Evidence for Blood–Brain Barrier Changes in Senile Dementia of the Alzheimer Type (SDAT)

H. M. WISNIEWSKI and P. B. KOZLOWSKI
New York State Institute for Basic Research in Developmental Disabilities
Staten Island, New York 10314

SENILE DEMENTIA of the Alzheimer type (SDAT) is the most common age-associated dementia. According to the recent Census Bureau data, there are about 25 million Americans aged 65 or older. Among those who are 65 and over, 11% have mild to moderate dementia and about 4.5% are severely demented. According to Tomlinson *et al.*[1], 55% of those people with clinical signs of dementia have SDAT, 15% are affected by multi-infarct dementia, and 22% by a combination of SDAT and multi-infarct dementia. These figures clearly show that SDAT constitutes one of the most important public health problems in this country. Among the pathological findings in SDAT, the neuritic (senile) plaques and the neurofibrillary changes are the most prominent lesions. Since the number of neuritic plaques and neurofibrillary changes appear to correlate with the degree of dementia, a considerable attention has been given to understanding the source of proteins making the amyloid fibers and the paired helical filaments (PHF) of the neurofibrillary tangles.[2,3]. During our studies on the pathogenesis of the neuritic and amyloid plaques in scrapie-infected mice, it was found that at the early stage of the disease the blood-brain barrier (BBB) permeability was changed.[4–6]

Recent studies[7,8] revealed that immunocytochemical techniques can be used to show indirectly the increased permeability of brain microvasculature by the visualization of extravasated serum proteins in the brain parenchyma. We applied this technique to study the brains from SDAT cases to determine whether there is evidence for BBB changes in SDAT.

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MATERIALS AND METHODS

The material used in the presented study consisted of seven cases of SDAT (persons aged 63 to 85 years), and five cases in which no clinical signs of dementia were present and neither senile plaques nor neurofibrillary changes were found by routine neuropathological examination. Two of these control cases were 77 years old, two were 49 years old, and one was 29 years of age. Autopsy was performed 3–4 hours after death, in three of the SDAT cases, in the other four cases from 12–24 hours after death. In control cases autopsy was done from 13 to 26 hours after death. Brains were routinely fixed in 10% formaline, cut and embedded in paraplast. The six micron sections were stained with hematoxylin-eosin, Kluver-Barrera, and combined Bodian-PAS techniques. For immunocytochemical study, serially cut 12-micron sections were used.

Sections were immunostained according to Sternberger's PAP technique.[9,10] Deparaffinized and rehydrated sections were washed in 0.05M Tris/HCL buffer, (pH 7.4) with 3% NaCL (Tris/NaCL) overnight at room temperature and immunostained in steps as follows: (1) The sections were incubated with 3% normal goat serum (NGS) for 10 minutes at room temperature; (2) They were then incubated with primary antisera; rabbit anti-human albumin (diluted 1 : 1000 and 1 : 2000); or rabbit anti-human globulins (in dilutions 1 : 500 and 1 : 1000) at 4°C overnight, in moist chambers. (3) The next day, sections were extensively washed in Tris/NaCL in large beaker, over a magnetic stirrer (two changes) for 15 minutes. (4) They were then incubated with goat anti-rabbit IgG antiserum (diluted 1 : 40) for 1 hour at room temperature and again (5) washed as in Step (3). (6) Incubation was then performed with a peroxidase antiperoxidase complex (PAP) of rabbit origin (dilution 1 : 40) for 1 hour at room temperature and (7) the sections were washed for 30 minutes in Tris/NaCL. (8) They were then allowed to react with dimethylaminoazobenzene (DAB) for 10 minutes [0.1% DAB (Sigma Chemicals) with 0.02% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6], (9) rinsed a few times with distilled water, and (10) post-stained for 1 minute with Harris hematoxilin and then dehydrated.

One percent NGS has been added to all primary antisera and PAP complex. Primary antisera was purchased from Cappel Lab, Cochranville, PA; goat anti-rabbit, PAP complex and NGS was purchased from Sternberger-Meyer Immunochemicals, Jarretsville, MD.

Control sections were treated in the same way as specifically stained

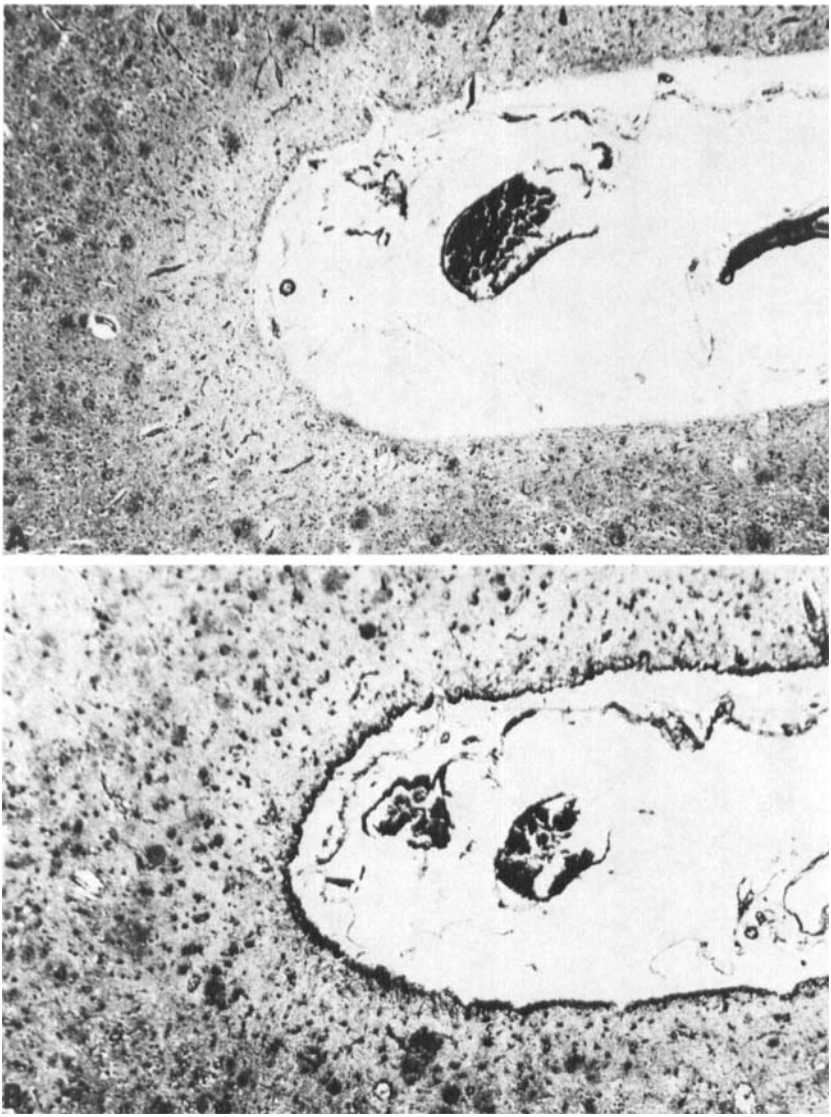

FIGURE 1. (A) Section of brain from an SDAT case. Numerous senile plaques in brain cortex. Bodian PAS stain. Original magnification: × 16; shown at 69% of original size.

(B) Another section from the same brain stained with antialbumin serum in dilution 1 : 2000. Numerous positively stained plaques and glial cells are seen lying within darkly stained neuropil. Magnification: same as 1A.

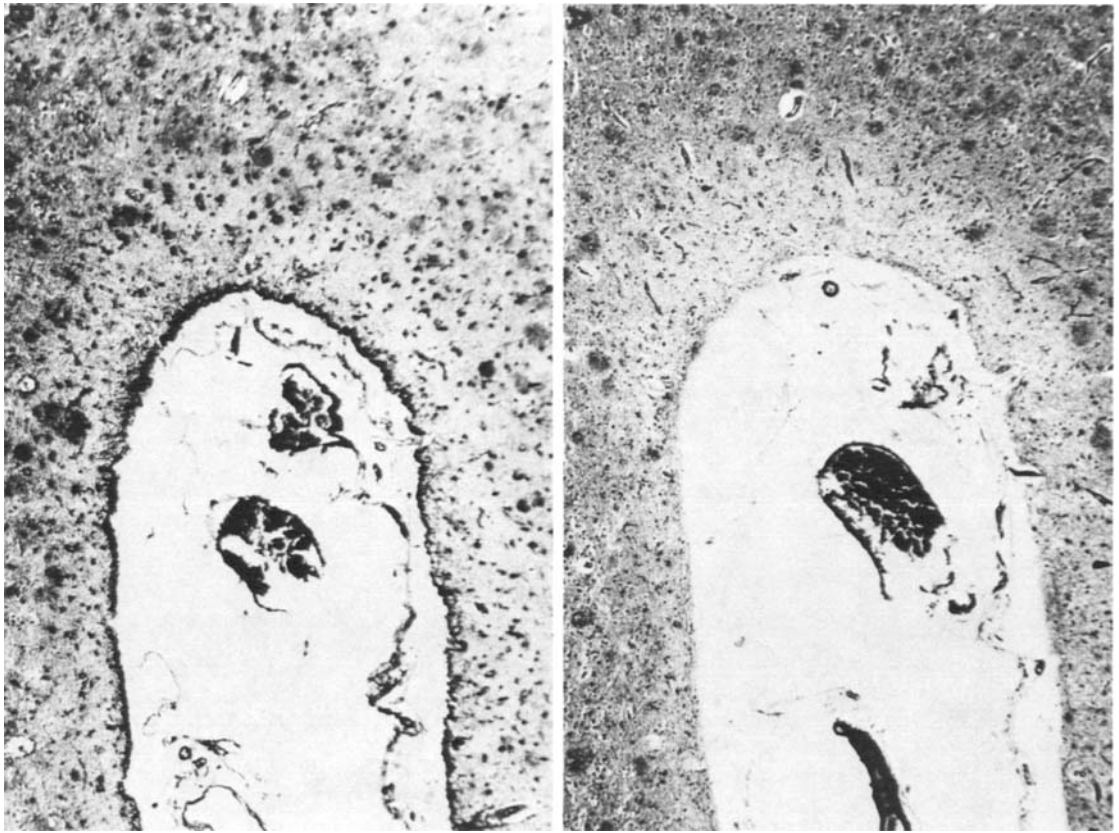

FIGURE 2. (A) Sections from another SDAT case. Cerebral cortex with numerous plaques positively stained (*arrows*) and glial cells surrounded by discrete halo (*arrowhead*). Almost all neurons in this section, especially large pyramidal neurons are strongly positive (*double arrows*). Antialbumin 1 : 3000. Original magnification: × 20; reduced to 72% of original size. (B) Positively stained, neurons in higher magnification. All neurons including axons are covered with reaction product. Few neuritic plaques are seen (*arrows*). Antiglobulin 1 : 500. Original magnification: × 100; reduced to 72% of original size.

sections with the exception that primary antisera was replaced by TBS, nonimmune rabbit serum (Sternberger-Meyer Immunochemicals), or by primary antiserum in which activity was blocked with antigen given in excess and absorbed for 30 minutes at 37°C. Chromatographically purified human albumins (Cappel Lab) were used for blocking antialbumin antiserum and chromatographically purified human immunoglobulins (Cappel Lab) were used for blocking antiglobulin antiserum.

The intensity of immunostaining was graded as very strong (+++) when a brownish-black reaction product was found, intensive (++) when dark brown staining was seen, weak (+) when brown staining was lighter but still greater than background staining; and nonsignificant (+/−) when the structures were faintly stained but the intensity of staining was only slightly greater than background staining.

RESULTS

In the non-SDAT cases only faint staining of the neuropil and some weakly stained neuronal perikarya in the fifth layer of the cortex with both antialbumin and anti-IgG were observed. White matter was also weakly positive, but only after treatment with antialbumin serum.

A few blood vessels, mostly small veins surrounded by brown reaction product forming perivascular halo, were found in all cases. These halos were seen in cortex and in white matter, and they were more intensively stained with antialbumin than with antiglobulin serum.

In all of the SDAT cases studied, the Bodian-PAS staining showed numerous neuritic and some amyloid plaques and neurons with neurofibrillary tangles (Fig. 1A).

In areas of the cortex where single plaques or no plaques were found the neuropil and the plaques were lightly stained. In the areas of the cortex where plaques were numerous all elements of the cortex and the plaques were heavily stained with both antialbumin and antiglobulin sera (Figs. 1B, 2A & B). Although the intensity of staining of the plaques stained varied, all plaques were stained with both antisera. The most intensive deposits of reaction product were seen in the corona of amyloid plaques (Fig. 3A). All plaques were surrounded with reactive astrocytes which were heavily stained (Figs. 3B & C, 4A & B). The majority of neurons found in plaque-rich areas, both with and without neurofibrillary changes, were heavily stained with both antisera (Fig. 2B). White matter and especially its numerous astrocytes was heavily stained with

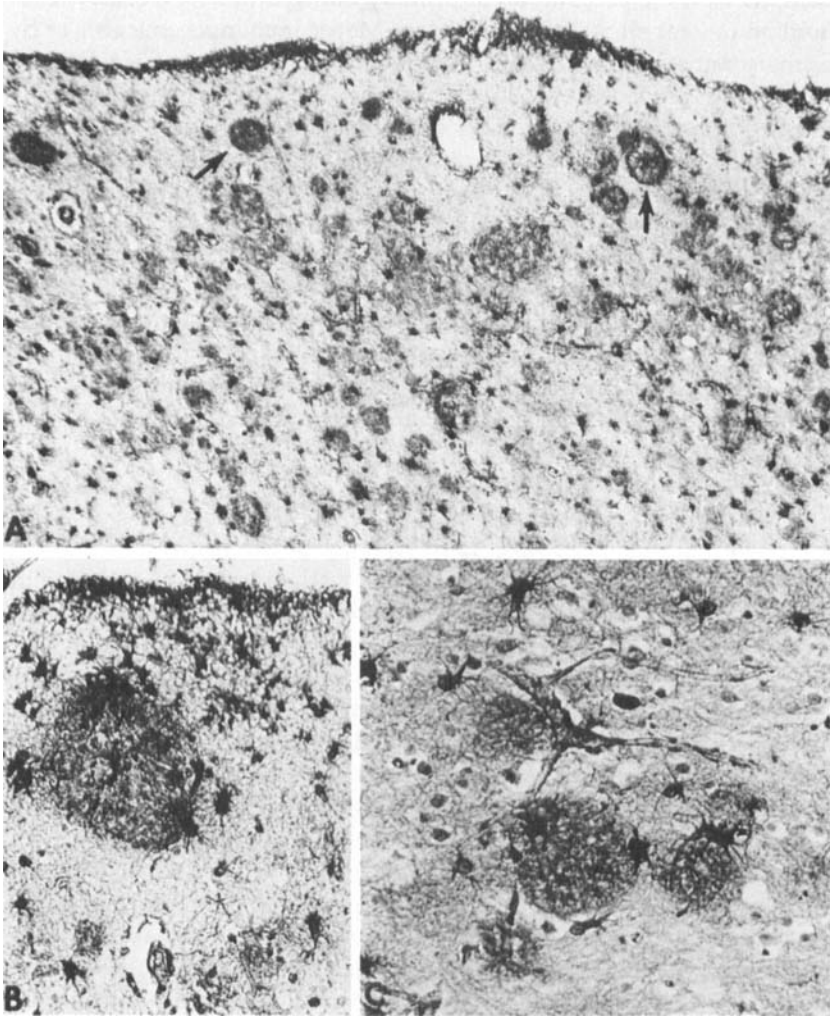

FIGURE 3. (A) Brain section from an SDAT case. Numerous neuritic and amyloid plaques in cerebral cortex. The corona of amyloid plaques is most intensively stained, numerous black astrocytes are also seen. Antiglobulin 1 : 500. Original magnification: × 40; reduced to 69% of original size.

(B) Large subpial plaque surrounded by black astrocytes. Antialbumin 1 : 2000. Original magnification: × 100; reduced to 69% of original size.

(C) Perivascular plaques with astrocytes. Antiglobulin 1 : 500. Original magnification: × 130; reduced to 69% of original size.

both antisera. The brown stained perivascular halos with prominent black stained astrocytes were much more numerous in the SDAT cases than in the control cases (FIG. 5A & B).

DISCUSSION

The presence of serum protein in the neuropil including the neuritic plaques and many cellular elements of the CNS is indirect evidence for a change in BBB permeability of microvessels in SDAT. Similar pictures using antialbumin and antiglobulin sera were observed in scrapie-infected mice.[11] However, in scrapie-infected mice, using HRP as a tracer, we were able to show the increased BBB permeability directly. Other investigators also reported the presence of serum proteins in neuritic plaques of SDAT cases.[12-15] These observations presented the possibility that the serum proteins, particularly the light chains of the gamma globulins can be the source of amyloid fibers.[16] Unfortunately, the small quantities of the amyloid material isolated from the SDAT brains did not allow us or other investigators to determine the origin of the protein(s) making the amyloid fibers. However, the preliminary data obtained so far indicate that the amyloid from SDAT plaques is not of the light chain immunoglobulin type.[17] The known decorative properties of the amyloid fibers are probably responsible for the presence of serum proteins within the neuritic and amyloid plaques.[5,6]

Other studies [18] have shown that dystrophic and degenerating neurites are part of the primitive and classical neuritic plaques and these profiles are involved also in the uptake and degradation of serum proteins. Other elements of the plaques that showed strongly positive reaction products were the astrocytes and some microglia cells and their processes. Outside the area of the plaques, strong positive staining of both astrocytes and neuronal elements gave the appearance of the resolution edema described by Klatzo *et al.*[19] It is interesting to note that the greatest accumulation of serum proteins appeared in the areas where many plaques were found. Also most of the "leaking" vessels were found in areas of amyloid deposits in mice infected with amyloid plaque-producing scrapie agent.[6]

A recent immunocytochemical study showed the presence of neurofilament polypeptides within the plaques amyloid.[15] This is probably also the result of unspecific binding by the amyloid fibers of brain proteins.

The functional consequences of the increased permeability of the brain microvasculature in SDAT are not known. However, one would

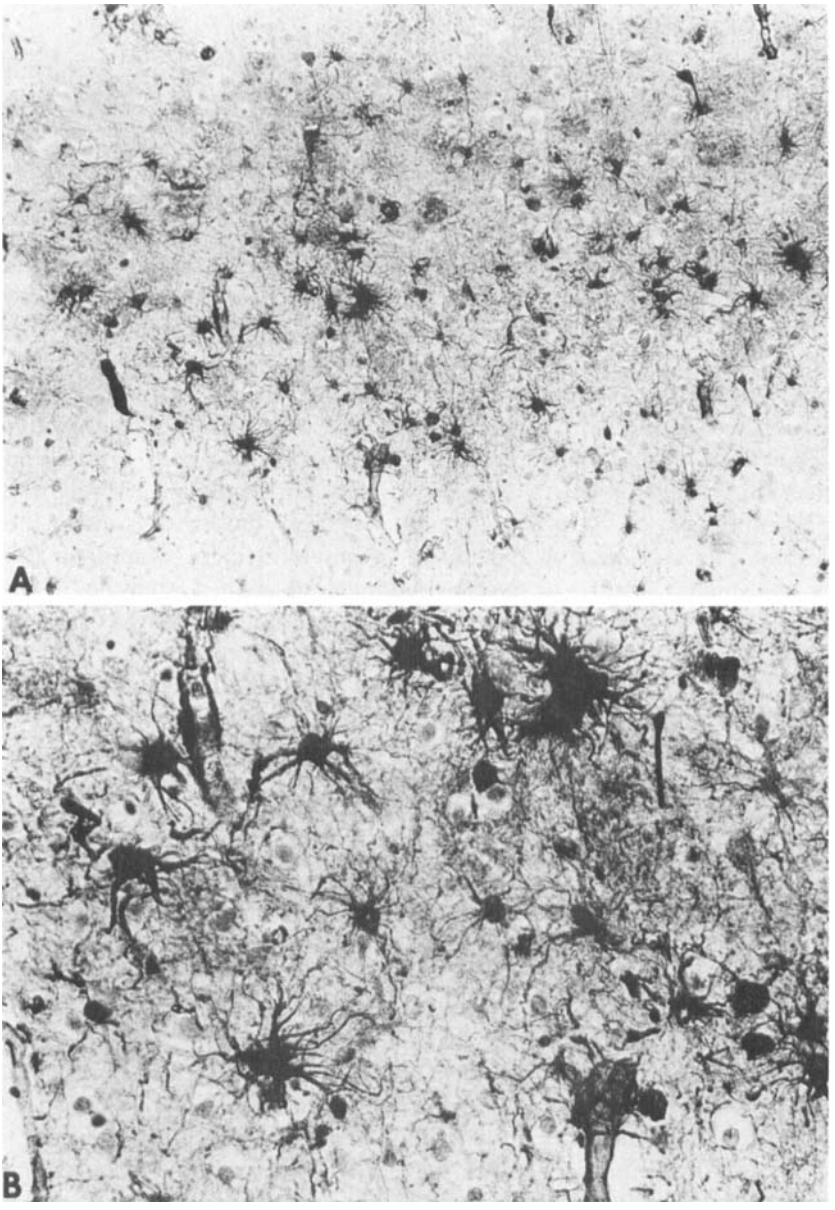

FIGURE 4. (A) Cerebral cortex with numerous plaques and monstrous protoplasmic astrocytes. Antiglobulin 1 : 1000. Original magnification: ×40; reduced to 71% of original size.

(B) Higher magnification of the same section. Note astrocytic processes running toward the capillary walls. Original magnification: × 100; reduced to 71% of original size.

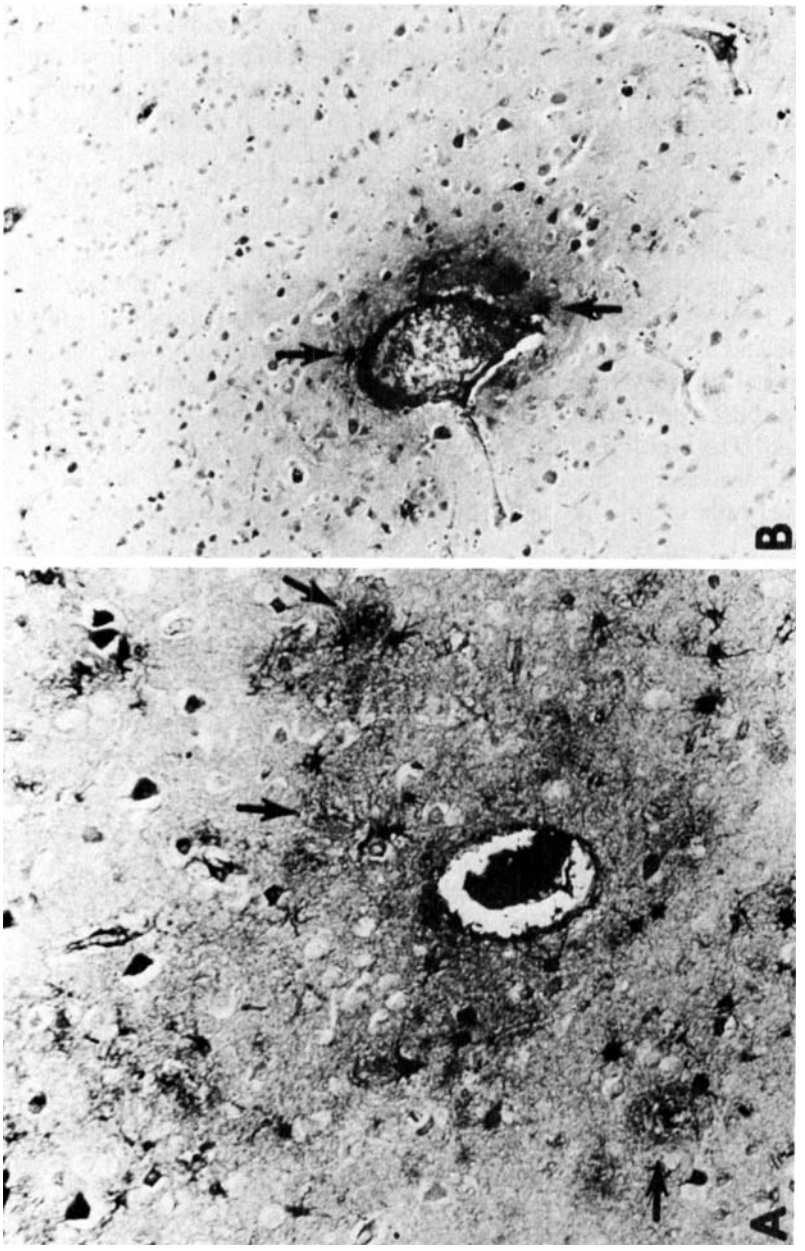

FIGURE 5. Cortical blood vessels surrounded by perivascular halo. (A) Blood vessels surrounded by senile plaques (*arrows*). Antiglobulin 1 : 500 Magnification: × 16. (B) Vessel in cortex of SDAT. Although no plaques are seen in the vicinity of this vessel, dark halo around the vessel is evident, and within halo black astrocytes are seen (*arrows*). Antialbumin 1 : 2000. Magnification × 20.

expect that the chronic "flooding" of the neuronal elements with serum proteins would affect their performance. As indicated above there is no evidence that the changes in BBB permeability lead to neuritic or amyloid plaque formation, and we do not have evidence that the serum proteins contribute to the intraneuronal formation of the paired helical filaments. The cause of the increased BBB permeability in SDAT is unknown. Since leaking vessels were found outside the plaque areas, it is unlikely that they are the only cause of increased BBB permeability. In mice the change of BBB is associated with scrapie infection of the brain. So far there is no evidence for the presence of a scrapie or C-J type of agent in SDAT. However, one of the leading pathological changes in SDAT – the neuritic and amyloid plaque – can be induced at the site of scrapie injection in genetically susceptible strain of mice. As indicated above, increased BBB permeability in scrapie-infected mice has been recently reported. The fact that, in SDAT, the brain microvessels also show increased permeability, in our opinion, supports the hypothesis that SDAT is caused by a scrapie-like agent in genetically susceptible humans.

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