Six dogs, three females and three males, ranging in age from 11 to 18 years, were used in this study (Dog Nos. 1-6 of this Chapter correspond to Dog Nos. 7, 11, 24, 31, and 33 of Chapter I, and Dog No. 26 of Chapter II, respectively). All the dogs were demonstrated to have prominent CA and SP formation. The dogs had no history of neurological disorders. Four of the 6 dogs were anaesthetized with sodium pentobarbital and perfused through the heart with 2 liters of 4% paraformaldehide in phosphate buffer (pH 7.2). During the perfusion, the descending aorta was clamped with a hemostat to save fixative. The dogs were decapitated and the exposed brain was re-immersed in perfusate for overnight at 4°C. The left cerebral hemisphere was used in this study.

For light microscopy a 5-mm-thick coronal slice cut at a level of the optic chiasma was dissected from the left hemisphere. The slice contained the cingulate and temporal cortices, where the highest density of SP was observed in the previous study (see Chapter I). The tissue block was then routinely processed and embedded in paraffin. Serial sections (6 μm) were stained with hematoxylin and eosin (HE), Nissl, Bodian, modified Bielschowsky [152] and thioflavin S. In the previous study on the brain amyloid deposits in aged dogs, SP and CA were well shown by the latter two stains (see Chapter I). Serial sections were also processed for immunohistochemistry using the peroxidase-antiperoxidase (PAP) method with diaminobenzidine as chromogen. The primary antibody was anti-human GFAP (rabbit polyclonal, Medac GmbH, West Germany). The sections were

counterstained with hematoxylin. Method specificity was evaluated by substituting normal rabbit serum with the primary antibody.

For electron microscopy small tissue blocks (1 mm^3) were taken from the cerebral cortex of the perfused dogs. These tissue blocks were post-fixed in phosphate-buffered 1% OsO_4 for 1 hr, dehydrated in ascending concentrations of ethanol and embedded in Epon. Semithin sections (1 μm) were stained with toluidine blue and were examined light microscopically. Thin sections were stained with lead citrate and uranyl acetate, and were examined in a JEM-100CXII.

At autopsy, tissue samples were taken from a variety of organs for routine light microscopic studies and fixed in 10% neutral buffered formalin. Paraffin sections (4 μm) were made and stained with HE.

III. RESULTS

The brains of the aged dogs showed normal gross-appearance except moderate to severe thickening of the meninges. The dogs were affected with a variety of disorders, including heart worm disease and tumor of visceral or genital organs.

The cerebral cortex of the aged dogs showed diffuse AG, in which prominent astrocytic reaction was shown around vessels irrespective of the intensity of amyloid deposition in the involved vessels (Fig. 21). In addition, such pattern of AG was consistent throughout the cerebral cortex, where CA was not always accompanied. As shown in the previous study, the majority of SP observed in the aged dogs were of diffuse type (see Chapter I). Diffuse AG was consistently observed throughout the cortex regardless of the intensity of SP formation in these lesions (Fig. 22). Serial sections stained with GFAP and modified Bielschowsky showed no sign of GFAP-positive astrocytes (GFAP-PA) surrounding diffuse SP; many mature SP were demonstrated to contain processes of GFAP-PA (Fig. 23). Electron microscopy frequently showed normal-looking capillaries wrapped with hypertrophic processes of astrocytes (Fig. 24).

IV. DISCUSSION

The findings obtained here showed that diffuse AG as well as perivascular AG appears not to be associated with amyloid deposits in vessels. Perivascular gliosis was also reported in normal aging and AD-affected human beings, and aged mice [6, 69-71]. It has been suggested that perivascular gliosis may be associated with either leakage of blood derived substances or thickening of the vascular wall, which may induce an impairment of exchange of oxygen and metabolites [69, 84]. In the present study, however, there were no signs of vascular pathology, including hemorrhage and ischemic changes. Thus, the cause of age-related perivascular AG is not fully understood at the present time.

The reactive astrocytes around SP have been reported in both humans and animals, although the significance of the relationship between them is still uncertain [6, 22, 23, 70, 106, 136]. Most of the astrocyte-associated SP observed in these studies might have been mature SP, because conventional staining methods used in the previous studies could not have demonstrated diffuse SP, which contain no compact amyloid deposits. Diffuse SP can be visualized when they are stained with AβP immunohistochemistry or improved silver staining methods (see Chapter I).

There are reports which suggest that astrocytes react simply to SP [22, 23]. Recent studies revealed that astrocytes express α_1- antichymotrypsin (ACT) mRNA and βAPP in the AD-affected brains of humans and the injured brains of mice, respectively [95, 116]. Both ACT and βAPP are considered to be associated with the pathogenesis of the core amyloid deposits in SP [1, 50,

57]. In addition, using AβP immunoelectron microscopy Yamaguchi et al. [150, 151] demonstrated astrocytic processes surrounding amyloid fibrils in diffuse SP and subpial AβP deposits in the AD-affected and SDAT-affected brains, respectively. Furthermore, acidic fibroblast growth factor, which has potent neurotrophic actions, was demonstrated in astrocytes surrounding SP in AD cases [129]. These findings may imply that astrocytes are involved in the evolution of SP. In the present study, mature plaques accompanied by a substantial number of GFAP-PA were often observed. In contrast, no obvious accumulation of GFAP-PA was detected around diffuse plaques. This may support the hypothesis that diffuse plaques are of early form of SP [150], because astrocytes respond to brain injuries in a timely fashion. Nevertheless, the possibility that astrocytes are associated with diffuse SP could exist because not all astrocytes necessarily reveal themselves by GFAP staining method [71]. Whether astrocytes react simply to degenerative neuritic components and/or deposited amyloid in neuropil as suggested by Mandybur [70] or actually participate in the process of SP formation should not be determined until the fine structural as well as physiological relationships between SP and astrocytes are elucidated.

V. SUMMARY

The topographic relationships between astrocytic gliosis (AG) and senile plaques (SP), and AG and cerebrovascular amyloidosis (CA) were studied in the cerebral cortex of six aged dogs using glial fibrillary acidic protein (GFAP) immunohistochemistry and electron microscopy. Coronal sections containing the cingulate and temporal cortices were stained with hematoxylin and eosin, Nissl, Bodian, modified Bielschowsky and thioflavin S in addition to GFAP immunohistochemistry. The cerebral cortex of the aged dogs showed diffuse AG, with which prominent astrocytic reaction around vessels was associated irrespective of the intensity of amyloid deposition in the involved vessels. Such pattern of AG was consistent throughout the cerebral cortex, where CA was not always accompanied. In addition, there was no difference in the pattern of AG between the area with SP and the area without SP, except occasional involvement of the processes of GFAP-positive astrocytes (GFAP-PA) in mature SP in the former area. It appears that an increase in GFAP-PA may not necessarily occur secondary to SP formation and CA.

CONCLUSION

1. The aged dogs developed senile plaques (SP) which were morphologically and immunohistochemically similar to those occurring in normal aged human beings and patients with Alzheimer's disease (AD). Based on the morphological characteristics demonstrated by the modified Bielschowsky stain, SP in the brains of the dogs were grouped into three types; diffuse, mature and perivascular plaques, of which diffuse plaques were predominant.

2. The broad similarities in staining properties between the SP of human beings and those of the dogs suggest a common mode of the formation and the evolution of the structure in these different species.

3. Most SP were observed in the cerebral cortex. Their densities were highest in the cingulate and temporal cortices. There were occasional SP in the subcortical nuclei, including the caudate nucleus and putamen, and hippocampus.

4. Mature and perivascular plaques, both of which contained compact amyloid deposits, always showed a close topographic relationship to blood vessels with cerebrovascular amyloidosis (CA). Whereas, most of the diffuse plaques showed no topographic relationship to CA. Cell bodies of neurons and/or glia were almost always involved in 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 and evolution of the different subtypes of SP in the brain of aged dogs.

5. Age-related astrocytic gliosis (AG) was observed in the dogs; the pattern was similar to that of aged human beings. The white matter, cortico-medullary junction, and subcortical nuclei in the cerebrum, central nuclei in the cerebellum, various nuclei in the brain stem, and grey matter of the spinal cord showed even and intense GFAP staining. AG was also prominent in the cerebral and cerebellar cortices and thalamus. Moderate AG was observed in the hippocampus and white matter of the cerebellum and spinal cord.

6. Electron microscopy demonstrated increased numbers of profiles of degenerative neural components in the vicinity of hypertrophic astrocytes in the cerebral cortex of the aged dogs. These findings imply that synaptic pathology may, like aged human beings, be also involved in the course of central nervous system (CNS) aging of dogs.

7. Moderate to severe AG was consistently shown in the CNS of the aged dogs. In contrast, young normal dogs showed minimum numbers of GFAP-positive astrocytes (GFAP-PA) in the CNS. These findings suggest that the observed AG in the CNS of the dogs may be a morphological expression of aging.

8. The cerebral cortex of the aged dogs showed diffuse AG, with which prominent astrocytic reactions around vessels were associated irrespective of the intensity of amyloid deposition in the involved vessels. Such pattern of AG was consistent throughout the cerebral cortex, where CA was not always accompanied. In addition, there was no difference in the pattern of AG between the area with SP and the area without SP, except occasional involvement of the processes of GFAP-PA in mature SP in the former area. It appears that an increase of GFAP-PA may not necessarily occur secondary to SP formation and CA.

9. Based on these findings, it is concluded that aged dogs may be suitable as an animal model for the study of the mechanisms involved in CNS aging of human beings.

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REFERENCES

1. Abraham, C.R., Selkoe, D.J., and Potter, H. 1988. Immunochemical identification of the serine protease inhibitor, α_1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell 52: 487-501.
2. Adams, I. and Jones, D.G. 1983. Synaptic remodelling and astrocytic hypertrophy in rat cerebral cortex from early to late adulthood. Neurobiol. Aging 3: 179-186.
3. Allsop, D., Haga, S., Haga, C., Ikeda, S., Mann, D.M.A., and Ishii, T. 1989. Early senile plaques in Down's syndrome brains show a close relationship with cell bodies of neurons. Neuropathol. Appl. Neurobiol. 15: 531-542.
4. Allsop, D., Landon, M., Kidd, M., Lowe, J.S., Reynolds, G.P., and Gardner, A. 1986. Monoclonal antibodies raised against a subsequence of senile plaque core protein react with plaque cores, plaque periphery and cerebrovascular amyloid in Alzheimer's disease. Neurosci. Lett. 68: 252-256.
5. Bahmanyar, S., Higgins, G.A., Goldgaber, D., Lewis, D.A., Morrison, J.H., Wilson, M.C., Shanker, S.K., and Gajdusek, D.C. 1987. Localization of amyloid β protein messenger RNA in brains from patients with Alzheimer's disease. Science 237: 77-80.
6. Beach, T.G., Walker, R., and McGeer, E.G. 1989. Patterns of gliosis in Alzheimer's disease and aging cerebrum. Glia 2: 420-436.
7. Bendotti, C., Forloni, G.L., Morgan, R.A., O'Hara, B.F., Oster-Granite, M.L., Reeves, R.H., Gearhart, J.D., and Coyle, J.T. 1988. Neuroanatomical

localization and quantification of amyloid precursor protein mRNA by in situ hybridization in the brains of normal, aneuploid and lesioned mice. Proc. Natl. Acad. Sci. U.S.A. 85: 3628-3632.

8. Bignami, A., Eng, L.F., Dahl, D., and Uyeda, C.T. 1972. Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res. 43: 429-435.

9. Bjorklund, H., Eriksdotter-Nillson, M., Dahl, D., Rose, G., Hoffer, B., and Olson, L. 1985. Image analysis of GFA-positive astrocytes from adolescence to senescence. Exp. Brain Res. 58: 163-170.

10. Bowman, C.L. and Kimelberg, H.K. 1984. Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. Nature (Lond.) 311: 656-659.

11. Braak, H., Braak, E., Grundke-Iqbal, I., and Iqbal. K. 1986. Occurrence of neuropil threads in the senile brain and in Alzheimer's disease: a third location of paired helical filaments outside the neurofibrillary tanlgles and neuritic plaques. Neurosci. Lett. 65: 351-355.

12. Card, J.P., Meade, R.P., and Davis, L.G. 1988. Immunocytochemical localization of the precursor protein for β-amyloid in the rat central nervous system. Neuron 1: 835-846.

13. Coria, F., Castano, E., Prelli, F., Larrondo-Lillo, M., van Duinen, S., Shelanski, M.L., and Frangione, B. 1988. Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. Lab. Invest. 58: 454-458.

14. Cork, L.C., Powers, R.E., Selkoe, D.J., Davies, P., Geyer, J.J., and Price, D.L. 1988. Neurofibrillary tangles and senile plaques in aged bears. J.

Neuropathol. Exp. Neurol. 47: 629-641.

15. Cork, L.C. and Price, D.L. 1990. Relationships of abnormal neuronal processes and amyloid in plaques in aged monkeys. J. Neuropathol. Exp. Neurol. 49: 309.

16. Cras, P., Kawai, M., Siedlak, S., Mulvihill, P., Gambetti, P., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., and Perry, G. 1990. Neuronal and microglial involvement in β-amyloid protein deposition in Alzheimer's disease. Am. J. Pathol. 137: 241-246.

17. Davies, C.A., Mann, D.M.A., Sumpter, P.Q., and Yates, P.O. 1987. A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease: correlation with cognitive severity. Ann. Neurol. 27: 457-464.

18. Dayan, A.D. 1970. Quantitative histological studies in the aged human brain. I. senile plaques and neurofibrillary tangles in 'normal' patients. Acta Neuropathol. (Berl.) 16: 85-94.

19. Dayan, A.D. 1971. Comparative neuropathology of ageing. Brain 94: 91-42.

20. de la Roza, C., Cano, J., and Reinoso-Suarez, F. 1985. An electron microscopic study of astroglia and oligodendroglia in the lateral geniculate nucleus of aged rats. Mech. Ageing Dev. 29: 267-281.

21. de Sauvage, F. and Octave, J.-N. 1989. A novel mRNA of the A4 amyloid precursor gene coding for a possibly secreted protein. Science 245: 651-653.

22. Dickson, D.W., Farlo, J., Davies, P., Crystal, H., Fuld, P., and Yen, S.C. 1988. Alzheimer disease. A double immunohistochemical study of senile

plaques. Am. J. Pathol. 132: 86-101.

23. Duffy, P.E., Rapport, M., and Graf, L. 1980. Glial fibrillary acidic protein and Alzheimer-type senile dementia. Neurology 30: 778-782.

24. Dyrks, T., Weidemann, A., Multhaup, G., Salbaum, J.M., Lemaire, H.-G., Kang, J., Muller-Hill, B., Masters, C.L., and Beyreuther, K. 1988. Identification, transmembrane orientation and biogenesis of the amyloid A4 precursor of Alzheimer's disease. EMBO J. 7: 949-957.

25. Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Cuwell, A.R., Oltersdorf, T., McClure, D., and Ward, P.J. 1990. Cleavage of amyloid β peptide during constitutive processing of its precursor. Science 248: 1122-1124.

26. Fierz, W., Endler, B., Reske, K., Wekerle, H., and Fontana, A. 1985. Astrocytes as antigen-presenting cells: I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effect on antigen presentation. J. Immunol. 134: 3785-3793.

27. Frei, K., Bodmer, S., Schwerdel, C., and Fontana, A. 1985. Astrocytes of the brain synthesize interleukin 3-like factors. J. Immunol. 135: 4044-4047.

28. Furukawa, S., Furukawa, Y., Satoyoshi, E., and Hayashi, K. 1986. Synthesis and secretion of nerve growth factor by mouse astroglial cells in culture. Biochem. Biophys. Res. Commun. 136: 57-63.

29. Geinisman, Y., Bondareff, W., and Dodge, J.T. 1978. Hypertrophy of astroglial processes in the dentate gyrus of the senescent rat. Am. J. Anat. 153: 537-544.

30. Giaccone, G., Tagliavini, F., Linoli, G., Bouras, C., Frigerio, L.,

Frangione, B., and Bugiani, O. 1989. Down's patients: extracellular preamyloid deposits precede neuritic degeneration and senile plaques. Neurosci. Lett. 97: 232-238.

31. Gibson, P.H. 1987. Ultrastructural abnormalities in the cerebral neocortex and hippocampus associated with Alzheimer's disease and aging. Acta Neuropathol. (Berl.) 73: 86-91.

32. Glenner, G.G. 1979. Congophilic microangiopathy in the pathogenesis of Alzheimer's syndrome (presenile dementia). Med. Hypotheses 5: 1231-1236.

33. Glenner, G.G. 1980. Amyloid deposits and amyloidosis. N. Engl. J. Med. 302: 1283-1291.

34. Glenner, G.G. and Wong, C.W. 1984. Alzheimer disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 120: 885-890.

35. Goedert, M. 1987. Neuronal localization of amyloid beta protein precursor mRNA in normal human brain and in Alzheimer's disease. EMBO J. 6: 3627-3632.

36. Goldgaber, D., Lerman, M.I., McBride, O.W., Saffiotti, U., and Gajdusek, D.C. 1987. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 235: 877-880.

37. Gonatas, N.K., Anderson, W.V., and Evangelista, I. 1967. The contribution of altered synapses in the senile plaque: an electron microscopic study in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 26: 25-39.

38. Goss, J.R., Finch, C.E., and Morgan, D.G. 1991. Age-related changes in

glial fibrillary acidic protein mRNA in the mouse brain. Neurobiol. Aging 12: 165-170.

39. Griffin, W.S.T., Stanley, L.C., Ling, C., White, L., MacLeod, V., Perrot, L.J., White, C.L.III, and Araoz, C. 1989: Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 86: 7611-7615.

40. Haga, S., Akai, K., and Ishii, T. 1989. Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. Acta Neuropathol. 77: 569-575.

41. Haga, C., Yamaguchi, H., Ikeda, K., and Kosaka, K. 1989. PAM-modified methenamine silver stain for senile plaques: comparison with β-protein immunostaining. Dementia 3: 417-422.

42. Hansen, L.A., Armstrong, D.M., and Terry, R.D. 1987. An immunohistochemical quantification of fibrous astrocytes in the aging human cerebral cortex. Neurobiol. Aging 8: 1-6.

43. Higgins, G.A., Lewis, D.A., Bahmanyar, S., Goldgaber, D., Gajdusek, D.C., Young, W.G., Morrison, J.H., and Wilson, M.C. 1988. Differential regulation of amyloid-β-protein mRNA expression within hippocampal neuronal subpopulations in Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 85: 1297-1301.

44. Ikeda, S., Yanagisawa, N., Allsop, D., and Glenner, G.G. 1989. Evidence of amyloid β protein immunoreactive early plaque lesions in Down's syndrome brains. Lab. Invest. 61:133-137.

45. Itagaki, S., McGeer, P.L., Akiyama, H., Zhu, S., and Selkoe, D.J. 1989. Relationship of microglia and astrocytes to amyloid deposits of

Alzheimer disease. J. Neuroimmunol. 24: 173-182.

46. Joachim, C.L., Duffy, L.K., Morris, J.H., and Selkoe, D.J. 1988. Protein chemical and immunocytochemical studies of meningovascular β-amyloid protein in Alzheimer's disease and normal aging. Brain Res. 474: 100-111.

47. Joachim, C., Games, D., Morris, J., Ward, P., Frenkel, D., and Selkoe, D. 1991. Antibodies to non-beta regions of the beta-amyloid precursor protein detected a subset of senile plaques. Am. J. Pathol. 138: 373-384.

48. Joachim, C.L., Mori, H., and Selkoe, D.L. 1989. Amyloid β-protein deposition in tissues other than brain in Alzheimer's disease. Nature (Lond.) 341: 226-230.

49. Joachim, C.L., Morris, J.H., and Selkoe, D.J. 1989. Diffuse amyloid plaques occur commonly in the cerebellum in Alzheimer's disease. Am. J. Pathol. 5: 309-319.

50. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature (Lond.) 325: 733-736.

51. Kawabata, S., Higgins, G.A., and Gordon, J.W. 1991. Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein. Nature (Lond.) 354: 476-478.

52. Kawai, M., Gras, P., Siedlak, S., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., Skelton, E., Gambetti, P., and Perry, G. 1990. Role of vascular smooth muscle cells in amyloid deposition in cerebral amyloid

angiopathy. J. Neuropathol. Exp. Neurol. 49: 339.

53. Kemper, T. 1984. Neuroanatomical and neuropathological changes in normal aging and in dementia. pp. 9-52. In: Clinical Neurology of Aging (Albert, M.L. ed.), Oxford University Press, New York.

54. Kesslak, J.P., Nieto-Sampedro, M., Globus, J., and Cotman, C.W. 1986. Transplants of purified astrocytes promote behavioral recovery after frontal cortex ablation. Exp. Neurol. 93: 377-390.

55. Khachaturian, Z.S. 1985. Diagnosis of Alzheimer's disease. Arch. Neurol. 42: 1097-1105.

56. Kimelberg, H.K. and Katz, D.M. 1985. High-affinity uptake of serotonin into immunocytochemically identified astrocytes. Science 228: 889-891.

57. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S., and Ito, H. 1988. Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. Nature (Lond.) 331: 530-532.

58. Kitamoto, T., Ogomori, K., Tateishi, J., and Prusiner, S.B. 1987. Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab. Invest. 57: 230-236.

59. Kitt, C.A., Price, D.L., Struble, R.G., Cork, L.C., Wainer, B.H., Becher, M.W. and Mobley, W.C. 1984. Evidence for cholinergic neurites in senile plaques. Science 226: 1443-1445.

60. Kitt, C.A., Struble, R.G., Cork, L.C., Mobley, W.C., Walker, L.C., Joh, T.H., and Price, D.L. 1985. Catecholaminergic neurites in senile plaques in prefrontal cortex of aged nonhuman primates. Neuroscience 16: 105-115.

61. Klier, F.G., Cole, G., Stallcup, W., and Schubert, D. 1990. Amyloid β-protein precursor is associated with extracellular matrix. Brain Res.

515: 336-342.

62. Koo, E.H., Sisodia, S.S., Archer, D.R., Martin, L.J., Beyreuther, K., Weidemann, A., and Price, D.L. 1989. Amyloid precursor protein (APP) undergoes fast anterograde transport. Soc. Neurosci. Abstr. 15: 23.

63. Koo, E.H., Sisodia, S.S., Archer, D.A., Martin, L.J., Weidemann, A., Beyreuther, K., Masters, C.L., Fischer, P., and Price, D.L. 1990. Precursor of amyloid protein in Alzheimer's disease undergoes fast anterograde axonal transport. Proc. Natl. Acad. Sci. U.S.A. 87: 1561-1565.

64. Landfield, P.W., McGaugh, J.L., and Lynch, G. 1978. Impaired synaptic potentiation processes in the hippocampus of aged, memory-deficient rats. Brain Res. 150: 85-101.

65. Landfield, P.W., Rose, G., Sandles, L., Wohlstadter, T.C., and Lynch, G. 1977. Patterns of astroglial hypertrophy and neuronal degeneration in the hippocampus of aged, memory-deficient rats. J. Gerontol. 32: 3-12.

66. Leuba, G. 1983. Aging of dendrites in the cerebral cortex of the mouse. Neuropathol. Appl. Neurobiol. 9: 467-475.

67. Lindholm, D., Heumann, R., Meyer, M., and Thoenen, H. 1987. Interleukin-1 regulates synthesis of nerve growth factor in nonneuronal cells of rat sciatic nerve. Nature (Lond.) 330: 658-659.

68. Lindsey, J.D., Landfield, P.W., and Lynch, G. 1979. Early onset and topographical distribution of hypertrophied astrocytes in hippocampus of aging rats: a quantitative study. J. Gerontol. 34: 661-671.

69. Mancardi, G.L., Liwnicz, B.H., and Mandybur, T.I. 1983. Fibrous astrocytes in Alzheimer's disease and senile dementia of Alzheimer's type. Acta Neuropathol. 61: 76-80.

70. Mandybur, T.I. 1989. Cerebral amyloid angiopathy and astrocytic gliosis in Alzheimer's disease. Acta Neuropathol. 78: 329-331.

71. Mandybur, T.I., Ormsby, I., and Zemlan, F.P. 1989. Cerebral aging: a quantitative study of gliosis in old nude mice. Acta Neuropathol. 77: 507-513.

72. Mann, D.M.A. 1985. The neuropathology of Alzheimer's disease: a review with pathogenetic, aetiological and therapeutic considerations. Mech. Ageing Dev. 31: 213-255.

73. Mann, D.M.A. 1989. Cerebral amyloidosis, aging and Alzheimer's disease: a contribution from studies on Down's syndrome. Neurobiol. Aging 10: 397-399.

74. Mann, D.M.A. and Esiri, M.M. 1989. The pattern of acquisition of plaques and tangles in the brains of patient under 50 years of age with Down's syndrome. J. Neurol. Sci. 89: 169-179.

75. Mann, D.M.A., Jones, D., Prinja, D., and Purkiss, M.S. 1990. The prevalence of amyloid (A4) protein deposits within the cerebral and cerebellar cortex in Down's syndrome and Alzheimer's disease. Acta Neuropathol. 80: 318-327.

76. Mann, D.M.A., Tucker, C.M., and Yates, P.O. 1987. The topographic distribution of senile plaques and neurofibrillary tangles in the brains of non-demented persons of different ages. Neuropathol. Appl. Neurobiol. 13: 123-139.

77. Masliah, E., Hansen, L., Albright, T., Mallory, M., and Terry, R.D. 1991. Immunoelectron microscopic study of synaptic pathology in Alzheimer's disease. Acta Neuropathol. 81: 428-433.

78. Masters, C.L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R.N., and Beyreuther, K. 1985. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J. 4: 2757-2763.

79. Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L., and Beyreuther, K. 1985. Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc. Natl. Acad. Sci. U.S.A. 82: 4245-4249.

80. Matsuyama, H. and Nakamura, S. 1978. Senile changes in the brain in the Japanese: Incidences of Alzheimer neurofibrillary changes and senile plaques. pp. 287-297. In: Alzheimer's Disease: Senile Dementia and Related Disorders (Katzman, R., Terry, R.D., and Bick, K.L. eds.), Raven Press, New York.

81. Mattice, L.A., Davies, P., Yen, S.-H., and Dickson, D.W. 1990. Microglia in cerebellar plaques in Alzheimer's disease. Acta Neuropathol. 80: 493-498.

82. McGeer, P.L., Akiyama, H., Itagaki, S., and McGeer, E.G. 1989. Immune system response in Alzheimer's disease. Can. J. Neurol. Sci. 16: 516-527.

83. Miller, D.L., Currie, J.R., Iqbal, K., Potempska, A., and Styles, J. 1988. Relationships among the cerebral amyloid peptides and their precursors. Alzheimer Disease and Associated Disorders 2: 253.

84. Miquel, J., Johnson, J.E.,Jr., and Cervous-Navarros, J. 1983. Comparison of CNS aging in humans and experimental animals. pp. 231-258. In: Brain Aging: Neuropathology and Neuropharmacology, Aging, vol. 21 (Cervous-Navarro, J. and Sarkander, J.-I. eds.), Ravan Press, New York.

85. Miyakawa, T., Katsuragi, S., Watanabe, K., Shimoji, A., and Ikeuchi, Y. 1986. Ultrastructural studies of amyloid fibrils and senile plaques in

human brain. Acta Neuropathol. (Berl.) 70: 202-208.

86. Miyakawa, T., Shimoji, A., Kuramoto, R., and Higuchi, Y. 1982. The relationship between senile plaques and cerebral blood vessels in Alzheimer's disease and senile dementia. Morphological mechanism of senile plaque production. Virchows Arch. [B] 40: 121-129.

87. Mountjoy, C.Q., Tomlinson, B.E., and Gibson, R.H. 1982. Amyloid and senile plaques and cerebral blood vessels. A semi-quantitative investigation of a possible relationship. J. Neurol. Sci. 57: 89-103.

88. Nolan, C.C. and Brown, A.W. 1989. Reversible neuronal damage in hippocampal pyramidal cells with triethyllead: the role of astrocytes. Neuropathol. Appl. Neurobiol. 15: 441-457.

89. O'Callaghan, J.P. and Miller, D.B. 1991. The concentration of glial fibrillary acidic protein increases with age in the mouse and rat brain. Neurobiol. Aging 12: 171-174.

90. Ogomori, K., Kitamoto, T., Tateishi, J., Sato, Y., Suetsugu, M., and Abe, M. 1989. β-Protein amyloid is widely distributed in the central nervous system of patients with Alzheimer's disease. Am. J. Pathol. 134: 243-251.

91. O'Kusky, J. and Colonnier, M. 1982. Postnatal changes in the number of astrocytes, oligodendrocytes, and microglia in the visual cortex (area 17) of the macaque monkey: a stereological analysis in normal and monocularly deprived animals. J. Comp. Neurol. 210: 307-315.

92. Oltersdorf, T., Fritz, L.C., Schenk, D.B., Lieberburg, I., Johnson-Wood, K.L., Beattie, E.C., Ward, P.J., Blacher, R.W., Dovey, H.F., and Sinha, S. 1989. The secreted form of the Alzheimer's amyloid precursor protein with Kunitz domain is protease nexin-II. Nature (Lond.) 341: 144-147.

93. Oltersdorf, T., Ward, P.J., Henriksson, T., Beattie, E.C., Neve, R., Lieberberg, I., and Fritz, L.C. 1990. The Alzheimer amyloid precursor protein. Identification of a stable intermediate in the biosynthetic/degradative pathway. J. Biol. Chem. 265: 4492-4497.

94. Palmert, M.R., Golde, T.E., Cohen, M.L., Kovacs, D.M., Tanzi, R.E., Gusella, J.F., Usiak, M.F., Younkin, L.H., and Younkin, S.G. 1988. Amyloid protein precursor messenger RNAs: differential expression in Alzheimer's disease. Science 241: 1080-1084.

95. Pasternack, J.M., Abraham, C.R., Van Dyke, B.J., Potter, H., and Younkin, S.G. 1989. Astrocytes in Alzheimer's disease gray matter express α_1-antichymotrypsin mRNA. Am. J. Pathol. 135: 827-834.

96. Paulson, O.B. and Newman, E.A. 1987. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science 237: 896-898.

97. Podlisny, M.B., Tolan, D., and Selkoe, D.J. 1991. Homology of the amyloid β-protein precursor in monkey and human supports a primate model for β-amyloidosis in Alzheimer's disease. Am. J. Pathol. 138: 1423-1435.

98. Politis, M.J. and Miller, J.E. 1985. The roles of reactive gliosis and mitosis on tropic factor production in traumatized nervous system tissue. Brain Res. 346: 186-189.

99. Ponte, P., Gonzalez-DeWhitt., P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F., and Cordell, B. 1988. A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. Nature (Lond.) 331: 525-527.

100. Prelli, F., Castano, E., Glenner, G.G., and Frangione, B. 1988. Differences between vascular and plaque core amyloid in Alzheimer's

disease. J. Neurochem. 51: 648-651.

101. Probst, A., Brunnschweiler, H., Lautenschlager, C., and Ulrich, J. 1987. A special type of senile plaque, possibly an initial stage. Acta Neuropathol. (Berl.) 74: 133-141.

102. Robakis, N.K., Ramakrishna, N., Wolfe, G., and Wisniewski, H.M. 1987. Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. Proc. Natl. Acad. Sci. U.S.A. 84: 4190-4194.

103. Rozemuller, J.M., Eikelenboom, P., Stam, F.C., Beyreuther, K., and Masters, C.L. 1989. A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J. Neuropathol. Exp. Neurol. 48: 674-691.

104. Saitoh, T., Sundsmo, M., Roch, J.-M., Kimura, N., Cole, G., Schubert, D., Oltersdorf, T., and Schenk, D.B. 1989. Secreted form of amyloid β protein precursor is involved in the growth regulation of fibrosis. Cell 58: 615-622.

105. Salbaum, J.M., Weidemann, A., Lemaire, H.G., Masters, C.L., and Beyreuther, K. 1988. The promoter of Alzheimer's disease amyloid A4 precursor gene. EMBO J. 7: 2807-2813.

106. Schechter, R., Yen, S.-H.C., and Terry, R.D. 1981. Fibrous astrocytes in senile dementia of the Alzheimer type. J. Neuropathol. Exp. Neurol. 40: 95-101.

107. Scheff, S.W., De Kosky, S.T., and Price D.A. 1990. Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol. Aging 11: 29-37.

108. Scheibel, M.E. and Scheibel, A.B. 1975. Structural changes in the aging brain. pp. 11-37. In: Clinical, Morphologic, and Neurochemical Aspects in the Aging Central Nervous System, vol. 1 (Brody, H., Harman, D., and Ordy, J.M. eds), Raven Press, New York.

109. Schubert, D., Jin, L.-W., Saitoh, T., and Cole, G. 1989. The regulation of amyloid β protein precursor secretion and its modulatory role in cell adhesion. Neuron 3: 689-694.

110. Selkoe, D.J. 1988. Molecular pathology of amyloidogenic proteins and the role of vascular amyloidosis in Alzheimer's disease. Neurobiol. Aging 10: 387-395.

111. Selkoe, D.J., Abraham, C.R., Podlisny, M.B., and Duffy, L.K. 1986. Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J. Neurochem. 146: 1820-1834.

112. Selkoe, D.J., Bell, D.S., Podlisny, M.B., Price, D.L., and Cork, L.C. 1987. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. Science 235: 873-877.

113. Selkoe, D.J., Davies, P., Geyer, J.J., and Price, D.L. 1988. Neurofibrillary tangles and senile plaques in aged bears. J. Neuropathol. Exp. Neurol. 47: 629-641.

114. Selkoe, D.J., Podlisny, M.B., Joachim, C.L., Vickers, E.A., Lee, G., Fritz, L.C., and Oltersdorf, T. 1988. β-amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. Proc. Natl. Acad. Sci. U.S.A. 85: 7341-7345.

115. Shivers, B.D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K.,

and Seeburg, P.H. 1988. Alzheimer's disease amyloidogenic glycoprotein: expression pattern in rat brain suggests a role in cell contact. EMBO J. 7: 1365-1370.

116. Siman, R., Card, J.P., Nelson, R.B., and Davis, L.G. 1989. Expression of beta-amyloid precursor protein in reactive astrocytes following neuronal damage. Neuron 3: 275-285.

117. Sinha, S., Dovey, H.F., Seubert, P., Ward, P.J., Blacher, R.W., Blaber, M., Bradshaw, R.A., Arici, M., Mobley, W.C., and Lieberburg, I. 1990. The protease inhibitory properties of the Alzheimer's β-amyloid precursor protein. J. Biol. Chem. 265: 8983-8985.

118. Smith, R.P., Higuchi, D.A., and Broze, G.J.,Jr. 1990. Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. Science 248: 1126-1128.

119. Snow, A.D., Mar, H., Nochlin, D., Kimata, K., Kato, M., Suzuki, S., Hassell, J., and Wight, T.N. 1988. The presence of heparan sulfate proteoglycans in the neuritic plaques and congophilic angiopathy in Alzheimer's disease. Am. J. Pathol. 133: 456-463.

120. Struble, R.G., Price, D.L.Jr., Cork, L.C., and Price, D.L. 1985. Senile plaques in cortex of aged normal monkeys. Brain Res. 361: 267-275.

121. Suenaga, T., Hirano, A., Llena, J.F., Yen, S.-H., and Dickson, D.W. 1990. Modified Bielschowsky stain and immunohistochemical studies on striatal plaques in Alzheimer's disease. Acta Neuropathol. 80: 280-286.

122. Tagliavini, F., Giaccone, G., Frangione, B., and Bugiani, O. 1988. Preamyloid deposits in cerebral cortex of patients with Alzheimer's disease and non demented individuals. Neurosci. Lett. 93: 191-196.

123. Tanzi, R.E., Gusella, J.F., Watkins, P.C., Bruns, G.A.P., St. George-Hyslop, P., Van Keuren, M.L., Patterson, D., Pagan, S., Kurnit, D.M., and Neve, R.L. 1987. Amyloid β protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science 235: 880-884.

124. Tanzi, R.E., McClatchey, A.I., Lamperti, E.D., Villa-Komaroff, L.L., Gusella, J.F., and Neve, R.L. 1988. Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. Nature (Lond.) 331: 528-530.

125. Terry, R.D., Gonatas, N.K., and Weiss, M. 1964. Ultrastructural studies in Alzheimer's presenile dementia. Am. J. Pathol. 44: 269-297.

126. Terry, R.D., Peck, A., Deteresa, R., Schechter, R., and Horoupian, D.S. 1981. Some morphometric aspects of the brain in senile dementia of the Alzheimer type. Ann. Neurol. 10: 184-192.

127. Tobo, M. 1984. Immunohistochemical study of gliosis in the brain of aging and dementia. Fukuoka Igaku Zasshi 75: 72-88. (in Japanese with English abstract)

128. Tomlinson, B.E., Blessed, G., and Roth, M. 1968. Observations on the brains of non-demented elderly persons. J. Neurol. Sci. 7: 331-356.

129. Tooyama, I., Akiyama, H., McGeer, P.L., Hara, Y., Yasuhara, O., and Kimura, H. 1991. Acidic fibroblast growth factor-like immunoreactivity in brain of Alzheimer patients. Neurosci. Lett. 121: 155-158.

130. Uchida, K., Miyauchi, Y., Nakayama, H., and Goto, N. 1990. Amyloid angiopathy with cerebral hemorrhage and senile plaque in aged dogs. Jpn. J. Vet. Sci. 52: 605-611.

131. Ulrich, J. 1985. Alzheimer changes in non-demented patients younger than sixty-five: possible early stages of Alzheimer's disease and senile dementia of Alzheimer type. Ann. Neurol. 17: 273-277.

132. Van Nostrand, W.E., Wagner, S.L., Suzuki, M., Choi, B.H., Farrow, J.S., Geddes, J.W., Cotman, C.W., and Cunningham, D.D. 1989. Protease nexin-II, a potent anti-chymotrypsin, shows identity to amyloid β-protein precursor. Nature (Lond.) 341: 546-549.

133. Vaughan, D.W. 1977. Age-related deterioration of pyramidal cell basal dendrites in rat auditory cortex. J. Comp. Neurol. 171: 501-516.

134. Vijayan, V.K. and Cotman, C.W. 1987. Hydrocortisone administration alters glial reaction to entorhinal lesion in the rat dentate gyrus. Exp. Neurol. 96: 307-320.

135. Walker, L.C., Kitt, C.A., Schwam, E., Buckwald, B., Garcia, F., Sepinwall, J., and Price, D.L. 1987. Senile plaques in aged squirrel monkeys. Neurobiol. Aging 8: 291-296.

136. Walker, L.C., Masters, C., Beyreuther, K., and Price, D.L. 1990. Amyloid in the brains of aged squirrel monkeys. Acta Neuropathol. 80: 381-387.

137. Weidemann, A., Konig., G., Bunke, D., Fischer, P., Salbaum, J.M., Masters, C.L., and Beyreuther, K. 1989. Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. Cell 57: 115-126.

138. Wisniewski, H.M. 1983. Neuritic (senile) and amyloid plaques. pp. 57-61. In: Alzheimer's Disease (Reisberg, B. ed.), The Free Press, New York.

139. Wisniewski, H.M., Ghetti, B., and Terry, R.D. 1973. Neuritic (senile) plaques and filamentous changes in aged rhesus monkeys. J.

Neuropathol. Exp. Neurol. 32: 566-584.

140. Wisniewski, H.M., Jonson, A.B., Raine, C.S., Kay, W.J., and Terry, R.D. 1970. Senile plaques and cerebral amyloidosis in aged dogs. Lab. Invest. 23: 287-296.

141. Wisniewski, H. and Terry, R.D. 1970. An experimental approach to the morphogenesis of neurofibrillary degeneration and the argyrophilic plaque. pp. 223-248. In: Ciba Foundation Symposium on Alzheimer's Disease and Related Conditions (Wolstenholme, G.E.W. and O'Connor, M. eds.), J. & A. Churchill, London.

142. Wisniewski, H.M. and Terry, R.D. 1973. Reexamination of the pathogenesis of the senile plaque. Prog. Neuropathol. 2: 1-26.

143. Wisniewski, H.M., Weigel, J., Wang., K.C., Kujawa, M., and Lach, B. 1989. Ultrastructural studies of the cells forming amyloid fibers in classical plaques. Can. J. Neurol. Sci. 16: 535-542.

144. Wisniewski, H.M., Wen, G.Y., and Kim, K.S. 1989. Comparison of four staining methods on the detection of neuritic plaques. Acta Neuropathol. 78: 22-27.

145. Wong, C.W., Quaranta, V., and Glenner, G.G. 1985. Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related. Proc. Natl. Acad. Sci. U.S.A. 82: 8729-8732.

146. Yamada, T., Sasaki, H., Furuya, H., Miyata, T., Goto, I., and Sakaki, Y. 1987. Complementary DNA for the mouse homolog of the human amyloid beta protein precursor. Biochem. Biophys. Res. Commun. 149: 665-671.

147. Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M., and Harigaya, Y. 1988. Diffuse type of senile plaques in the brains of Alzheimer-type dementia.

Acta Neuropathol. 77: 113-119.

148. Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M., and Ihara, Y. 1988. A variety of cerebral amyloid deposits in the brain of the Alzheimer-type dementia demonstrated by β protein immunostaining. Acta Neuropathol. 76: 541-549.

149. Yamaguchi, H., Nakazato, Y., Hirai, S., and Shoji, M. 1990. Immunoelectron microscopic localization of amyloid β protein in the diffuse plaques of Alzheimer-type dementia. Brain Res. 508: 320-324.

150. Yamaguchi, H., Nakazato, Y., Hirai, S., Shoji, M., and Harigaya, Y. 1989. Electron micrograph of diffuse plaques: initial stage of senile plaque formation in the Alzheimer brain. Am. J. Pathol. 135: 593-597.

151. Yamaguchi, H., Nakazato, Y., Yamazaki, T., Shoji, M., Kawarabayashi, T., and Hirai, S. 1991. Subpial β/A4 amyloid deposition occurs between astroglial processes in Alzheimer-type dementia. Neurosci. Lett. 223: 217-220.

152. Yamamoto, T. and Hirano, A. 1986. A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. Neuropathol. Appl. Neurobiol. 12: 3-9.

NH_2-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Gln-Val-

His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val---COOH

Fig. 1 Sequence of cerebrovascular amyloid protein (Glenner & Wong, 1984) [34]

a

A Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-
1 5 10

Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe
11 15 20

Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala.
21 25 30

Ile-Xxx-Xxx-Leu-Met-Val
31 35 36

B Gly-Ala-Ile-Xxx-Xxx-Leu-Met-Val
29 36

C Val-Gly-Gly-Val-Val-Ile-Ala
36 42

D Val-Gly-Gly-Val-Val-Ile-Ala-Thr
36 43

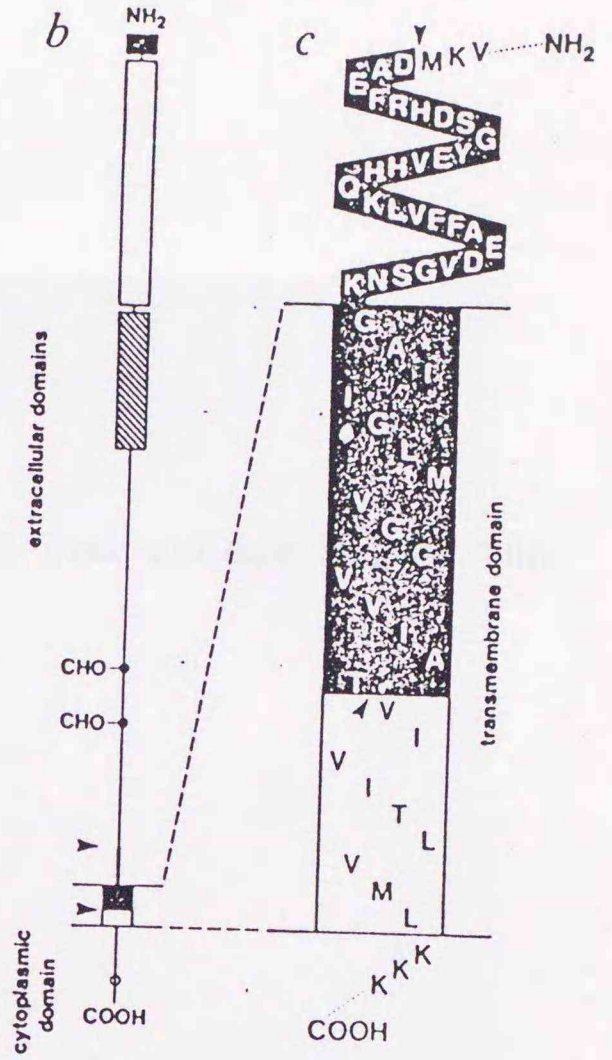


Fig. 2. Schematic diagram of βAPP as a cell-surface glycoprotein.

(Kang et al. 1987) [50]

Fig. 3. Gross appearance of the brain from a 16-year-old dog (Dog No. 29). Note the thickening of the meninges (arrows).

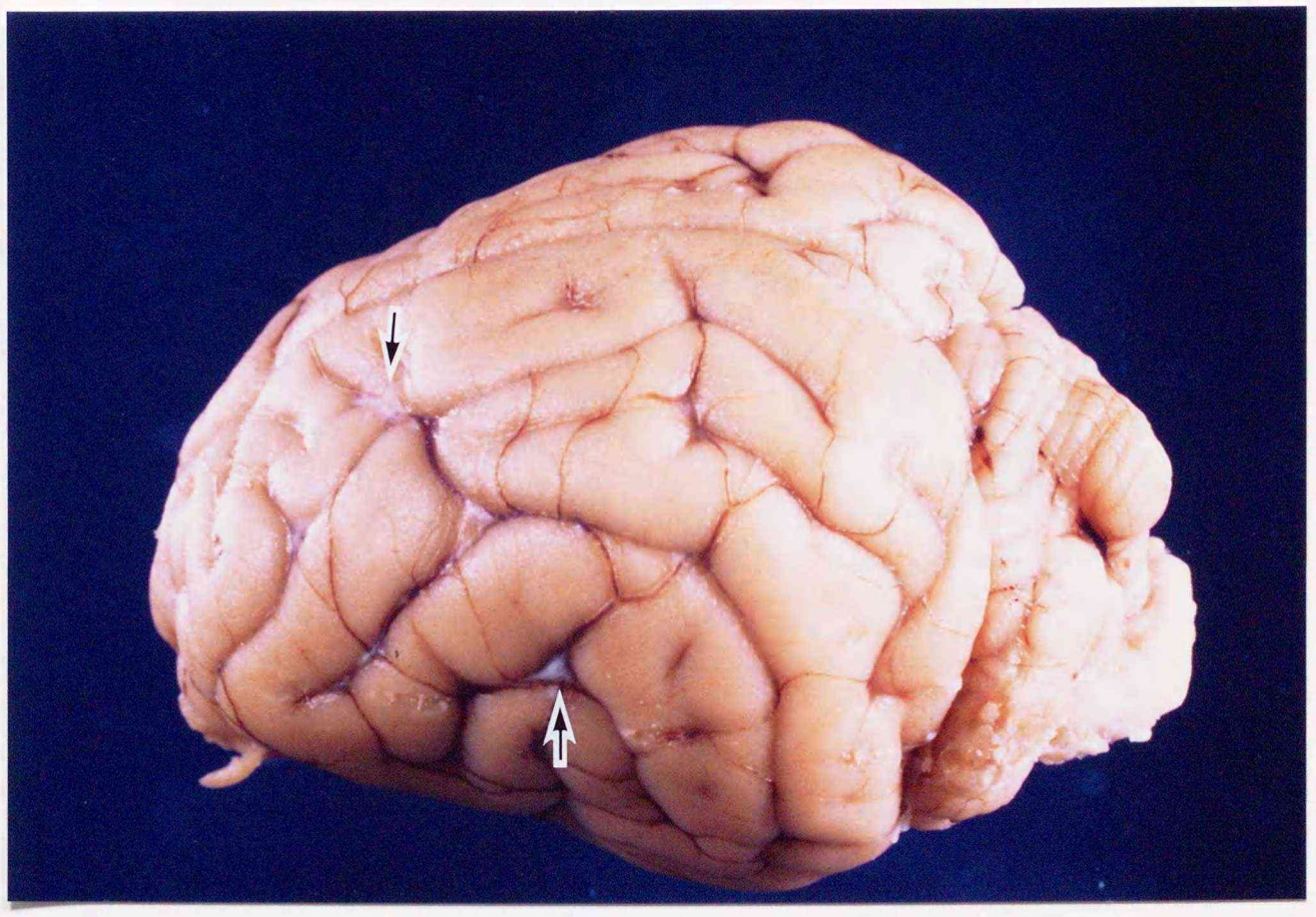


Fig. 4. Temporal cortex stained with modified Bielschowsky stain. Senile plaques (SP), most of which are diffuse plaques, are distributed throughout the cortical layers, especially in the cellular layer. Dog No. 33, x60.

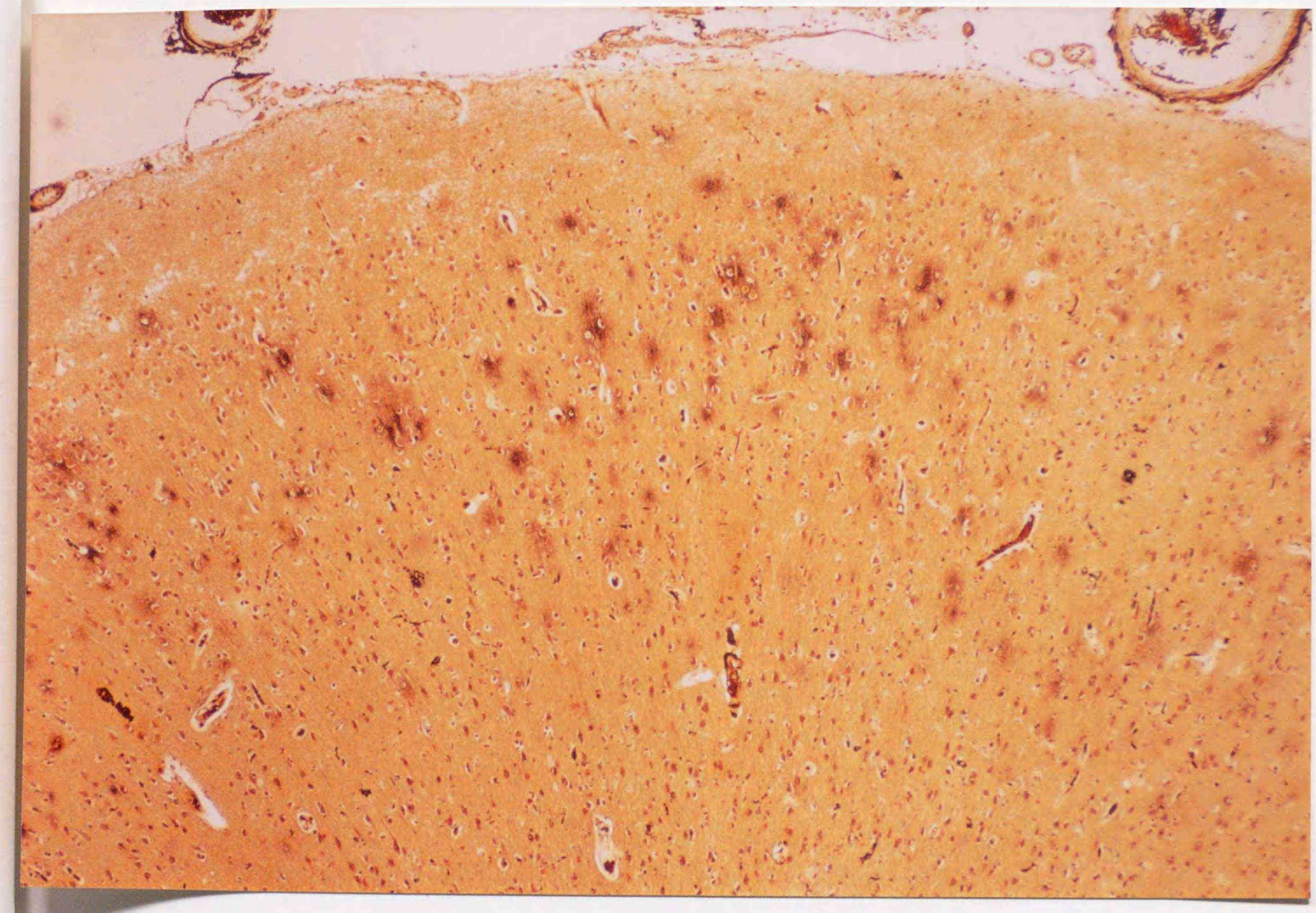


Fig. 5. Serial sections of the temporal cortex stained with amyloid β-protein (AβP) immunostain (a), modified Bielschowsky stain (b) and thioflavin S stain (c). Arrow indicates cerebrovascular amyloidosis (CA). Dog No. 16, x180.

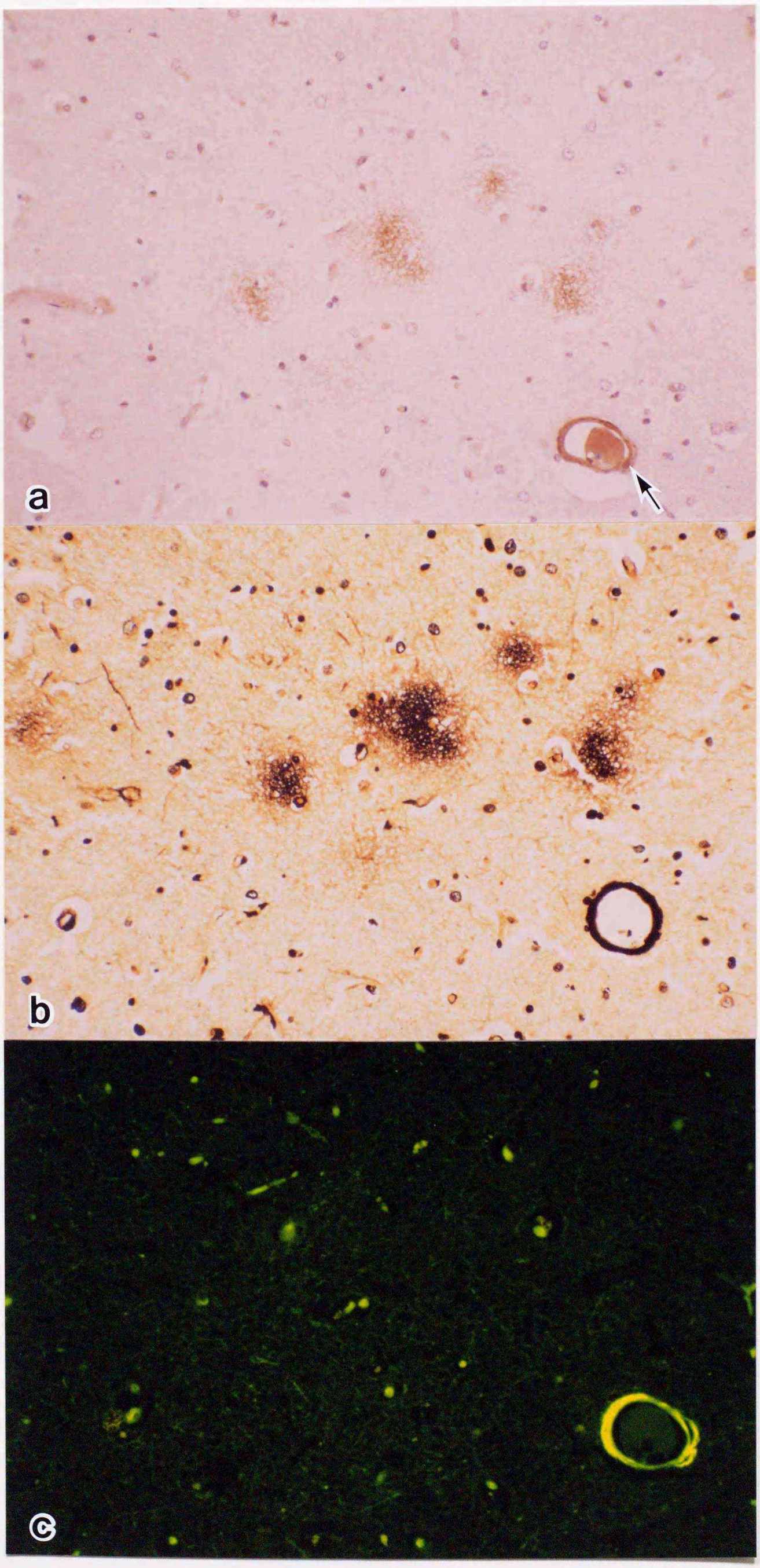

a
b
c

Fig. 6. Schematic diagram of the distributions of CA and three subtypes of SP in four coronal sections at different levels through one cerebral hemisphere of a 17-year-old dog (Dog No. 33). Dots represent CA in sections A-D and diffuse plaques in sections E-H, respectively. Mature plaques are represented by ▲ and perivascular plaques by △. H in C; hippocampus, NC in B; caudate nucleus, P in B; putamen, T in C; thalamus.

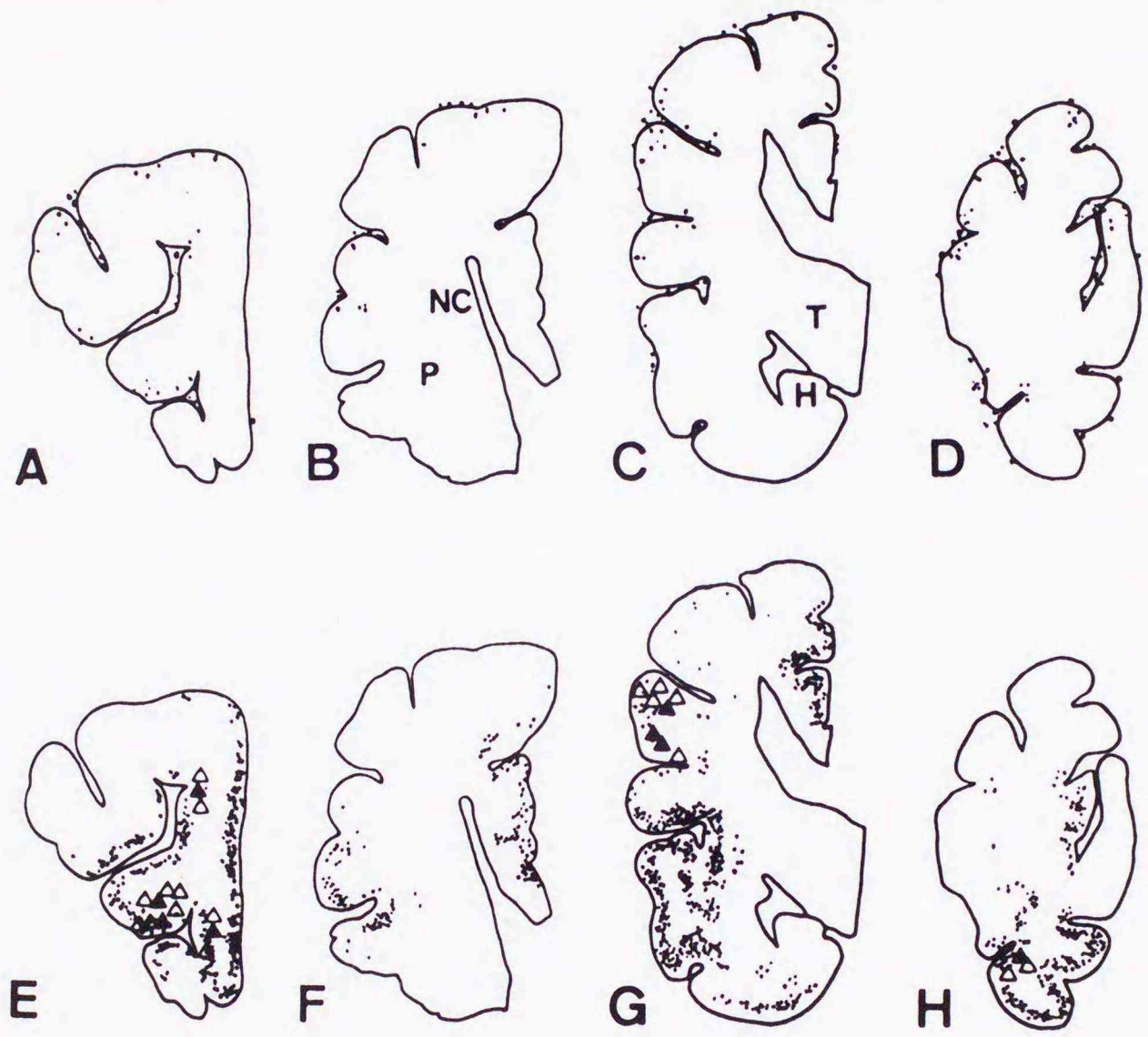
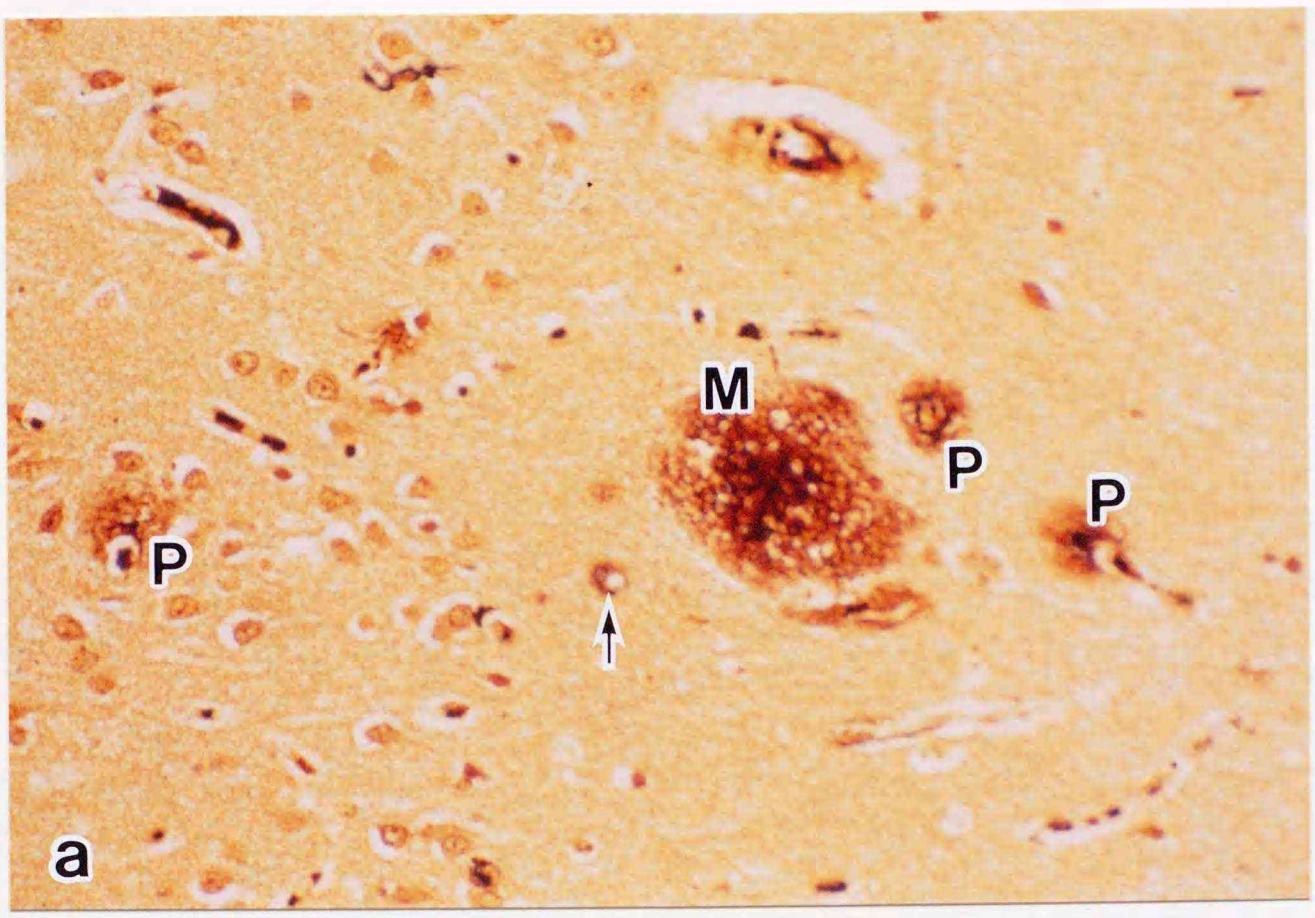

NC
P
T
H
A
B
C
D
E
F
G
H

Fig. 7. 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 topographically related. Dog No. 33, x180.


M
P
P
P
a

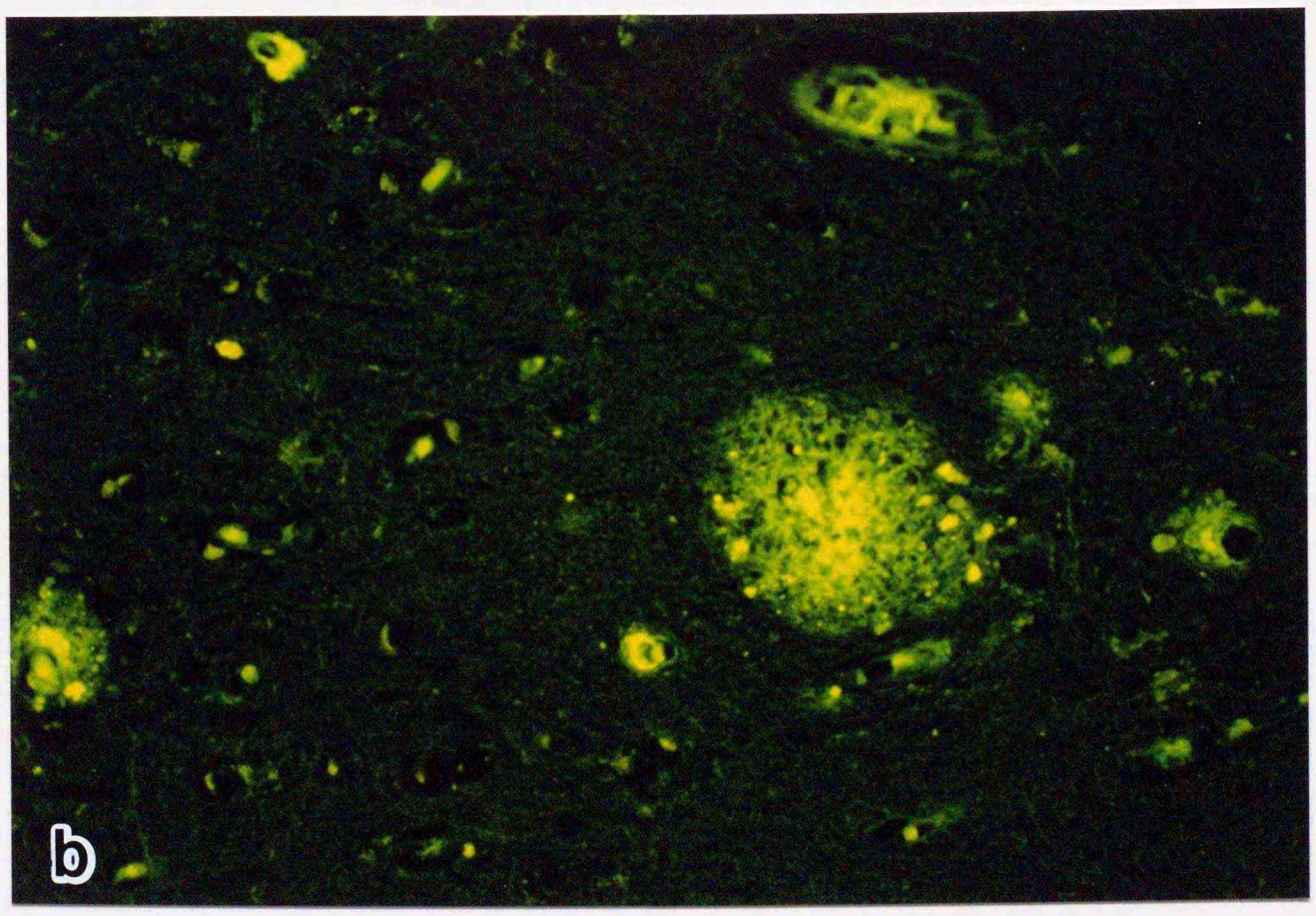

b

Fig. 8. Serial sections of the frontal cortex stained with periodic acid-methenamine silver (a), modified Bielschowsky (b), Bodian (c), and thioflavin S (d) stains. 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, x360.

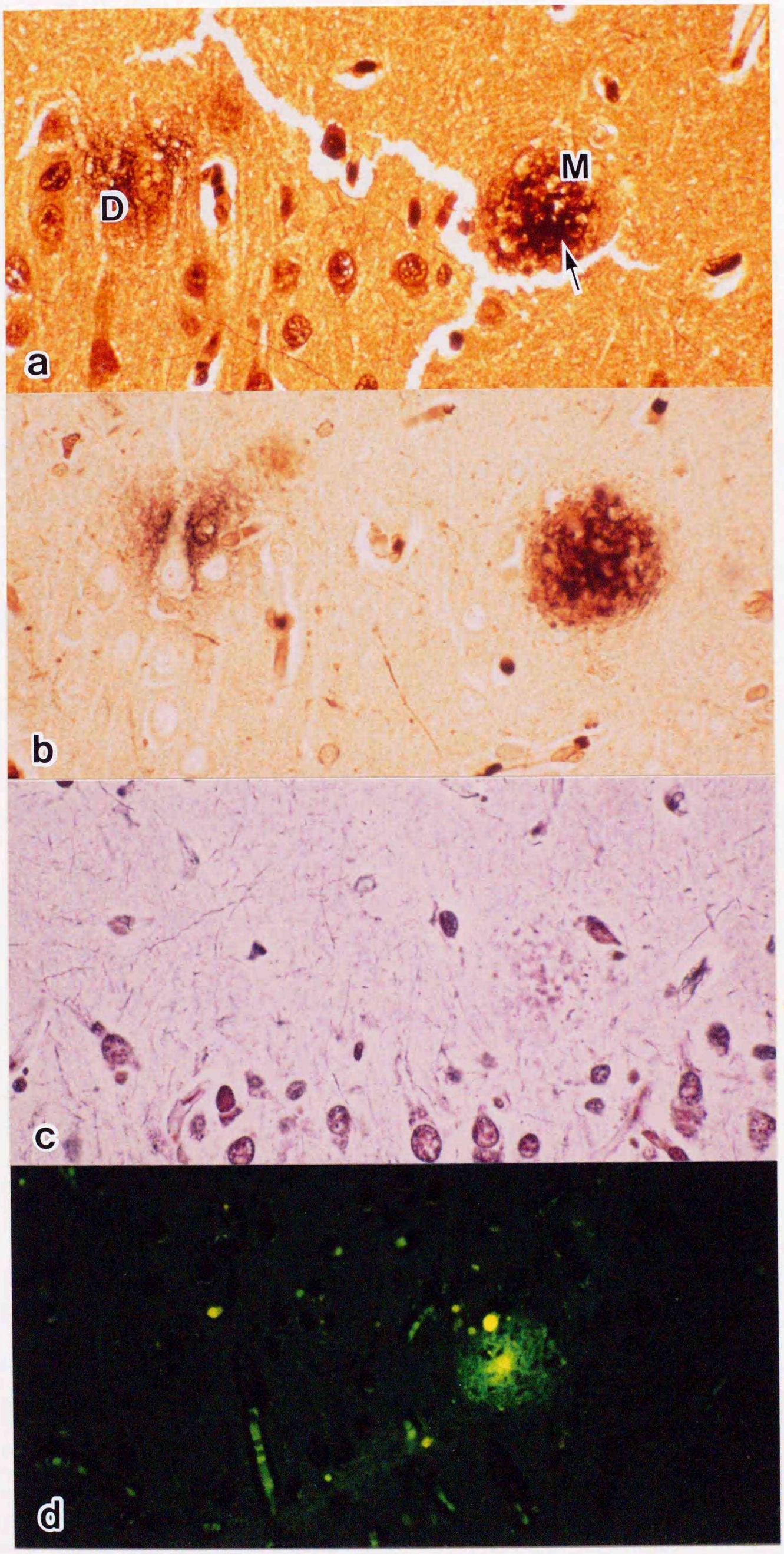

D
M
a
b
c
d

Fig. 9. 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, x360.

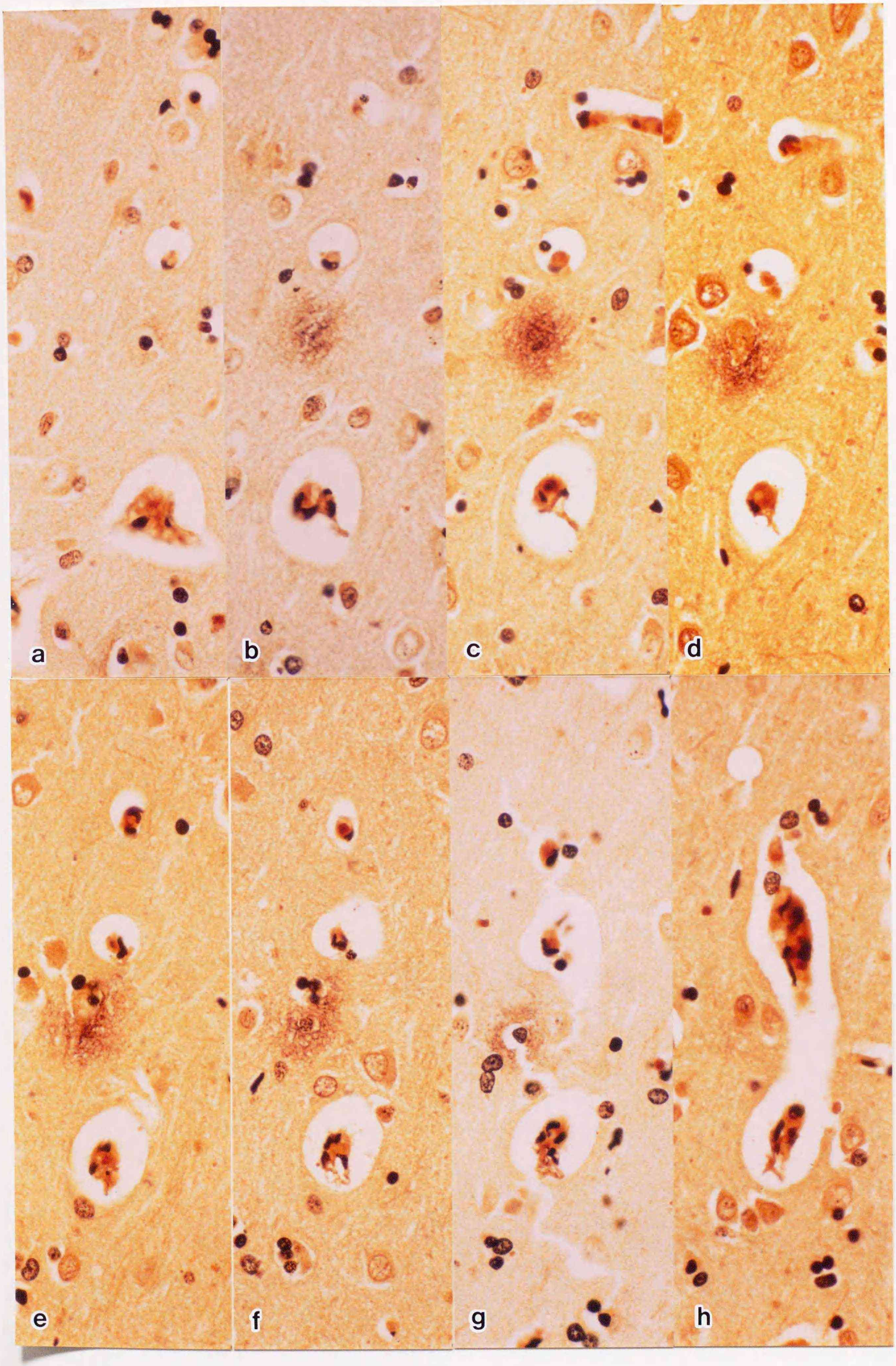

a
b
c
d
e
f
g
h

Fig. 10. AβP immunostain demonstrating amyloid deposits on the periphery of an apparently normal neuron in the temporal cortex. Dog No. 16, x540.

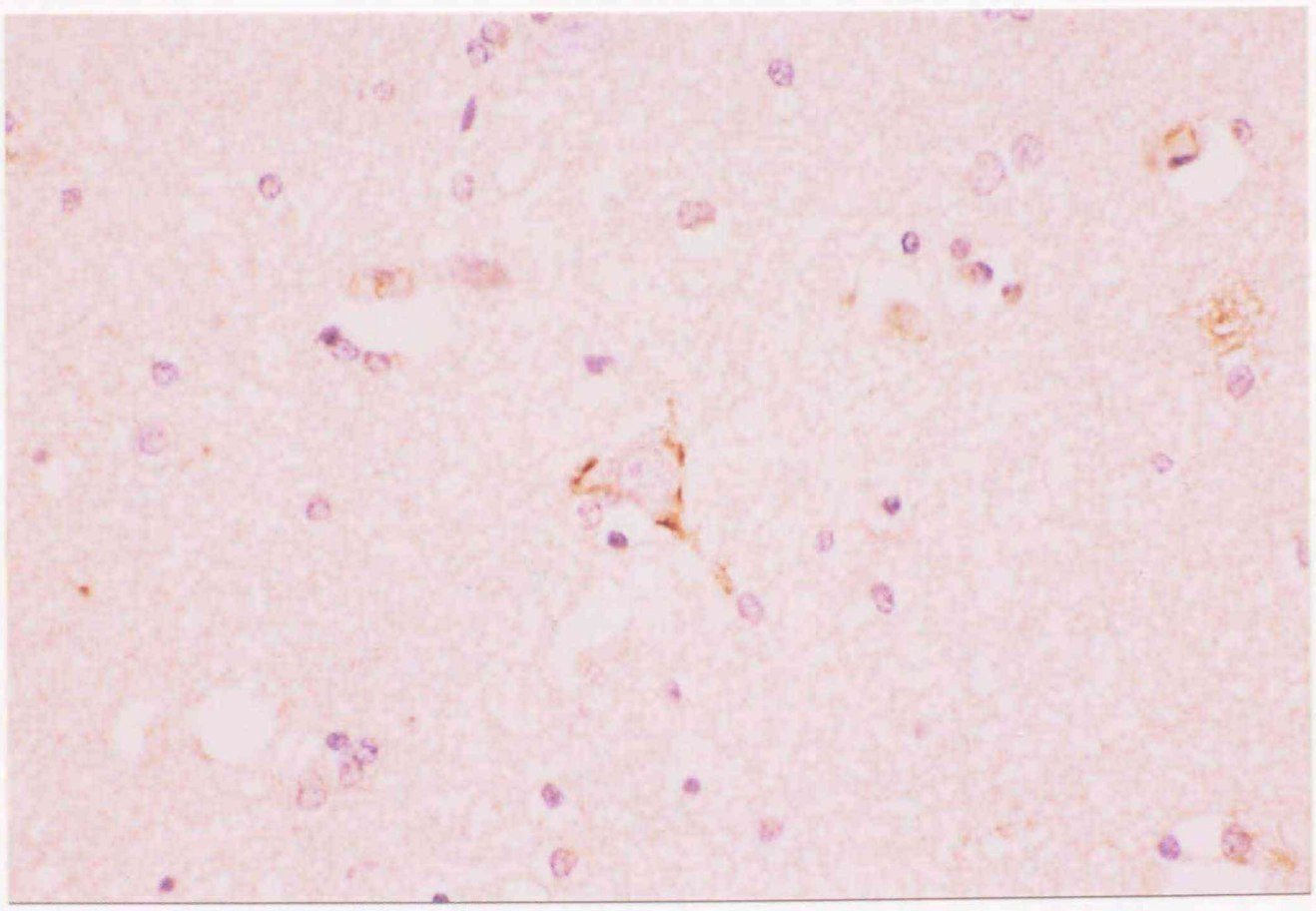
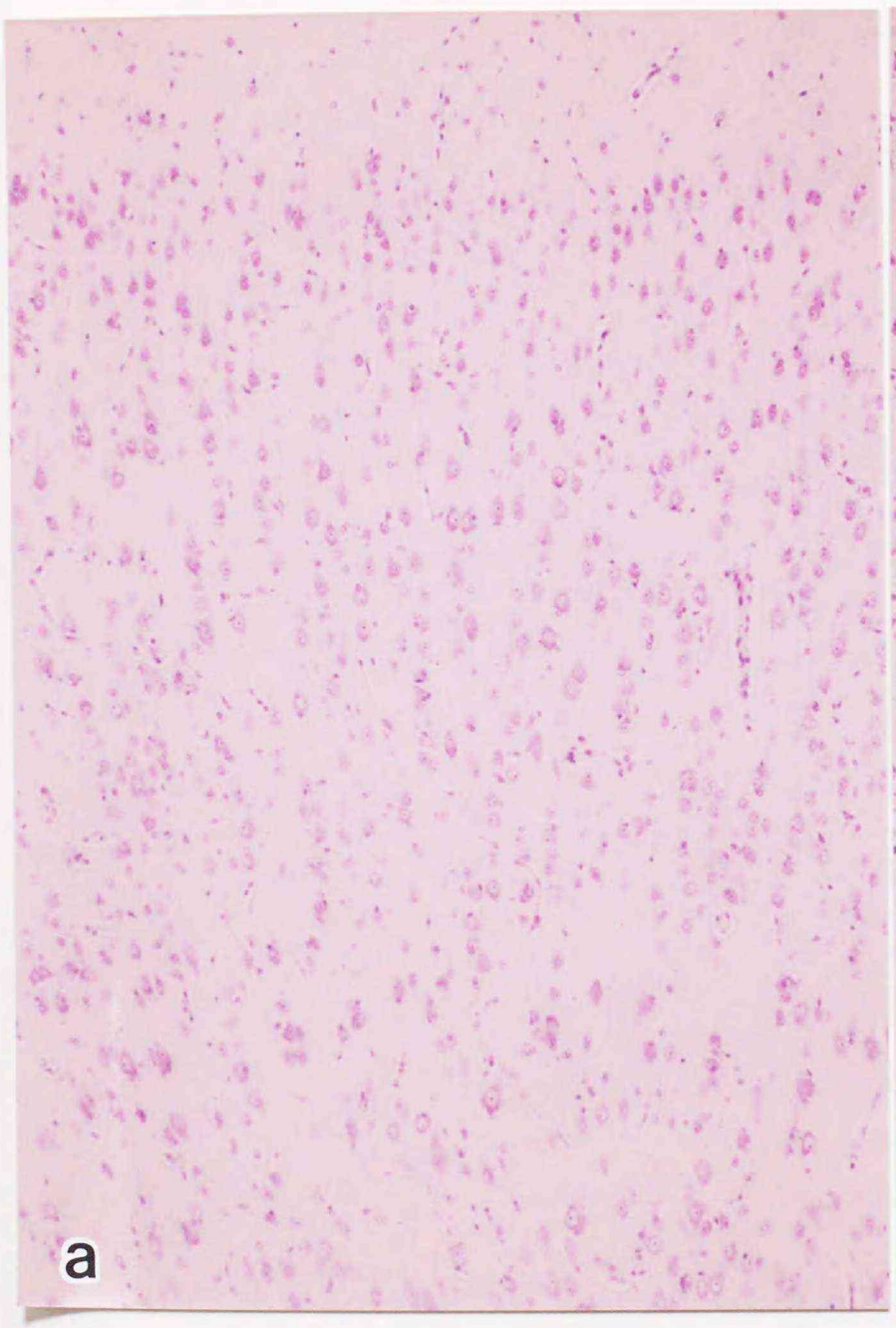


Fig. 11. Cerebral cortices from a 8-month-old dog (Dog No. 5) (a) and a 17-year-old dog (Dog No. 25) (b). Note the apparent neuronal loss in b. Nissl stain, x75.


a

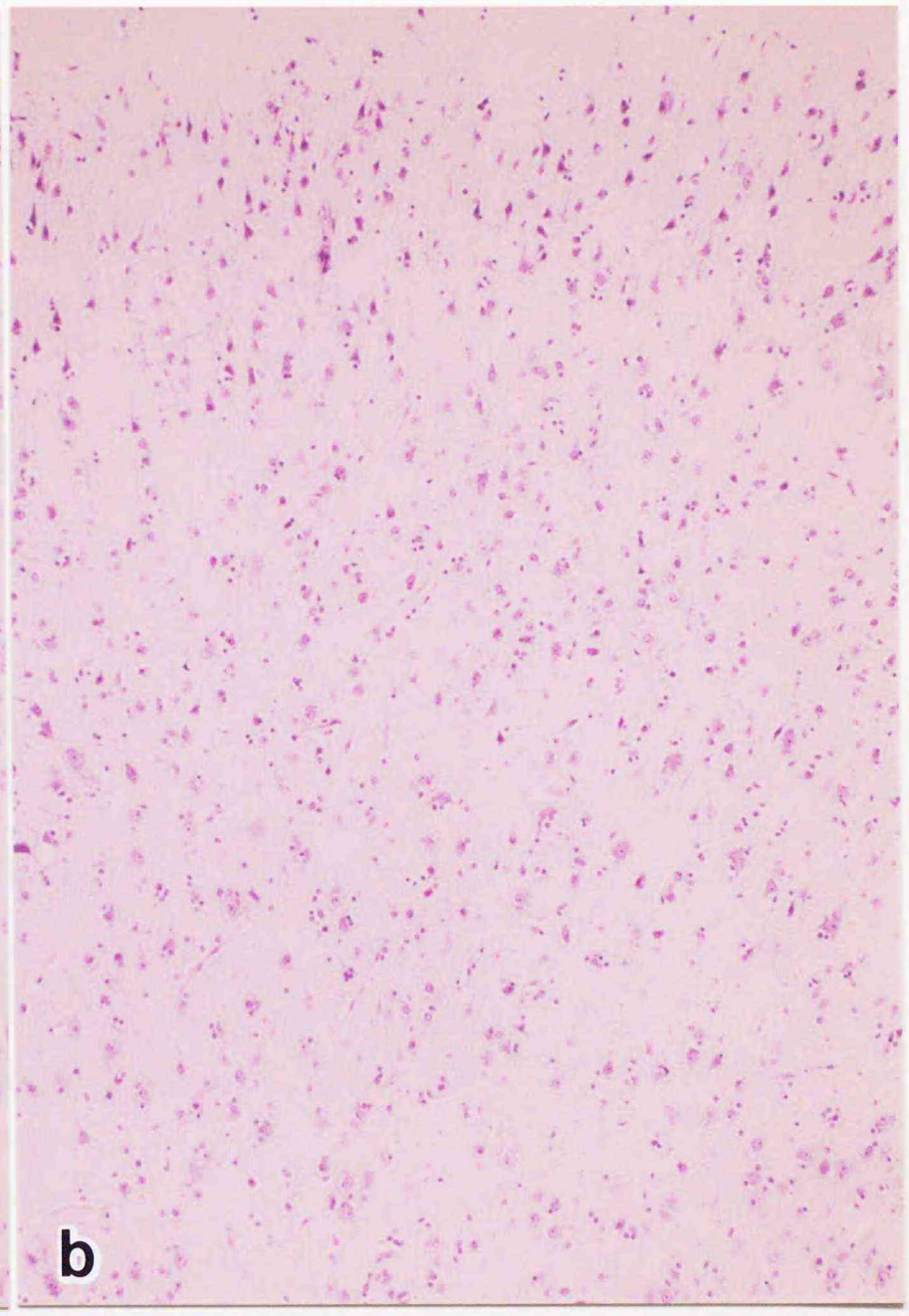

b

Fig. 12. Cerebral cortex from a 17-year-old dog (Dog No. 25), showing a considerable increase in glial fibrillary acidic protein (GFAP)-positive astrocytes (PA). GFAP immunostain, x120.

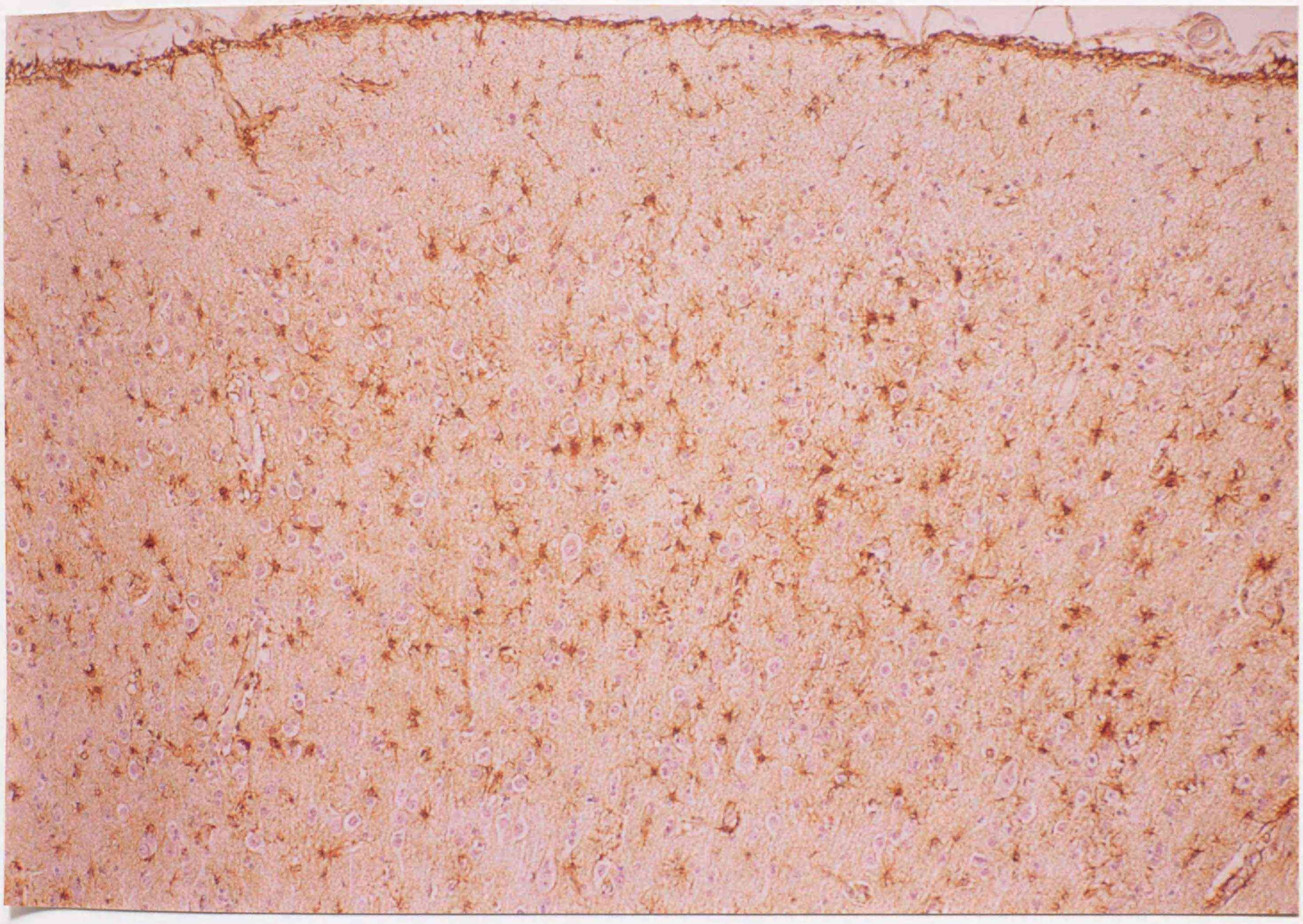


Fig. 13. Transmission electron micrograph of the cerebral cortex from a 14-year-old dog (Dog No. 17). Hypertrophic astrocytes contain lipofuscin pigments (L) and bundles of intermediate filaments (F). x15,000. Bar = 1 μm.

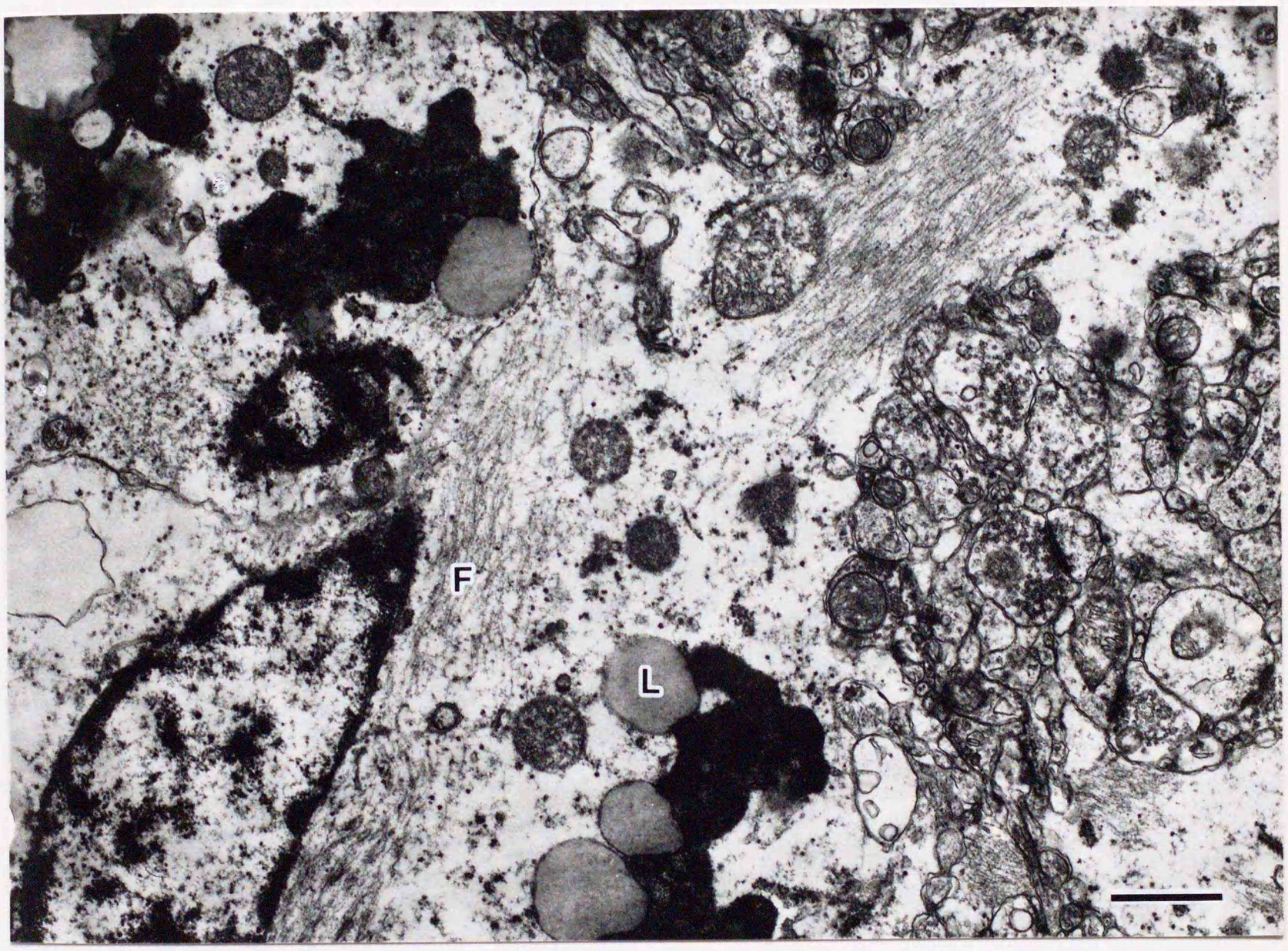
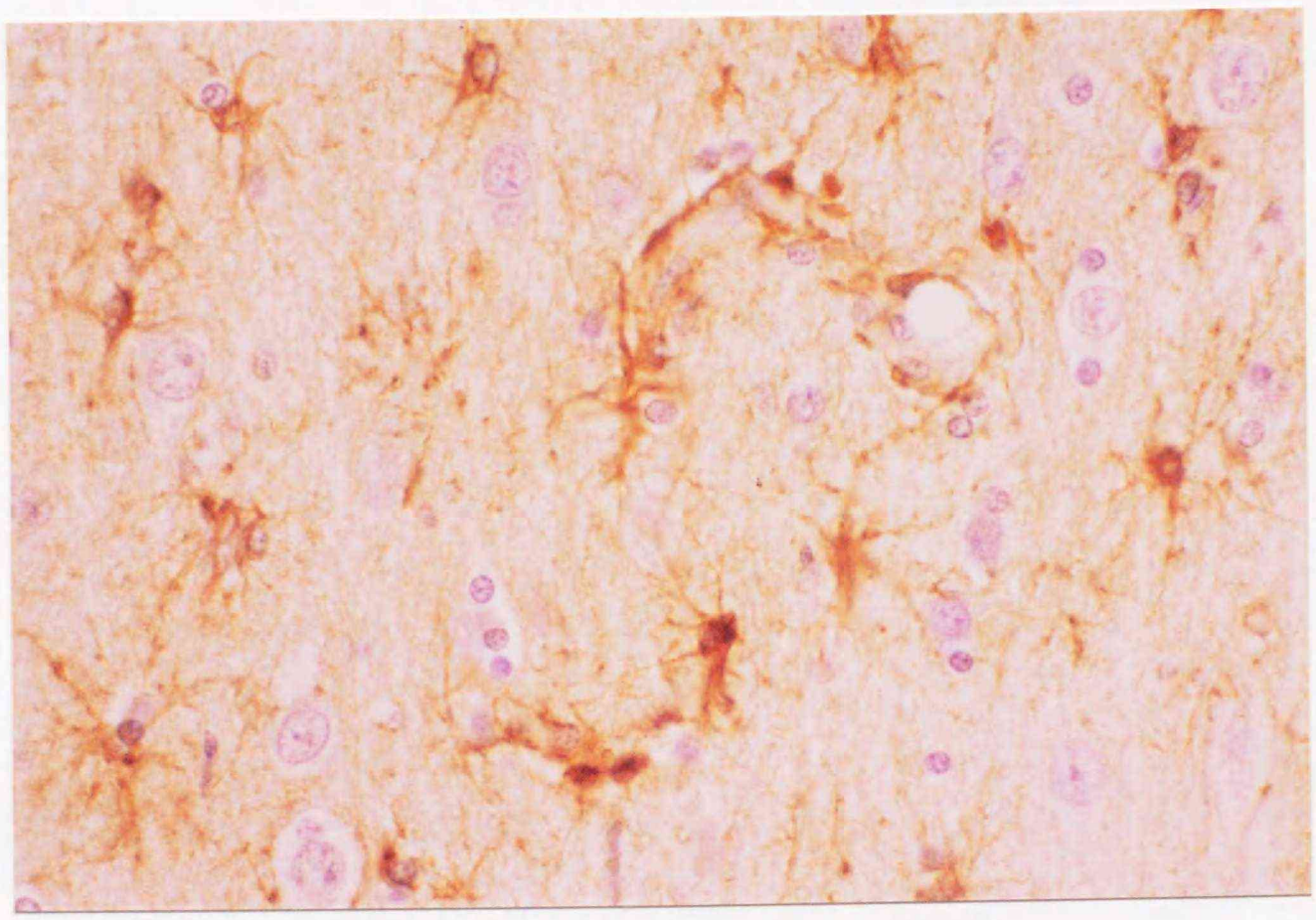

F
L

Fig. 14. Cerebral cortex from a 17-year-old dog (Dog No. 25), showing an interstitial and perivascular accumulation of GFAP-PA. GFAP immunostain, x240.

Fig. 15. Cerebral cortex from a 17-year-old dog (Dog No. 25), showing hypertrophic GFAP-PA. Note the GFAP-PA surrounding the nerve cell body (arrow). GFAP immunostain, x240.



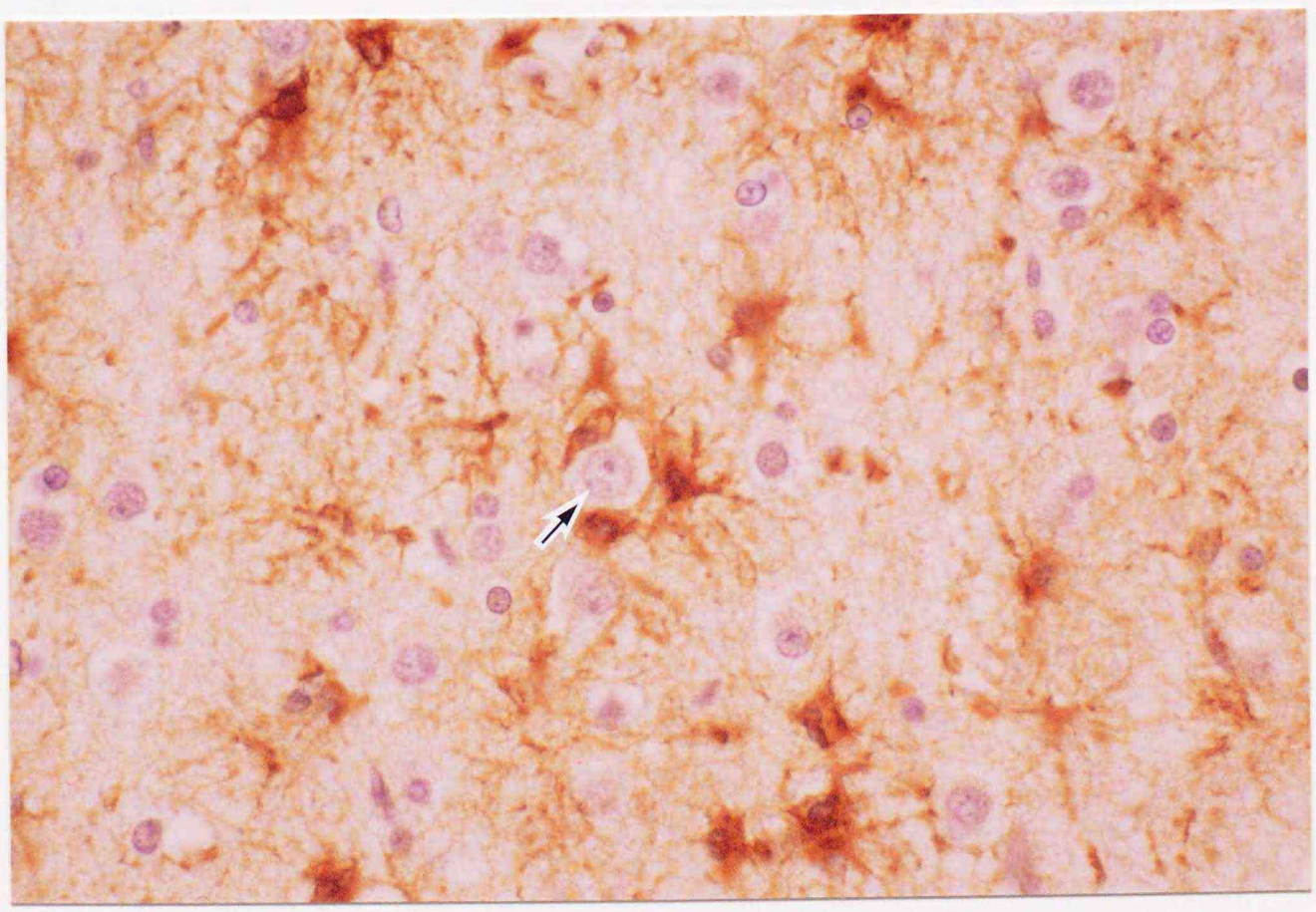
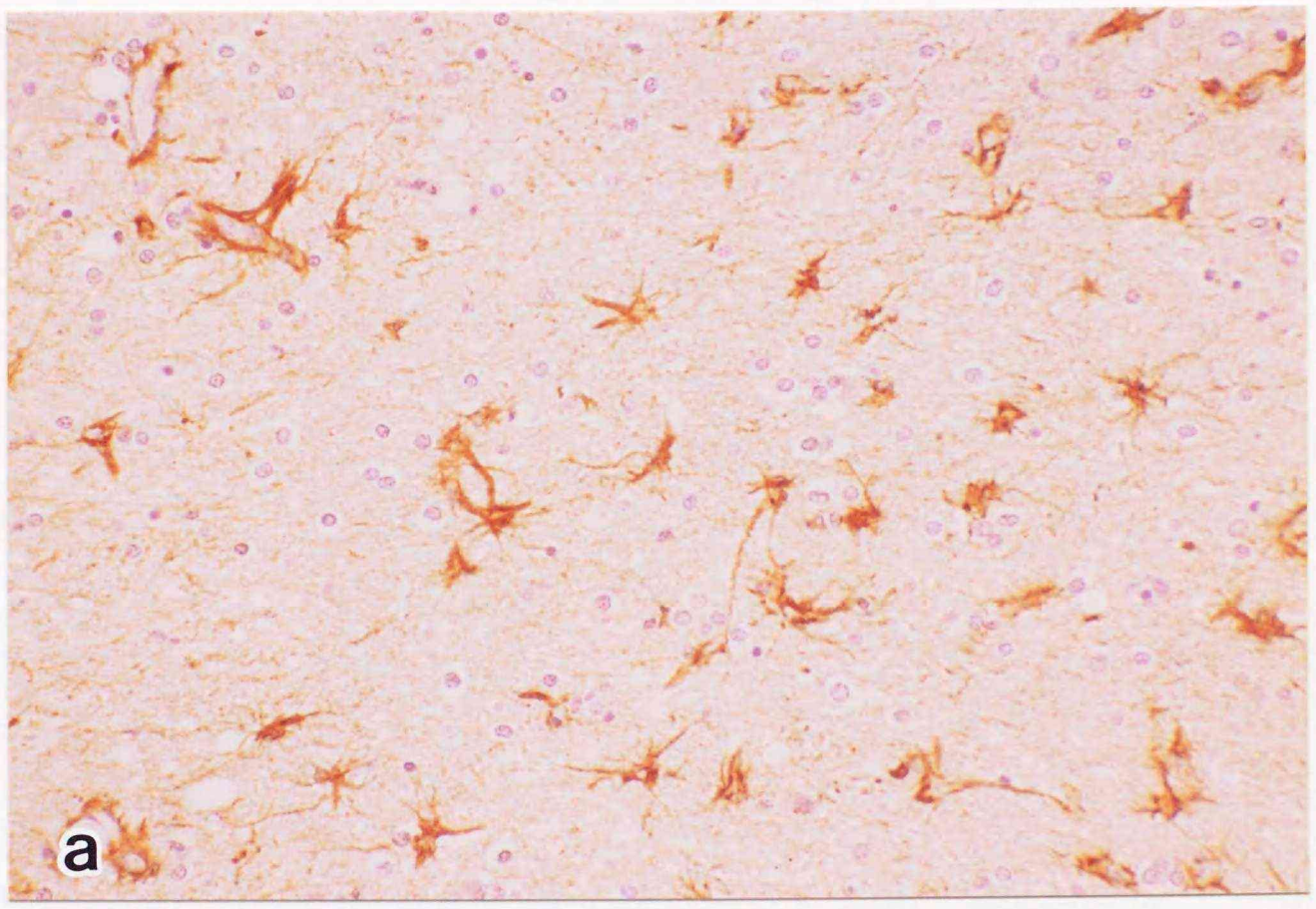
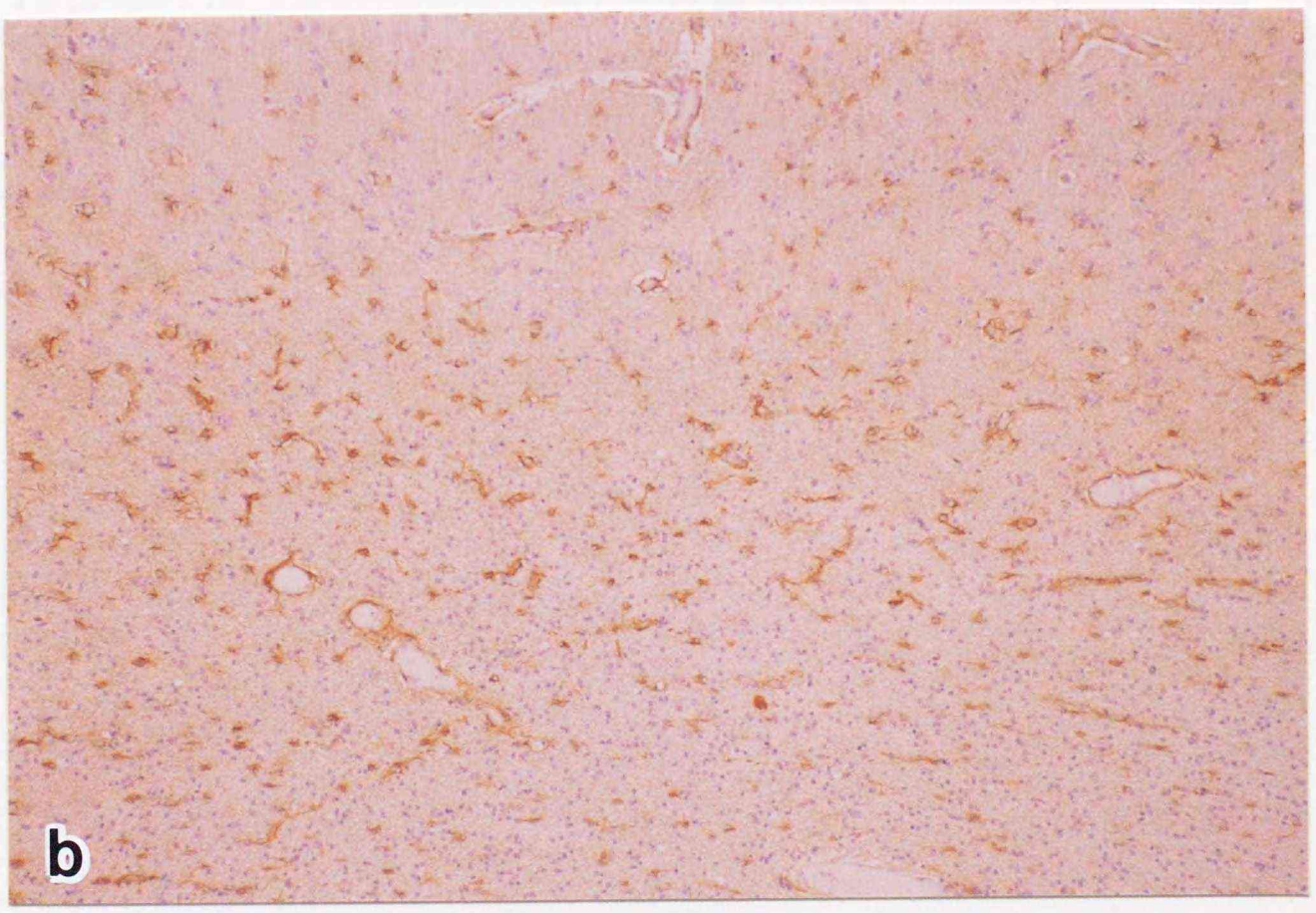
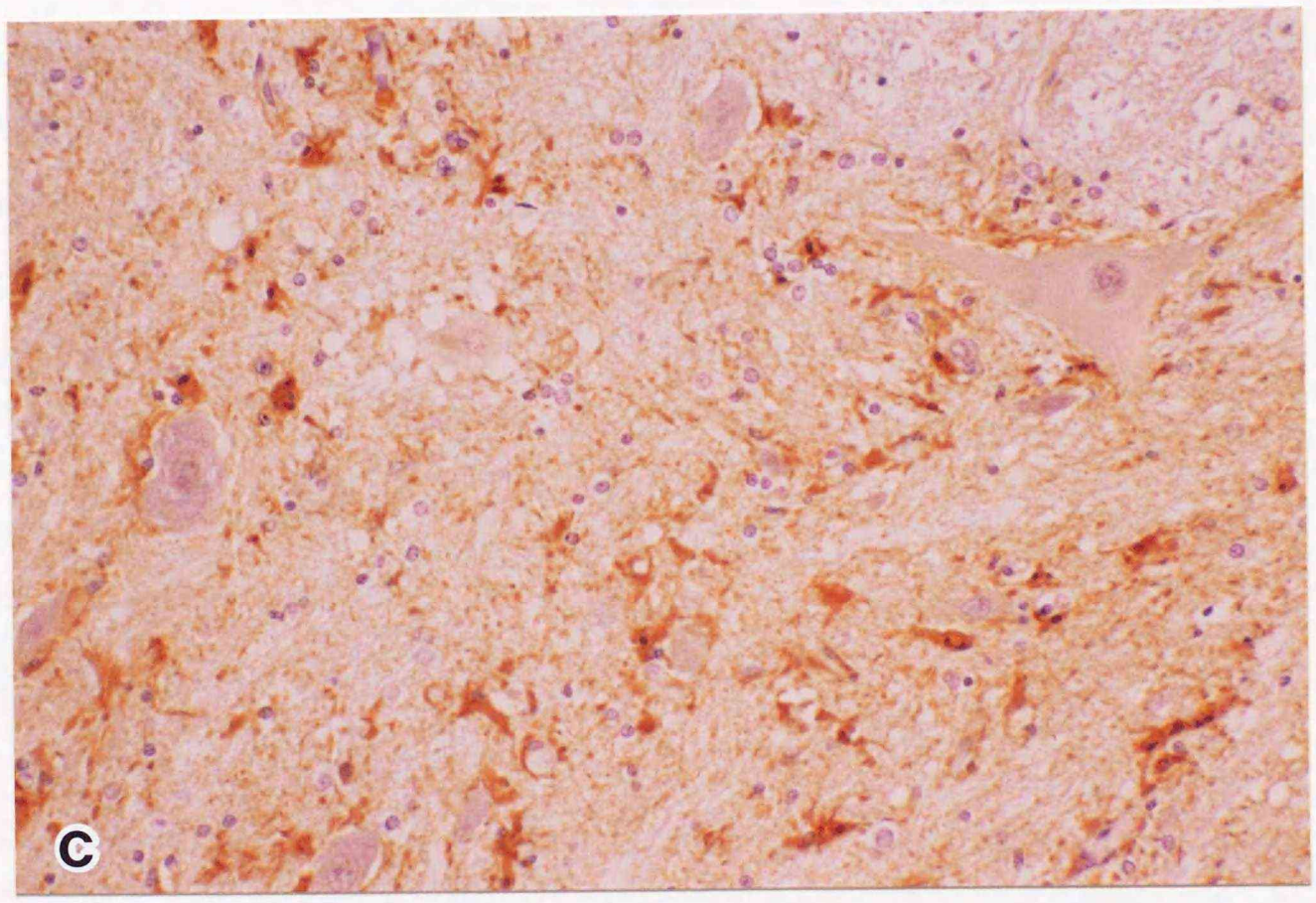
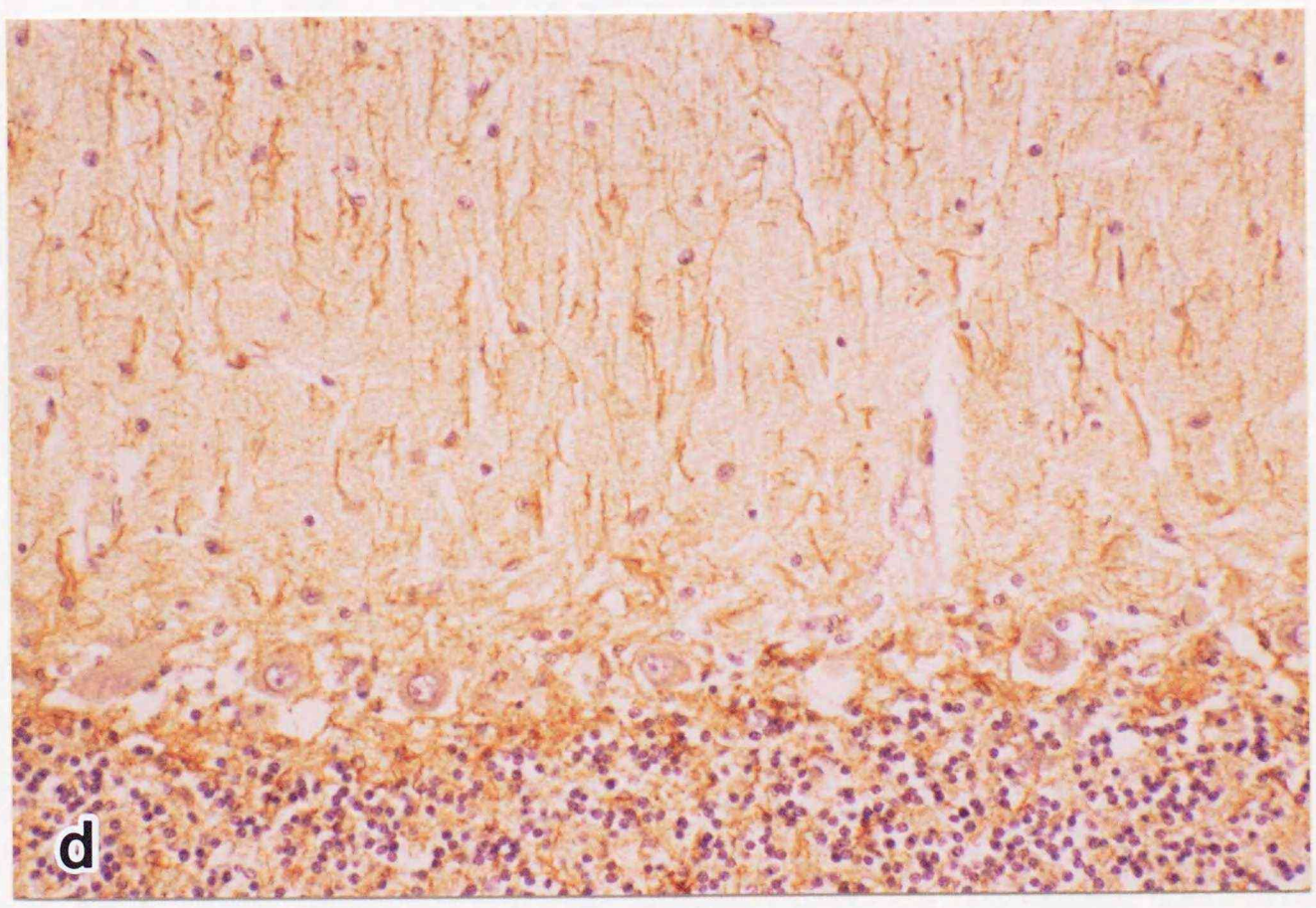


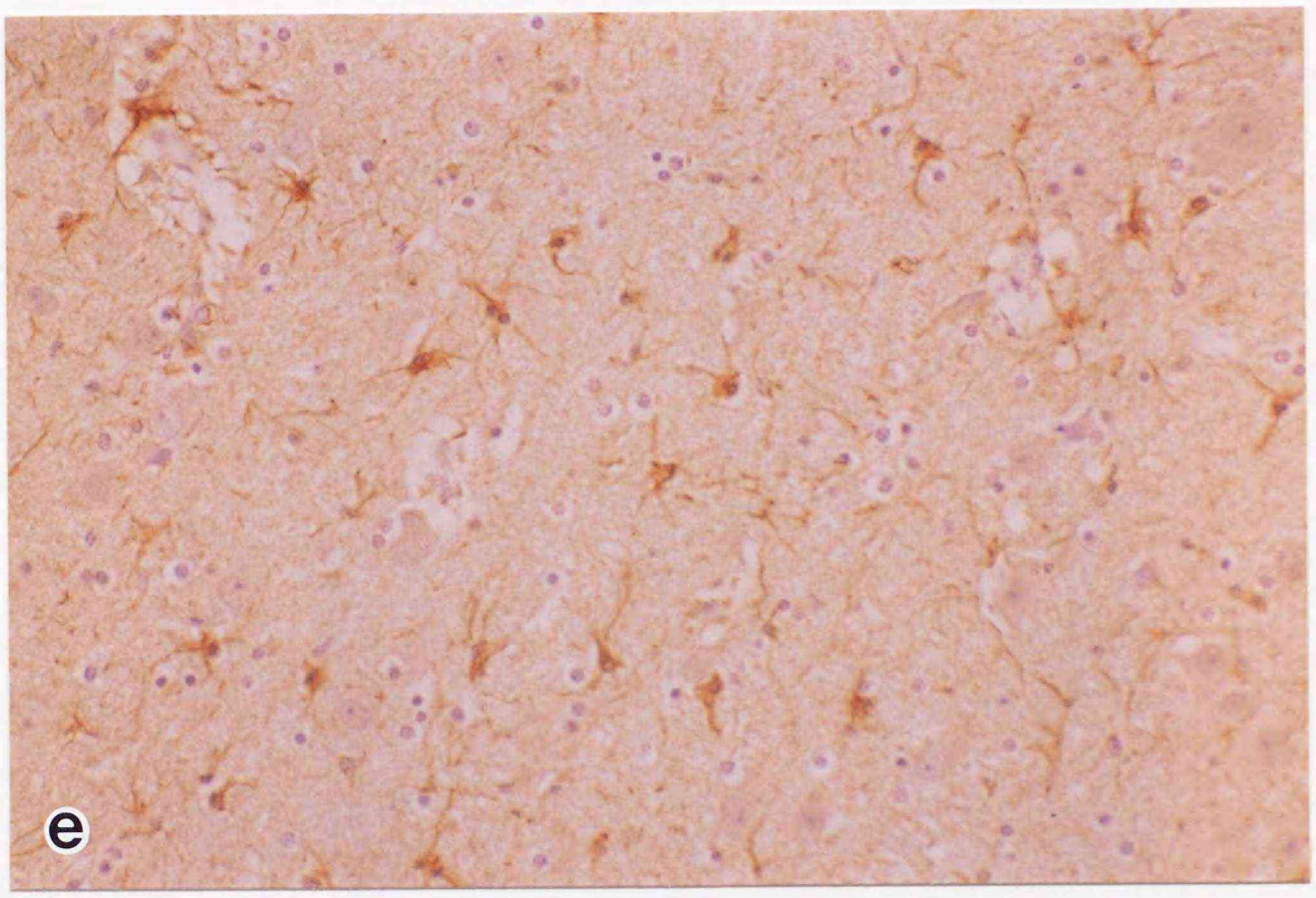
Fig. 16. GFAP immunohistochemistry of the central nervous system of the dogs. Note the moderate to severe astrocytic gliosis in the aged dogs: subcortical white matter of the cerebrum (a, Dog No. 21, x150); cortico-medullary junction of the cerebrum (b, Dog No. 23, x110); spinal cord (c, Dog No. 21, x150); cerebellum (d, Dog No. 24, x150); thalamus (e, Dog No. 20, x150); hippocampus (f, Dog No. 16, x150); subependymal area of the cerebrum (g, Dog No. 16, x150). Only occasional GFAP-positive fibers (arrows) are demonstrated in the cerebral cortex of a 6-month-old dog (h, Dog No. 5, x110).

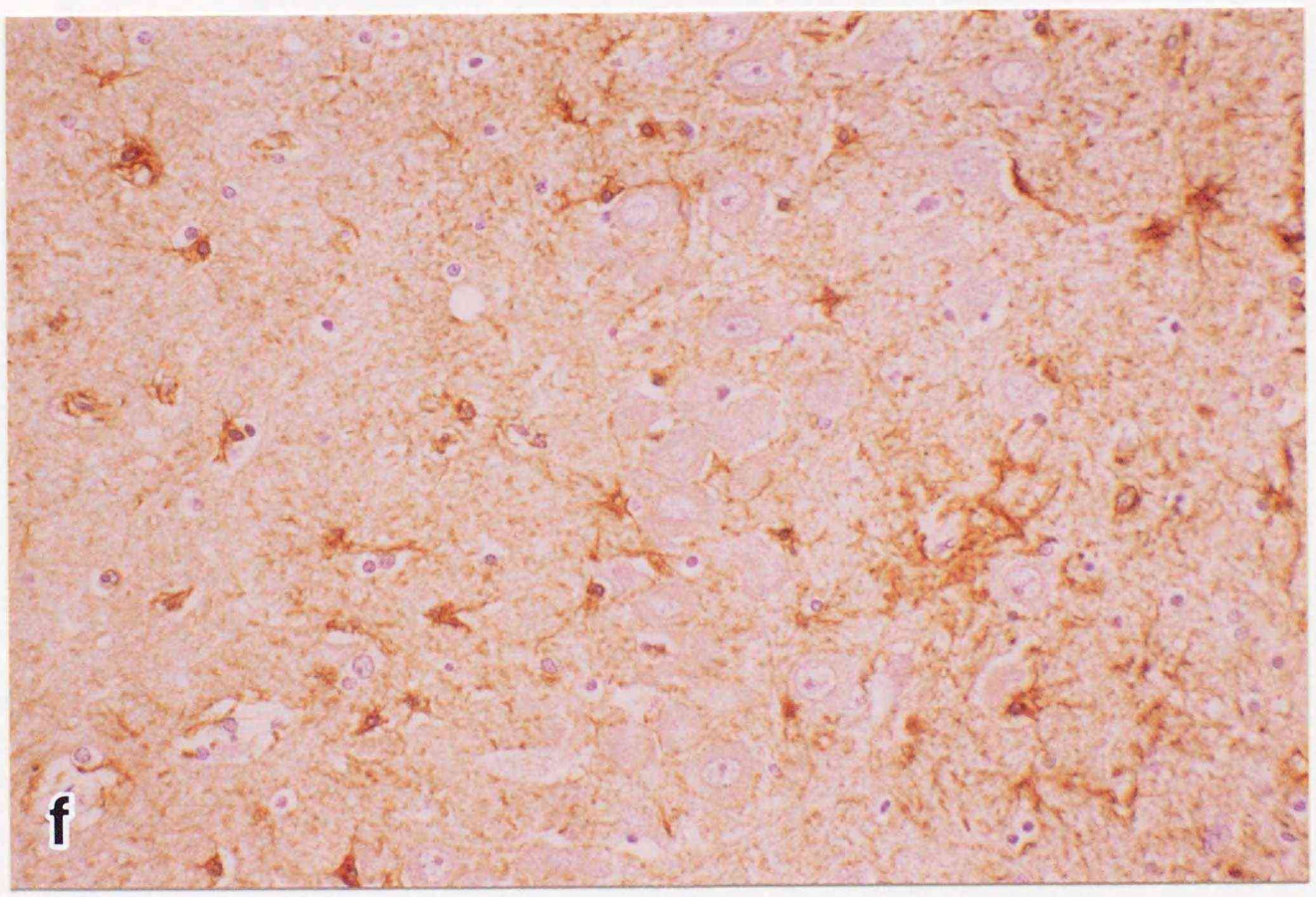

a

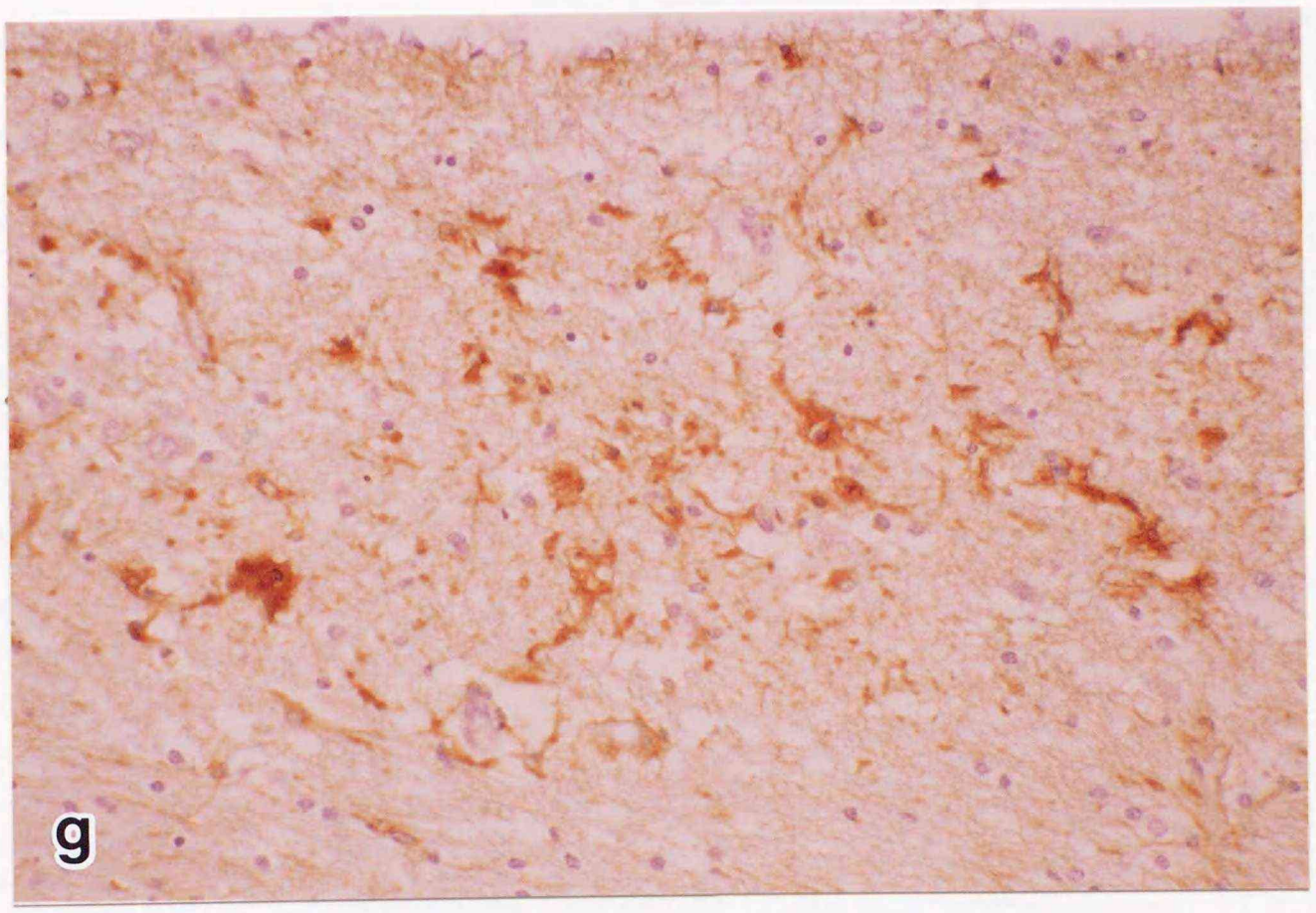

b


c


d


e


f


g

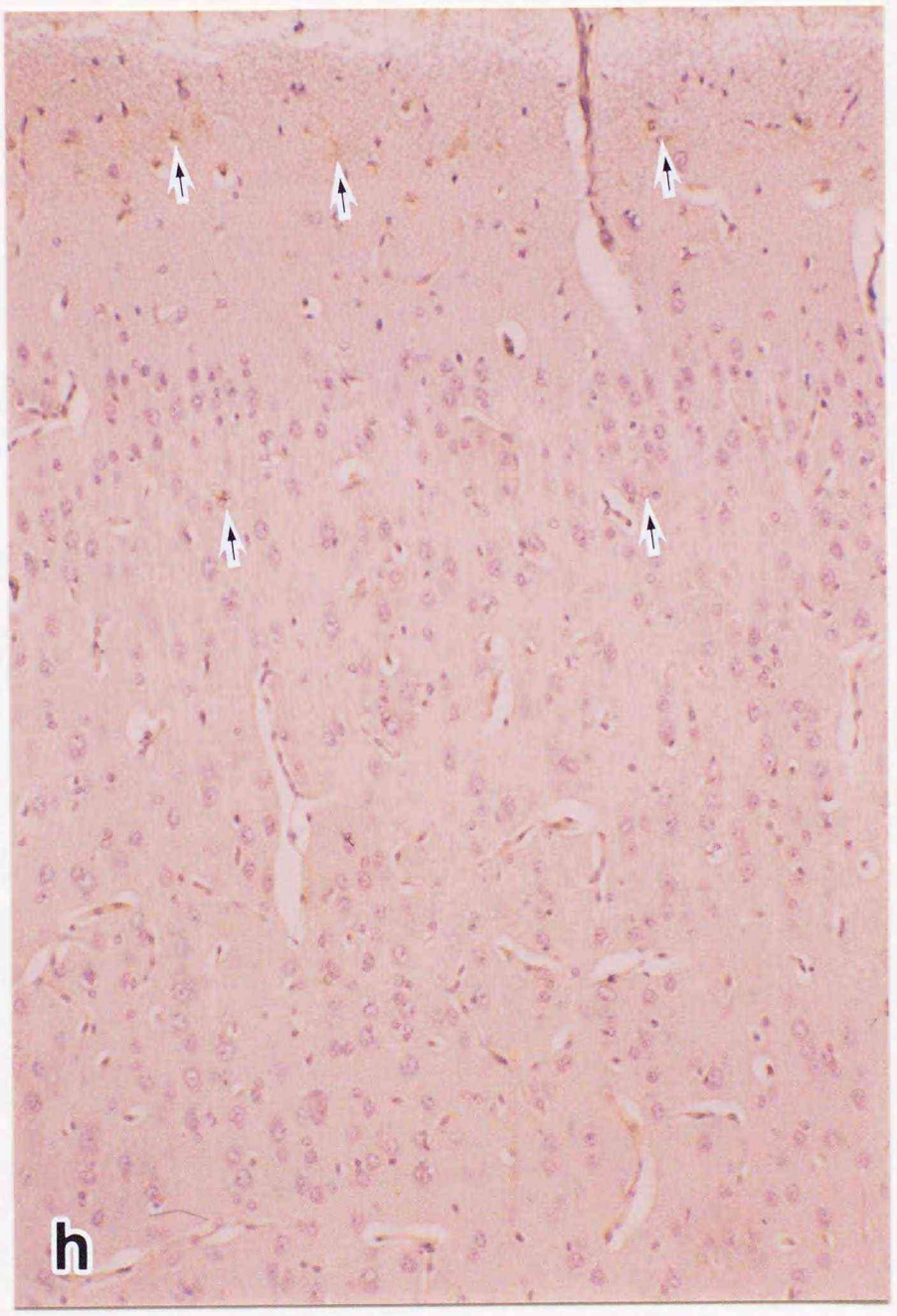
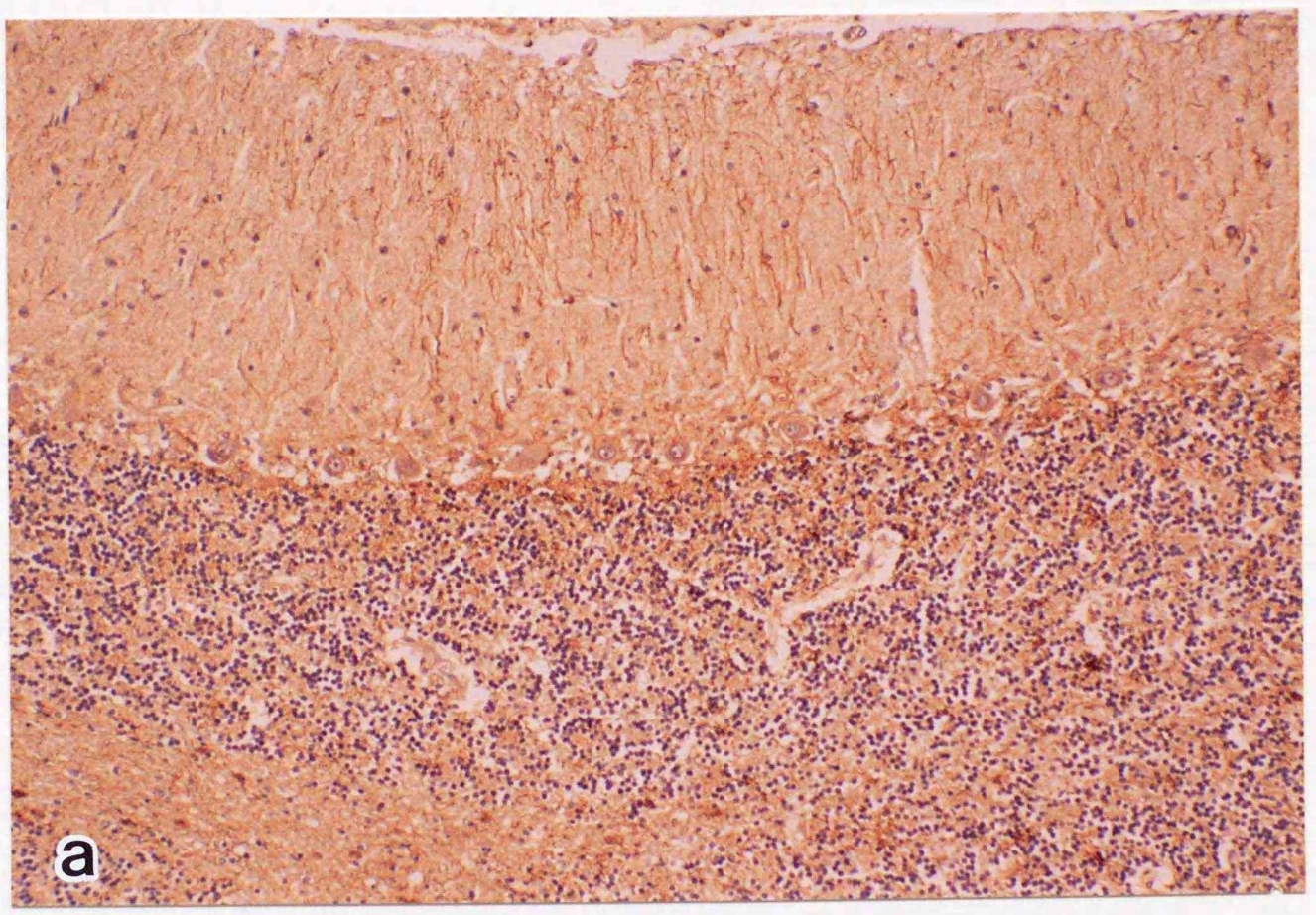

h

Fig. 17. Serial sections of the cerebellum stained with GFAP immunostain (a) and Bodian stain (b). No obvious degenerative changes of the neural architecture are observed in b. Dog No. 25, x75.


a

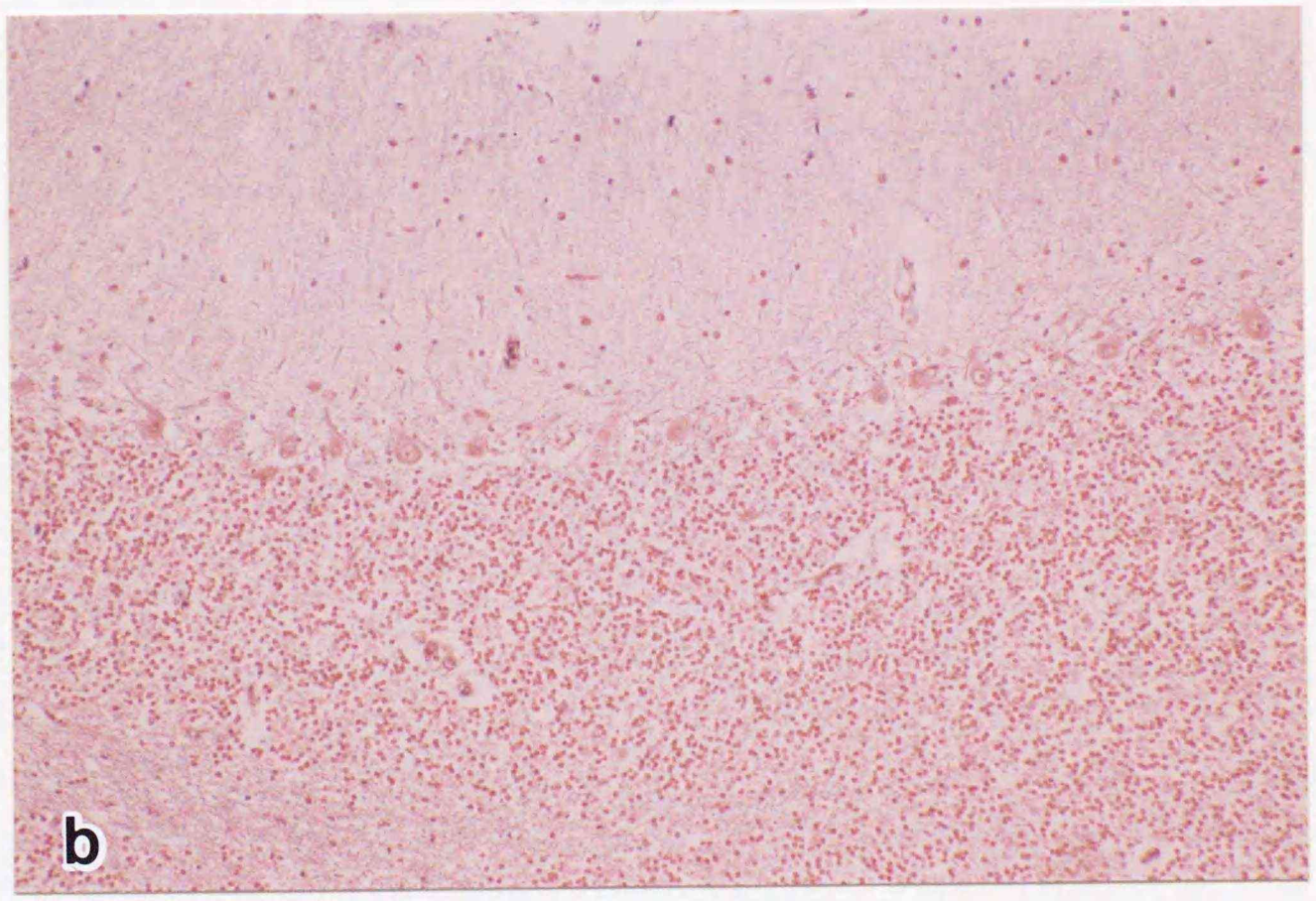
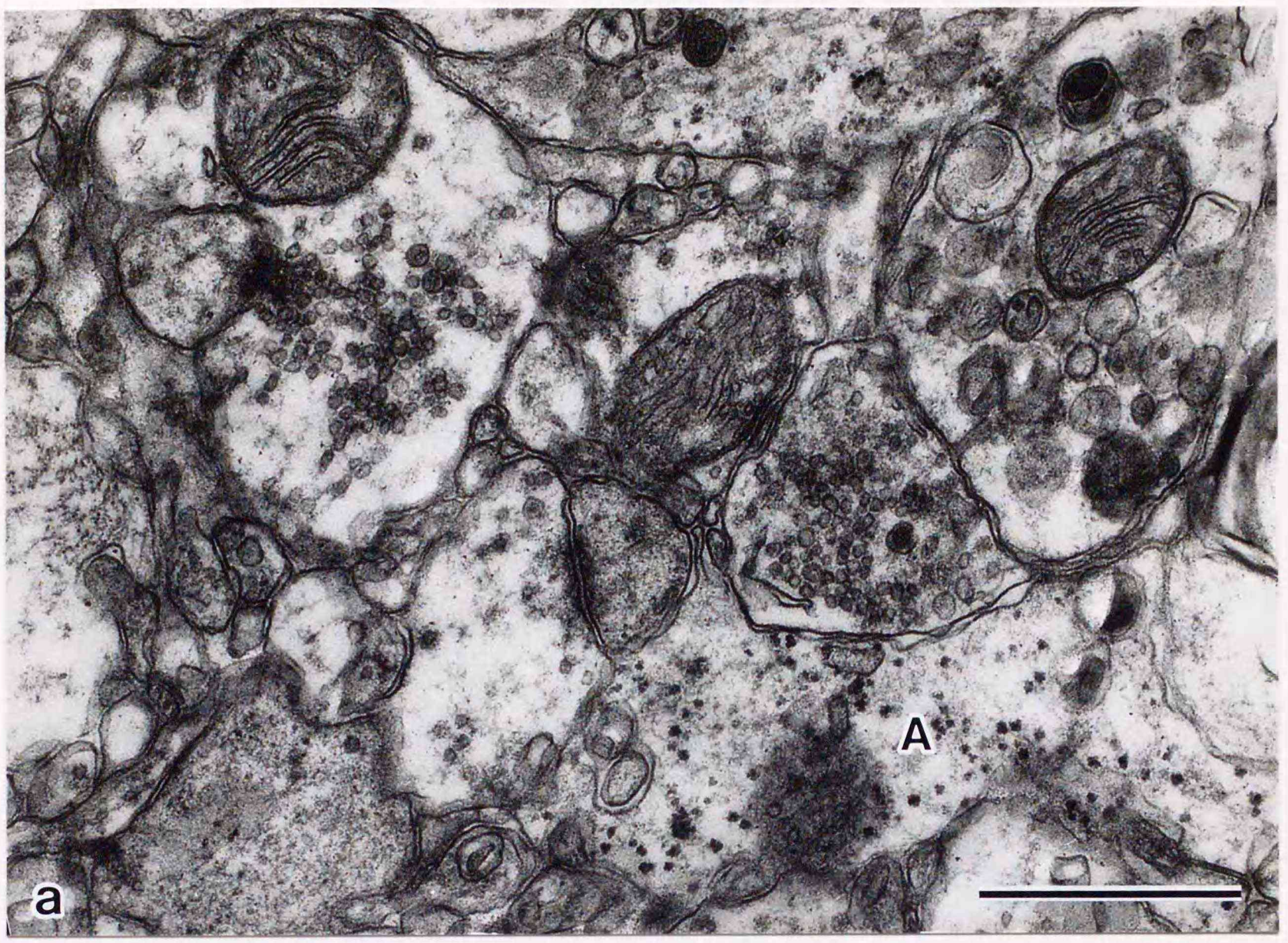
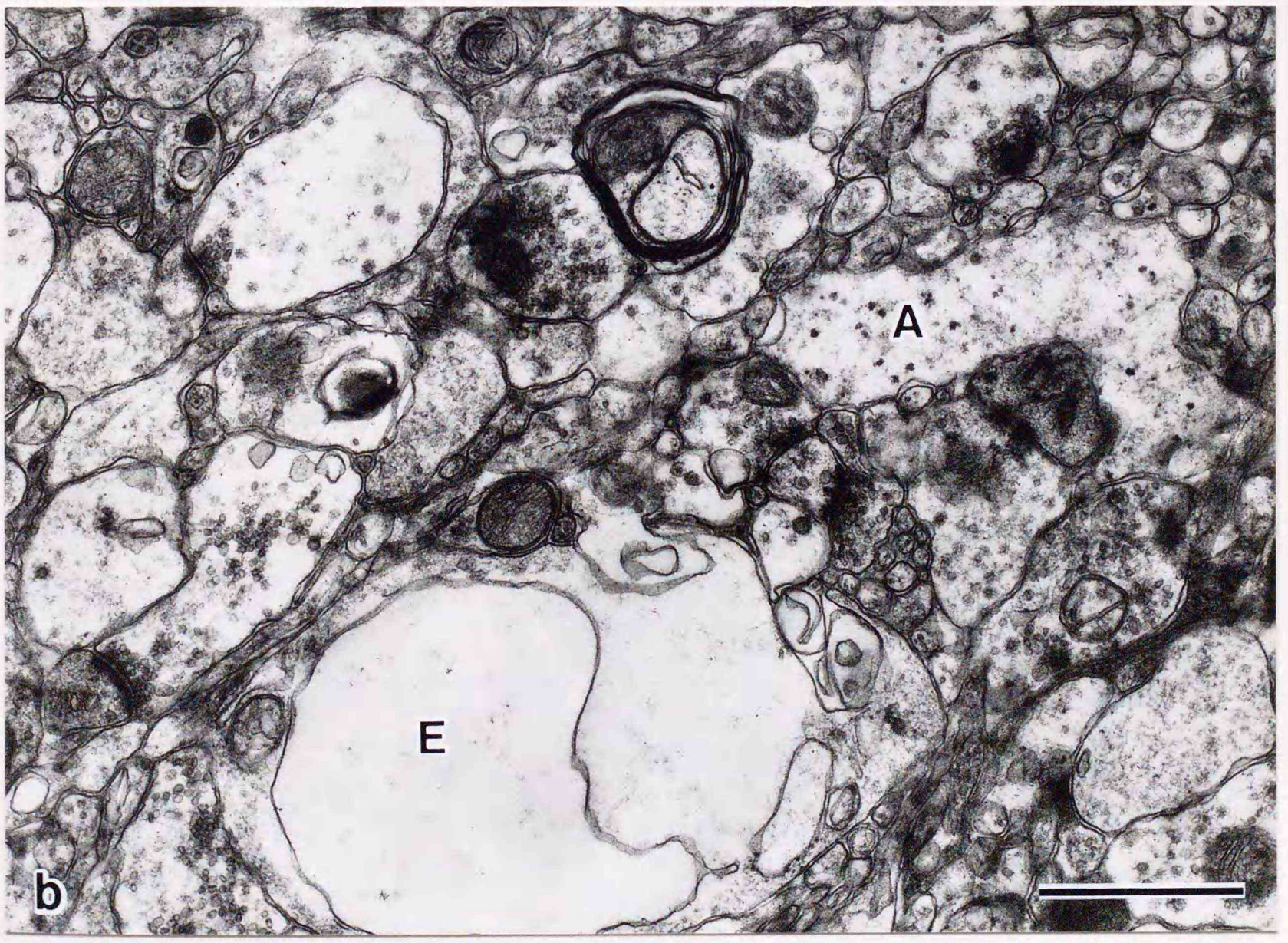
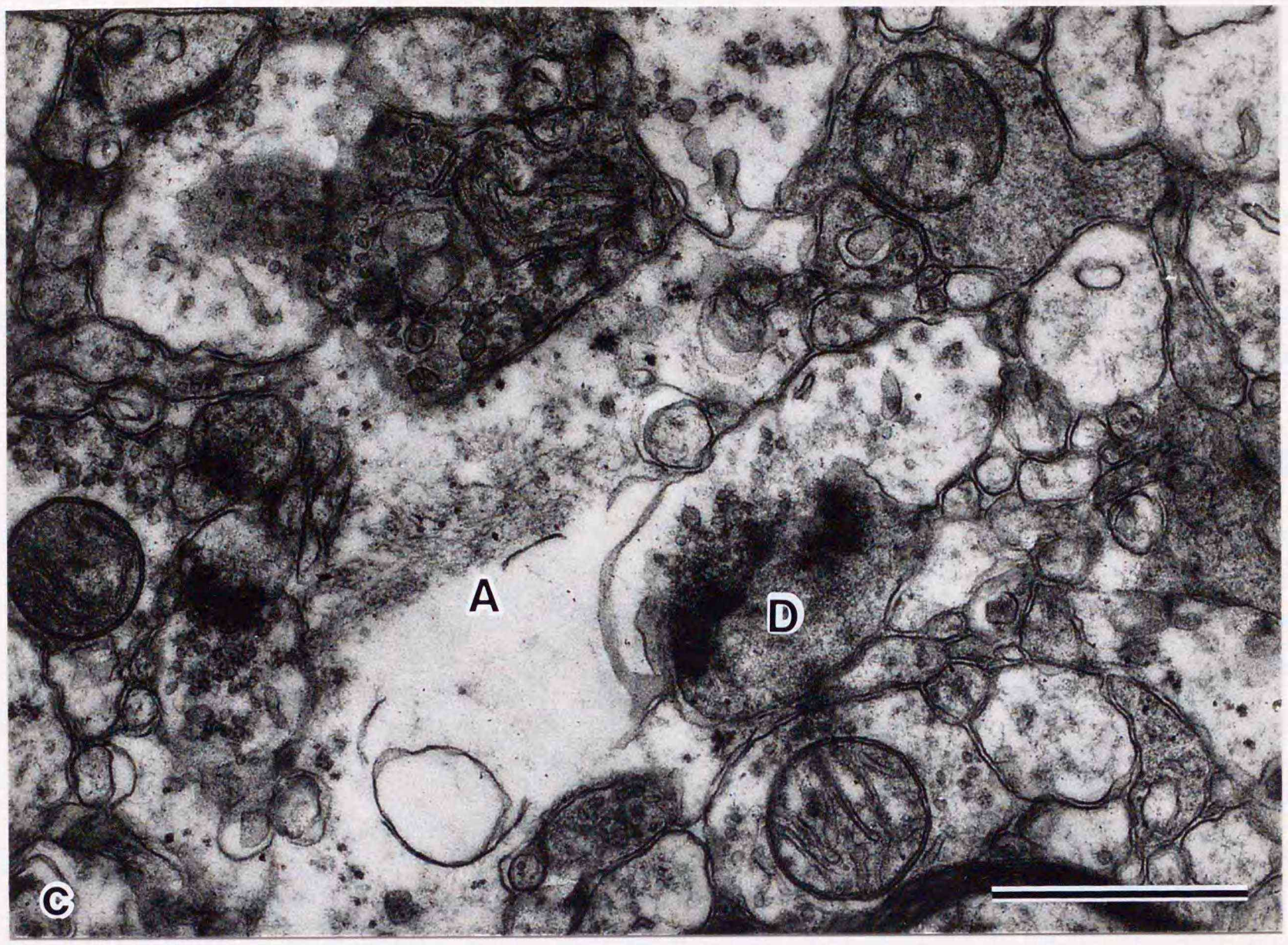

b

Fig. 18. Transmission electron micrographs of the cerebral cortex from the aged dog (Dog No. 17), showing a variety of degenerative profiles of neural components: swollen synapse-like vesicles with dense contents (a, x21,000); a dilated endoplasmic reticulum-like structure (E) (b, x15,000); heterogeneous dense bodies (D) (c, x22,000); dark axon terminals (d, x24,000). Note the increased number of glycogen granules in the astrocytic processes (A). Bar = 1 μm.


A
a


A
E
b


A
D
C

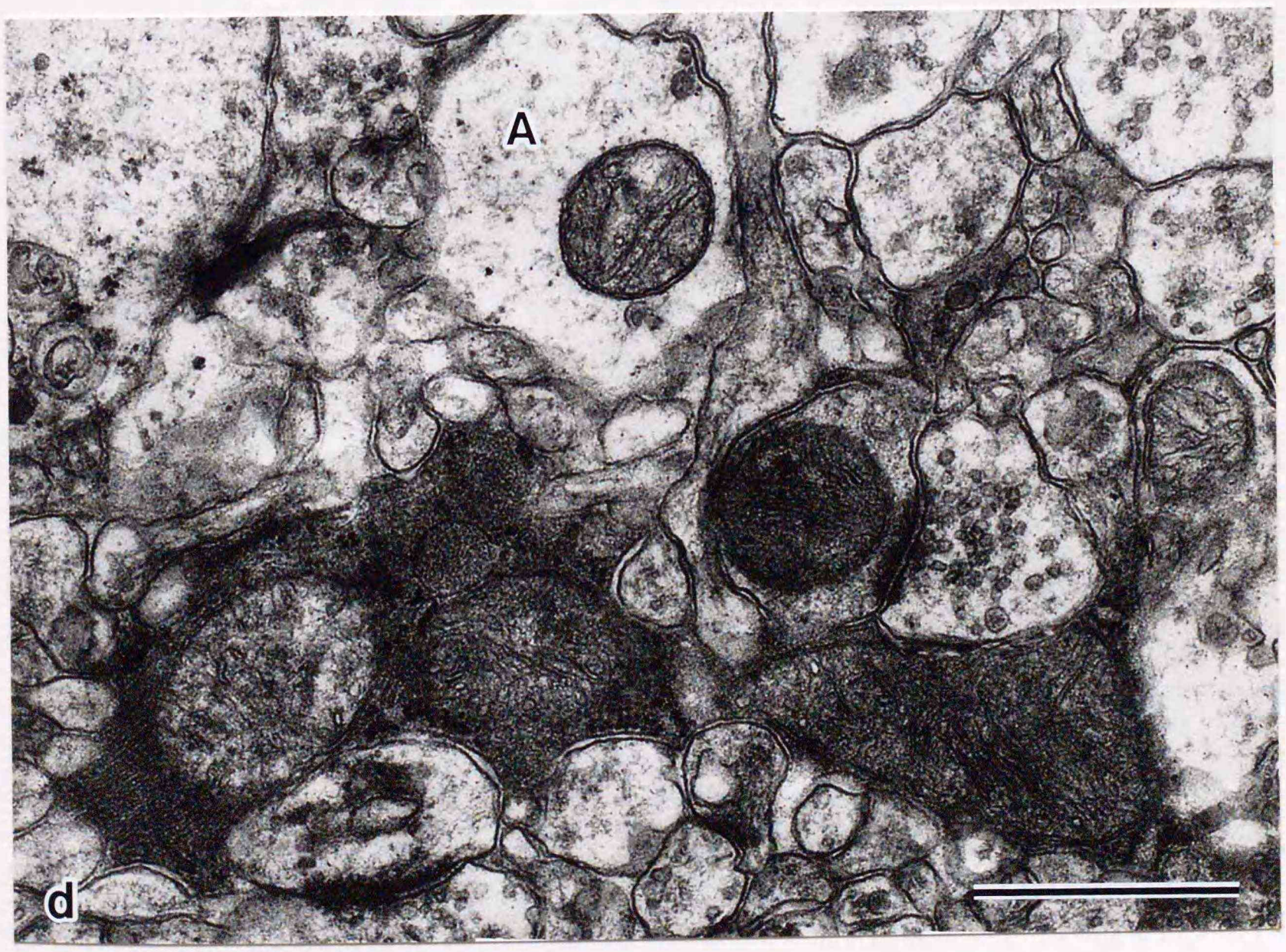

A
d

Fig. 19. Transmission electron micrograph of the cerebral cortex from a 18-year-old dog (Dog No. 26). Note the bundles of intermediate filaments (F) in the process of a hypertrophic astrocyte surrounding the degenerative neural components. x30,000. Bar = 1 μm.

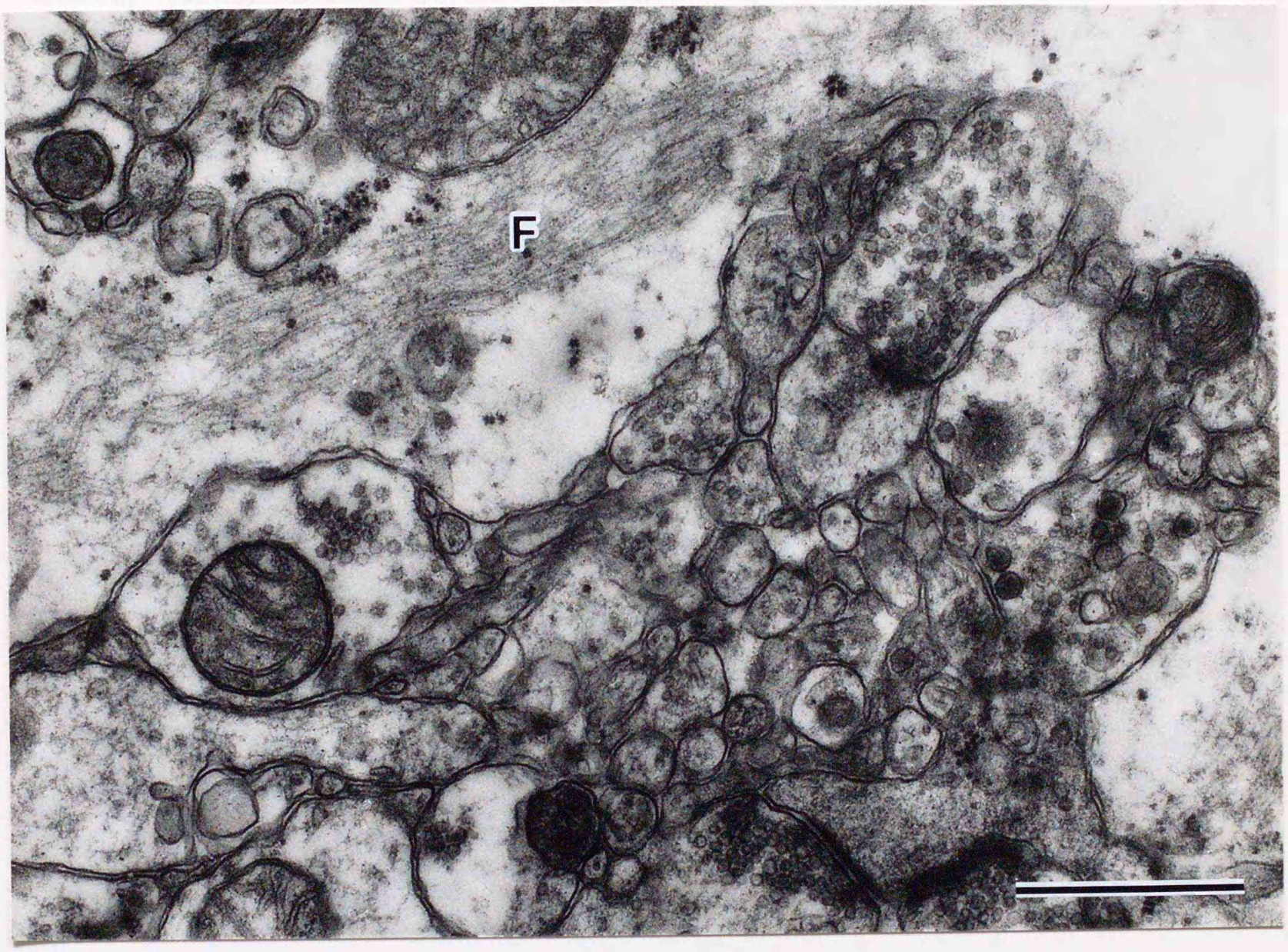

F

Fig. 20. Transmission electron micrograph of the cerebral cortex from a 12-month-old dog (Dog No. 6). No degenerative profiles of neural components are observed. x13,000. Bar = 1 μm.

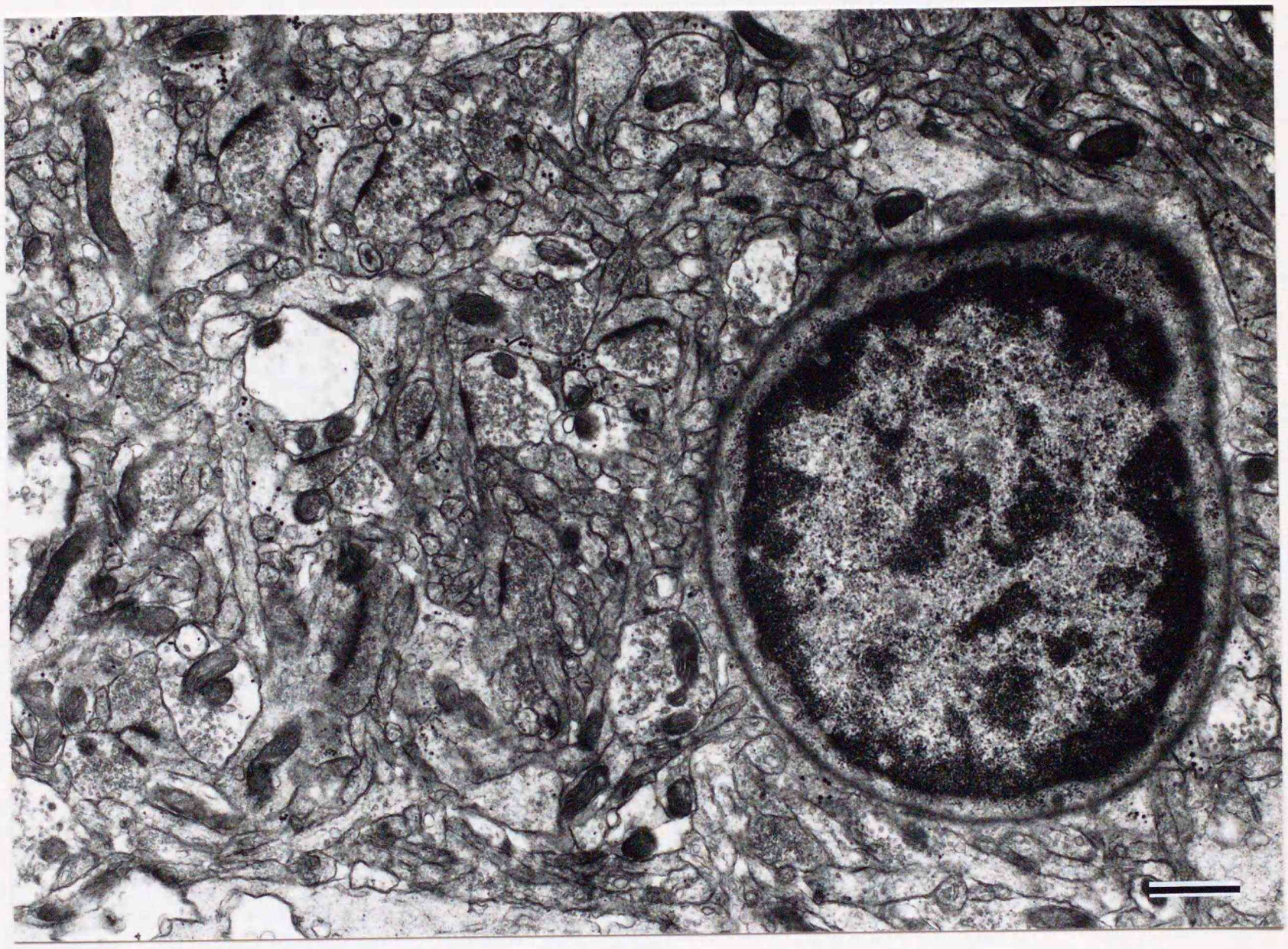
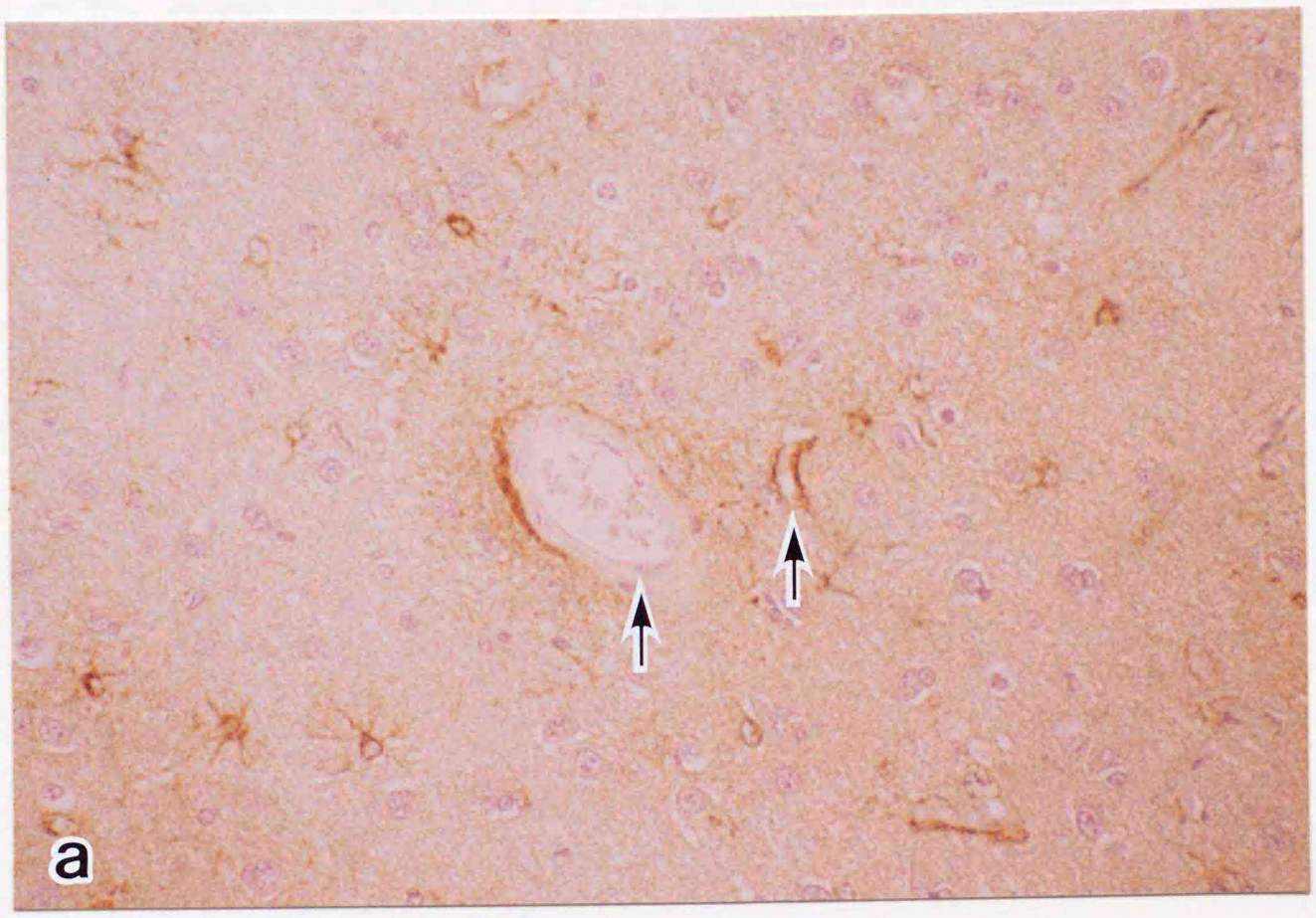


Fig. 21. a; Cerebral cortex from a 17-year-old dog (Dog No. 5), showing a diffuse astrocytic gliosis with blood vessels (arrows) surrounded by a GFAP positive network. GFAP immunostain, x175. b; Amyloid deposits are not necessarily demonstrated in the vessels with astrocytic reaction when a GFAP-stained section counterstained with thioflavin S is observed on a fluorescent microscope. b is the same slide as a and visualized with fluorescein filters. Thioflavin S stain, x175.


a

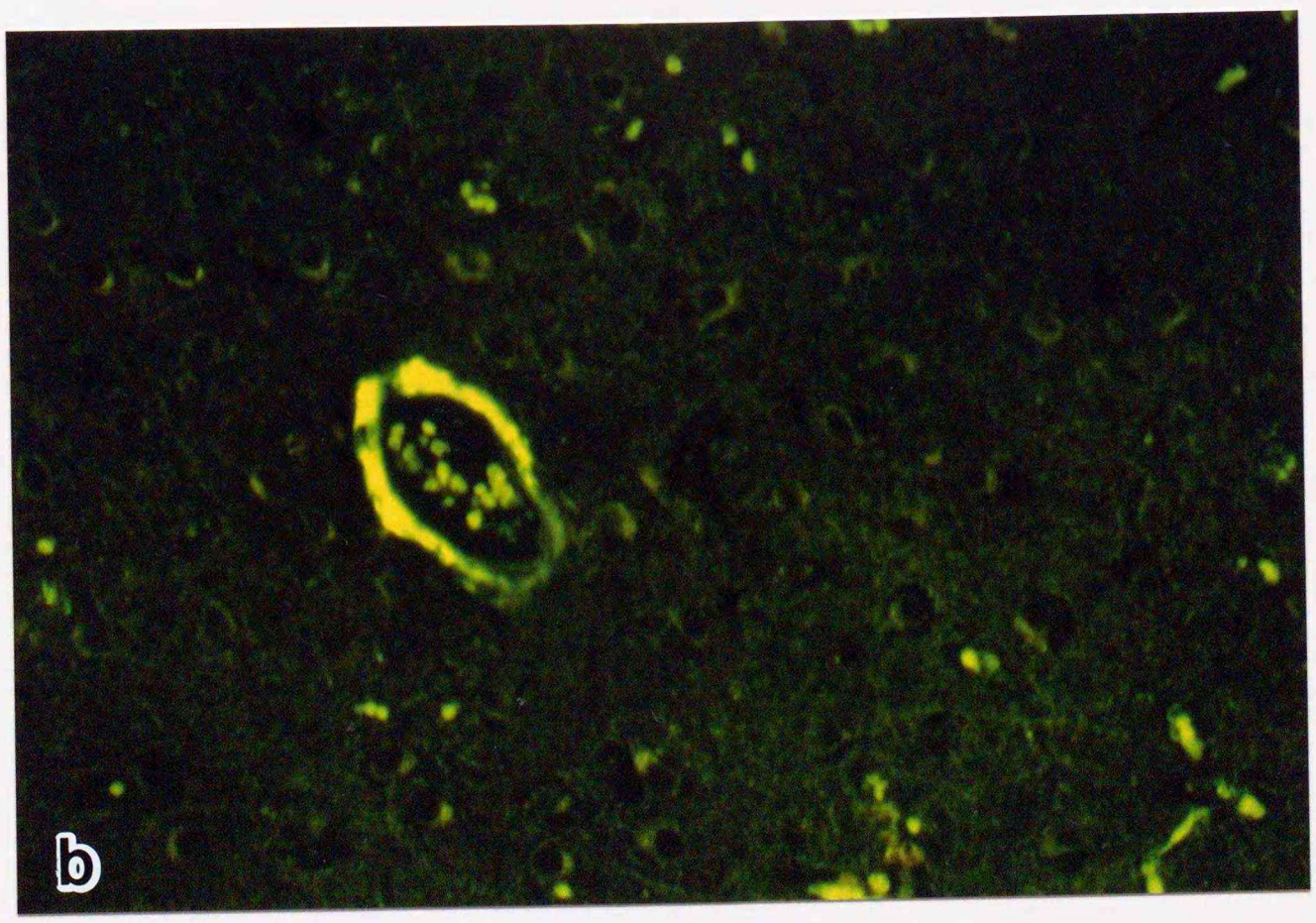
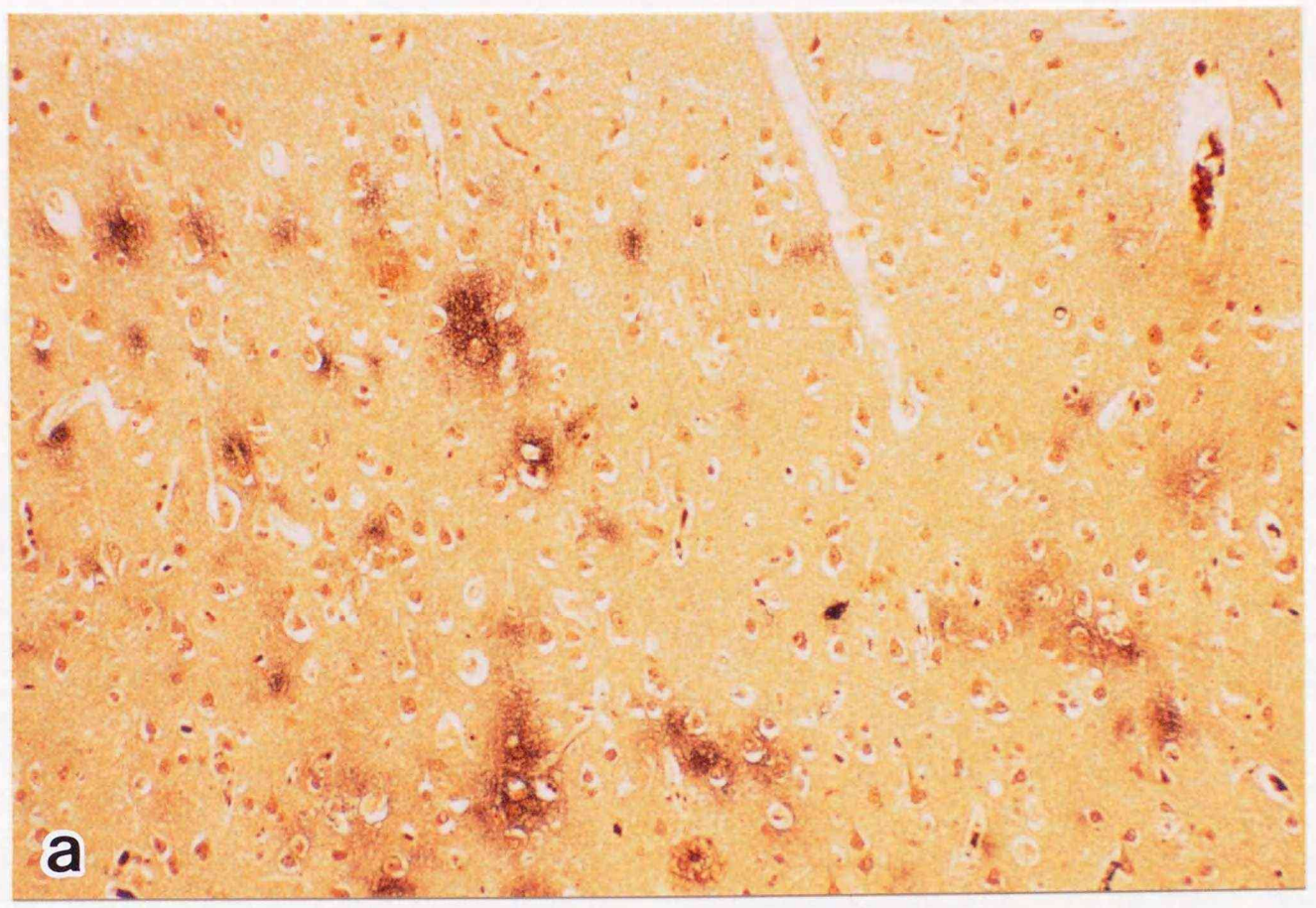
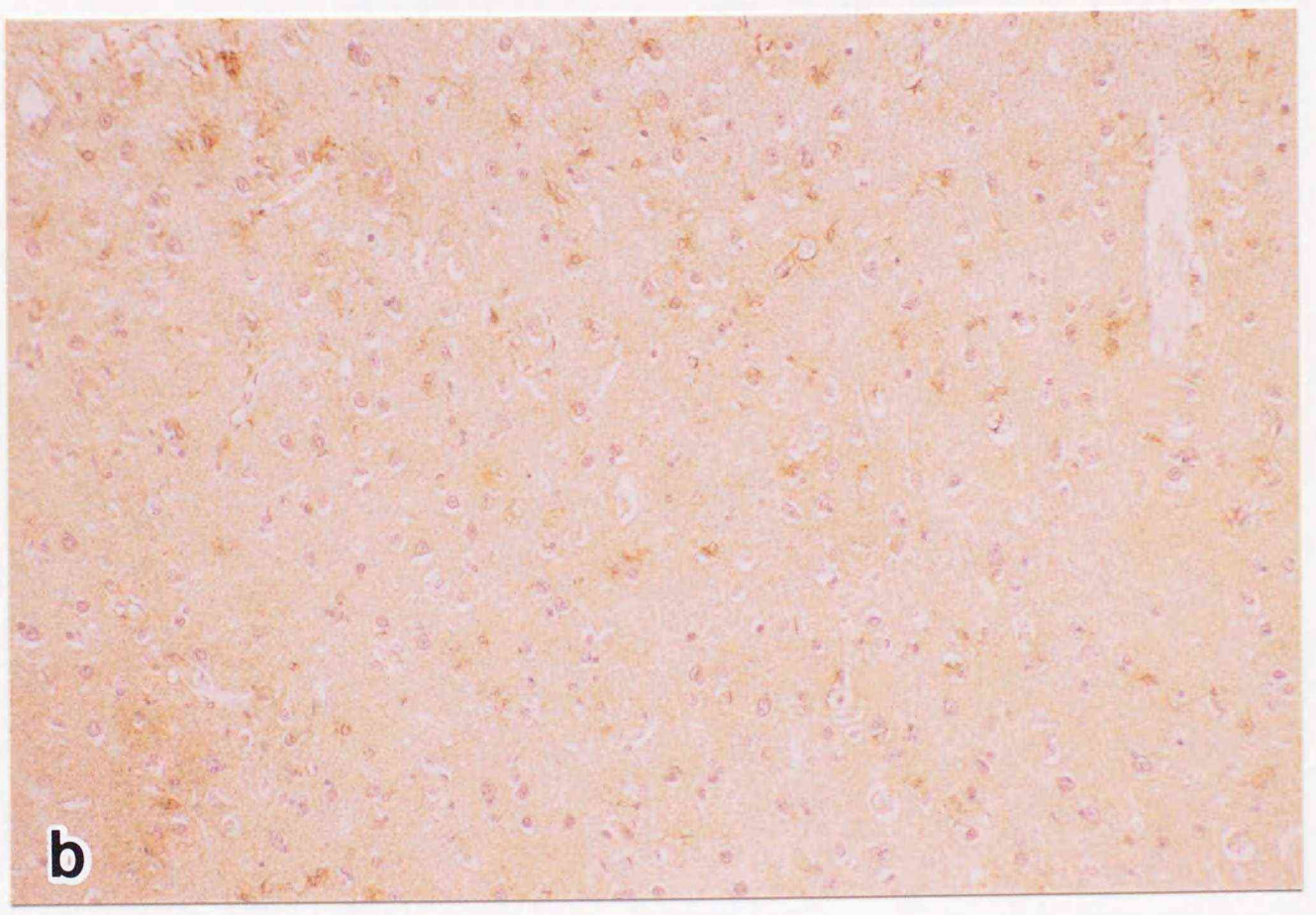
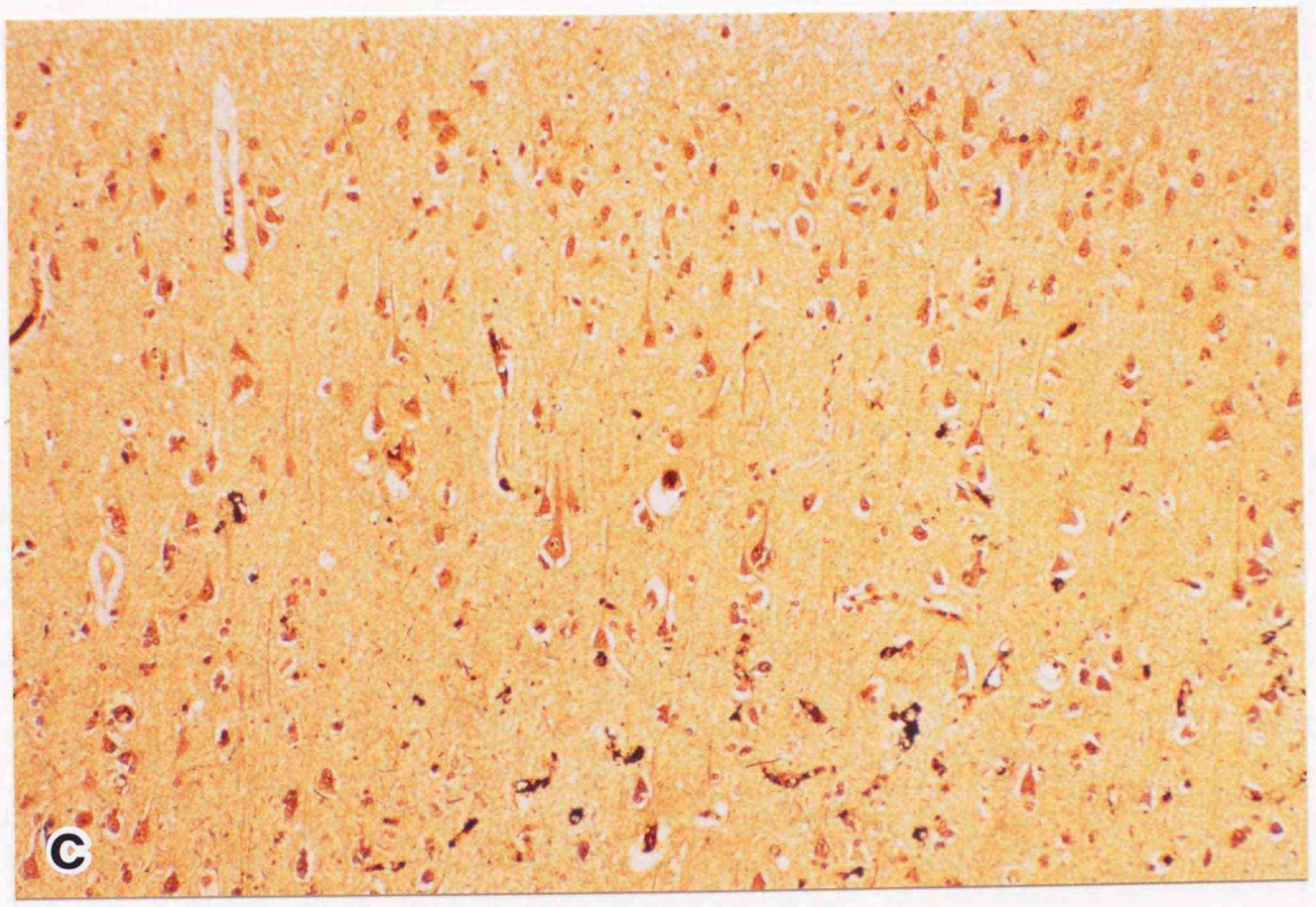

b

Fig. 22. Serial sections from the cingulate cortex (a, b) and parietal cortex (c, d) of a 17-year-old dog (Dog No. 5). Note no obvious difference in the pattern and intensity of astrocytic gliosis between the area with senile plaques (SP) (a, b) and the area without SP (c, d). a and c; modified Bielschowsky stain, x120. b and d; GFAP immunostain, x120.


a


b


c

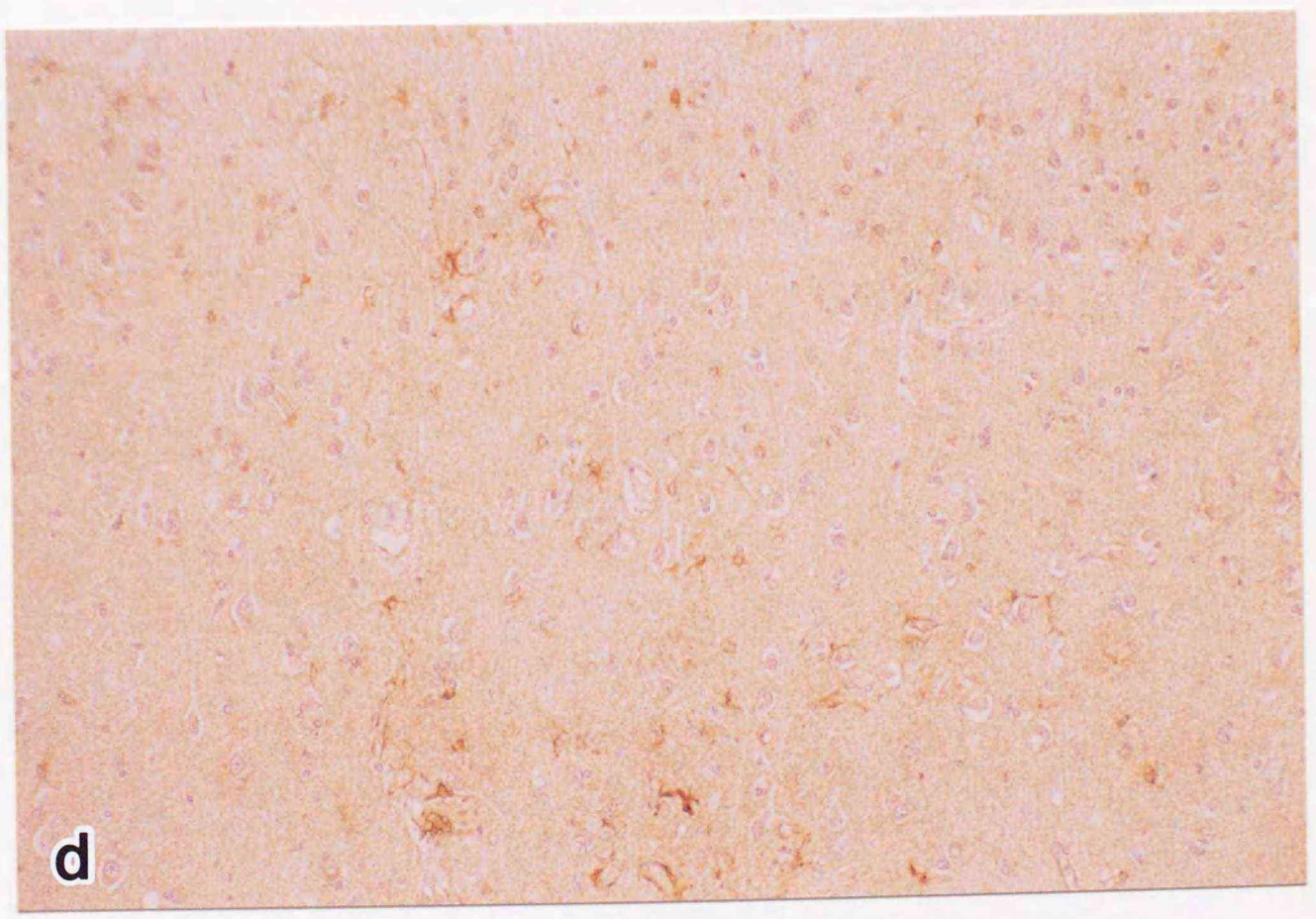
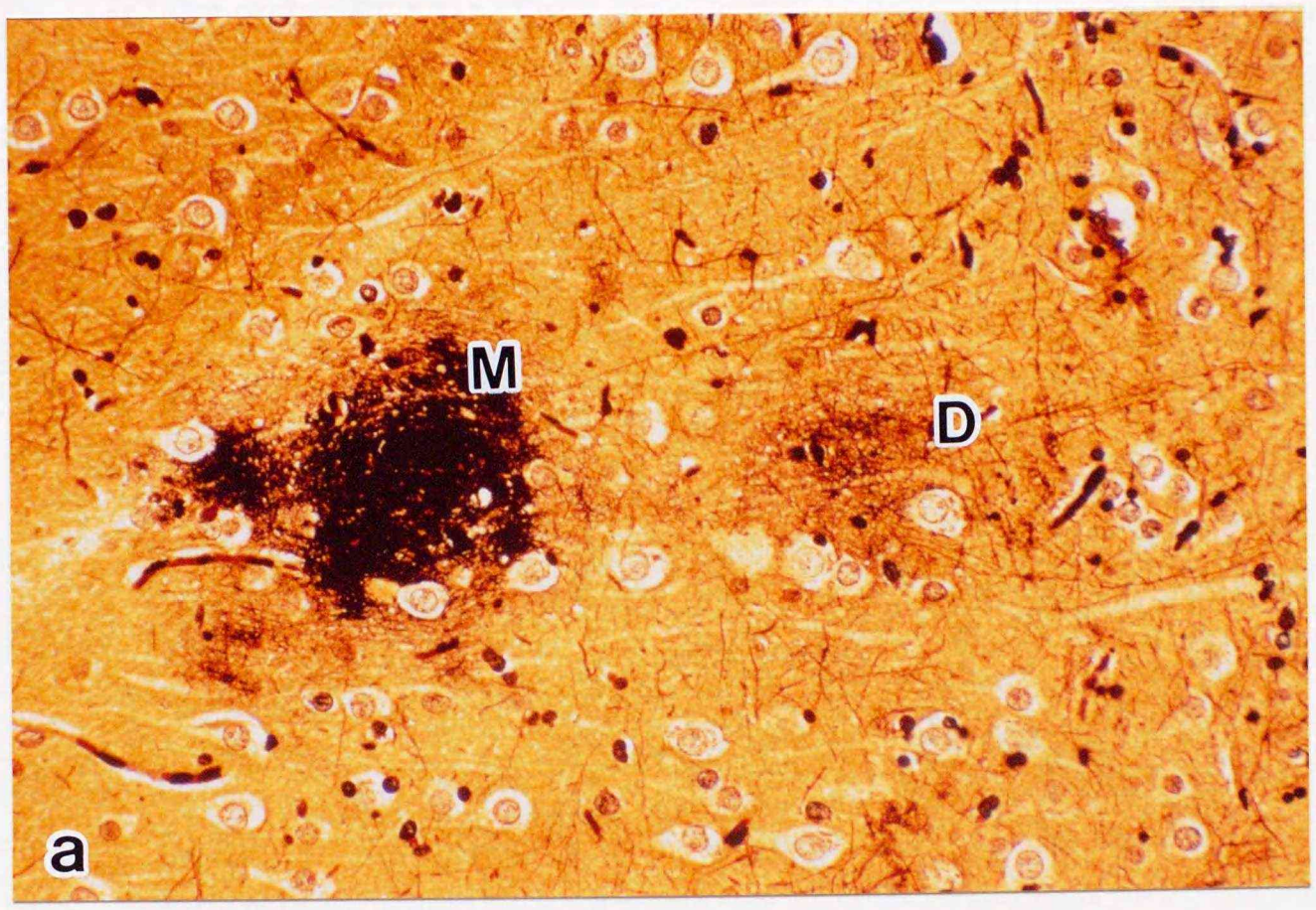

d

Fig. 23. Serial sections from the cingulate cortex of a 17-year-old dog (Dog No. 4). A diffuse plaque (D) and a mature plaque (M) are stained with modified Bielschowsky stain (a). Note processes of GFAP-positive astrocytes involved in the mature plaque (M) in a section stained with GFAP immunostain (b). x175.


M
D
a

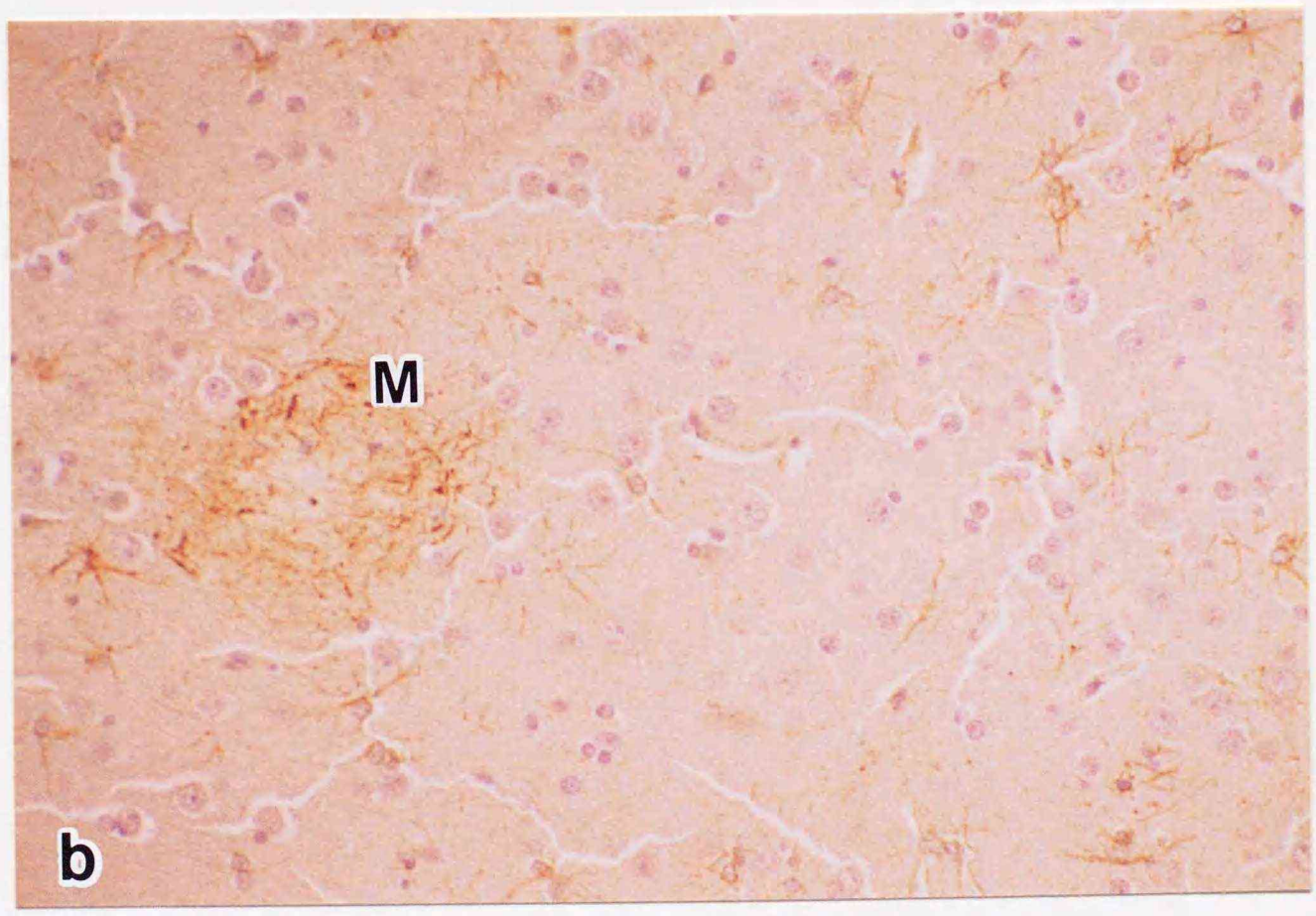
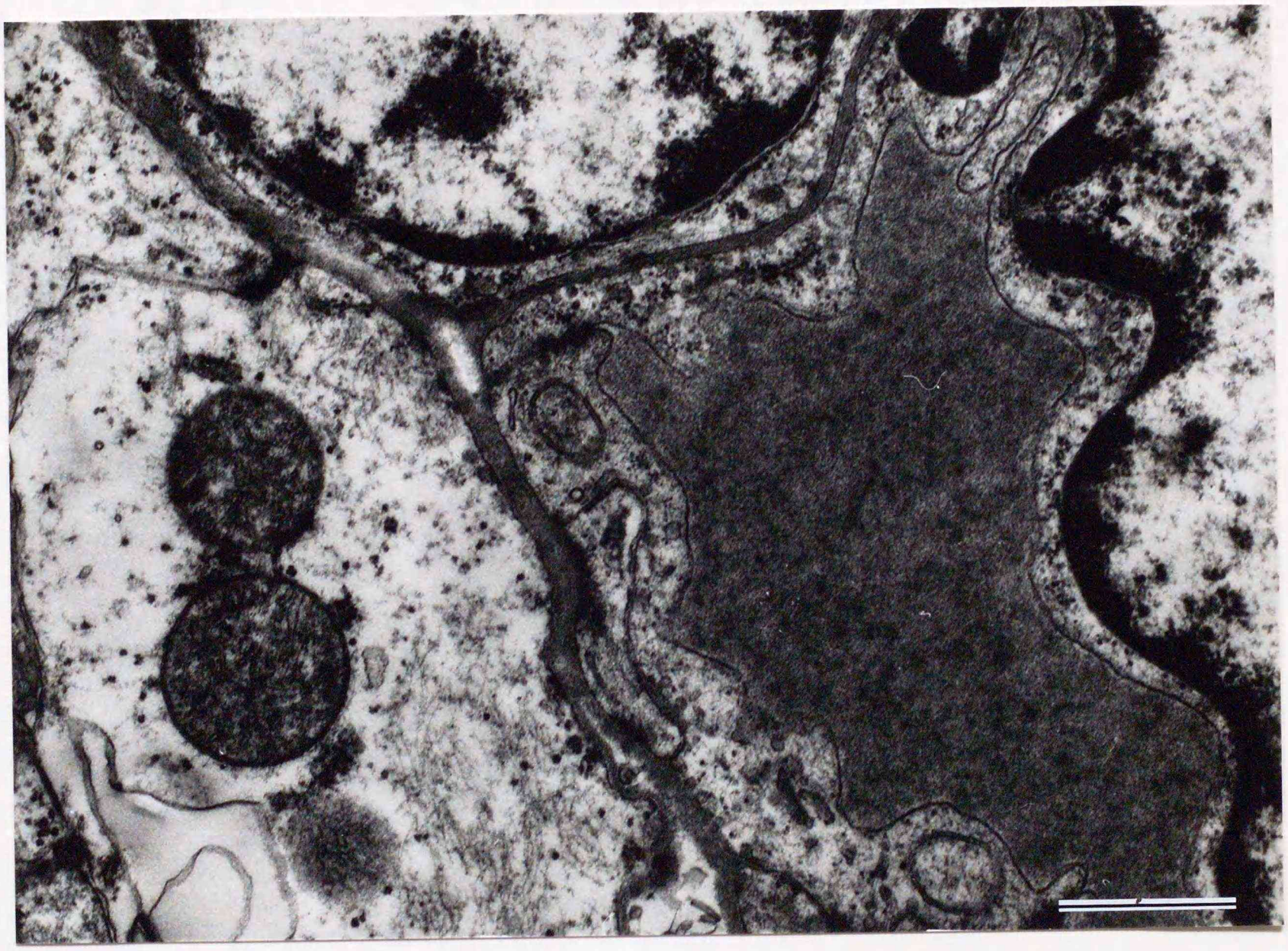

M
b

Fig. 24. Electron micrograph of an apparently normal-looking capillary wrapped with a process of astrocytes. Cingulate cortex. Dog No. 4, x12,000. Bar = 1 μm.



inches 1 2 3 4 5 6 7 8

cm 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Kodak Color Control Patches

Blue Cyan Green Yellow Red Magenta White 3/Color Black

Kodak Gray Scale

C Y M

A 1 2 3 4 5 6 **M** 8 9 10 11 12 13 14 15 **B** 17 18 19