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Ultrastructural Studies of Amyloid Fibrils and Senile Plaques in Human Brain

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Summary. Amyloid fibrils and senile plaques in brains with Alzheimer's disease, senile dementia and Down's syndrome were examined by light and electron microscopy. In addition, replicas of amyloid fibrils, made by a quick freezing method from a brain with Down's syndrome, were examined. All amyloid masses forming the cores of senile plaques consisted of numerous amyloid fibrils spreading from the walls of small blood vessels to the surrounding parenchyma. The amyloid fibrils ran in various directions, forming bundle-like groups in a geometrical array. They appeared as rods with hollow structures consisting of an array of globular units in the replicas, while they showed bead-like structure in the tissue specimens of 500-nm thick sections. The ultrastructure of replicas reveals a new finding on the structure of amyloid fibrils in the human brain.

Key words: Senile plaques – Amyloid fibrils – Quick freezing method

Introduction

Amyloid fibrils are observed in senile plaques and around blood vessels as a mass of deposits. However, as far as we are aware, the ultrastructure of amyloid fibrils in the brain has not yet been studied by a quick freezing method.

In the present study, in addition to the light and electron microscope examination of tissue specimens, replicas of amyloid fibrils produced by a quick freezing method (Heuser et al. 1979) were examined.

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Materials and Methods

The material consisted of five cases of Alzheimer's disease, two cases of senile dementia and one case of Down's syndrome. The clinical and histopathological findings were consistent with the diagnosis given (Table 1).

Parts of the cerebral cortex of the parieto-occipital and temporal lobes were removed from the brains immediately after death, cut into small pieces and immersed for 2 h in 3% glutaraldehyde in phosphate buffer (pH 7.4). They were washed in phosphate buffer (pH 7.4) for 10 min and immersed for 2 h in 2.5% osmium tetroxide in phosphate buffer (pH 7.4). The tissues were dehydrated in alcohol and embedded in epon. Thick sections were stained with toluidine blue for light microscopy. After observation of toluidine blue preparations, thin sections 30–50 nm and thick sections 300–500 nm were stained with alkaline-bismuth solution, and examined with a Hitachi 12 A (100 kV) and Jeol 2000 EX (200 kV) electron microscope.

Fresh material from the brain with Down's syndrome was cut into small pieces and immersed in 2% formaldehyde for 12 h and was used for producing replicas by a quick freezing method (Heuser et al. 1979). The replicas were also examined with the Jeol 2000 EX (200 kV) electron microscope.

Serial sections were cut from paraffin blocks of each case and stained with PAS and silver solution for examination by light microscopy.

Results

Deposits of amyloid masses were observed around small blood vessels (*drusige Entartung der Hirnarterien*) and all of the amyloid masses forming the central cores of typical senile plaques were those of the amyloid deposited around small blood vessels (Fig. 1 A, B).

In specimens of 300–500 nm thickness observed using the 200 kV electron microscope, the amyloid masses, seen around the blood vessels by light microscopy, consisted of numerous amyloid fibrils spreading as a radial structure from the walls of the blood vessels to the parenchyma (Fig. 2). Amyloid fibrils forming the core of senile plaques existed around the blood vessels (Fig. 3).

Table 1. Material

Case no.	Clinical diagnosis	Sex	Age (years)	Onset (years)	Clinical course of disease	Histopathological findings
1.	Alzheimer's disease	male	56	48	8 years	Alzheimer's disease
2.	Alzheimer's disease	female	52	49	3 years	Alzheimer's disease
3.	Alzheimer's disease	male	63	56	7 years	Alzheimer's disease
4.	Senile dementia	male	84	73	11 years	Senile dementia
5.	Senile dementia	male	78	70	8 years	Senile dementia
6.	Alzheimer's disease	male	65	60	10 years	Alzheimer's disease
7.	Alzheimer's disease	male	70	65	15 years	Alzheimer's disease
8.	Down's syndrome	male	39	0	39 years	Down's syndrome

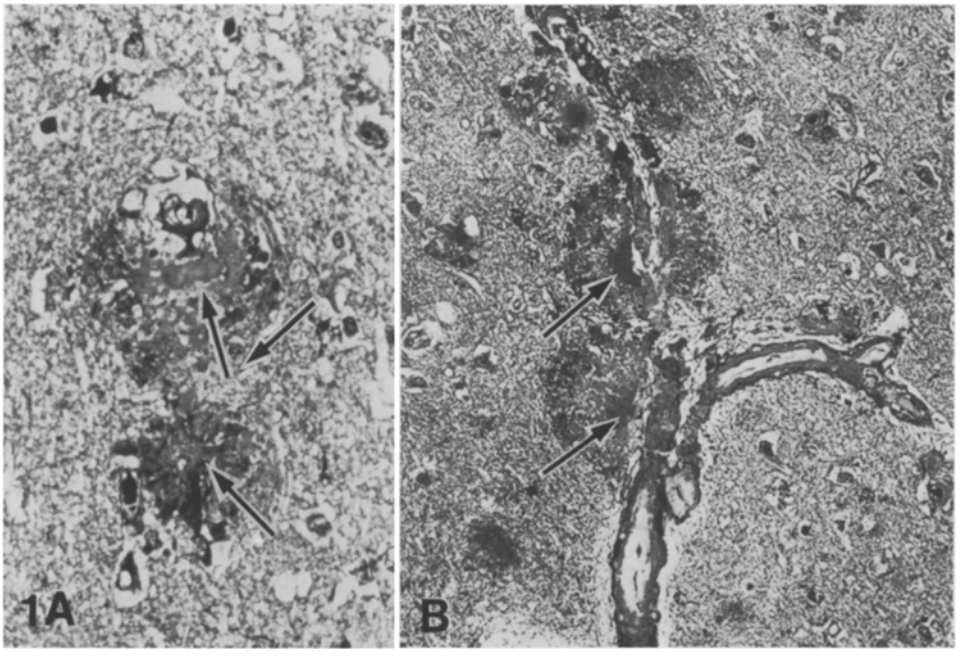

Fig. 1. A Typical senile plaques (case 2). Central cores of amyloid deposits (*arrow*) around blood vessels. Toluidine blue stain, × 390. **B** Blood vessel passes through senile plaques (case 5). Amyloid deposits (*arrow*) around the blood vessel exist in the center of the senile plaques. Toluidine blue stain, × 320

Detailed examination of the amyloid fibrils in the thick specimens showed that they ran in various directions (Fig. 4). In thin 50-nm sections, amyloid fibrils showed paired arrayed filaments 7–10 nm, which consisted of granular units (Fig. 5A). On the other hand, in thick 500-nm sections the amyloid fibrils showed bead-like structures (Fig. 5B).

In amyloid masses replicas obtained by the quick freezing method from the brain with Down's syndrome amyloid masses were attached to the capillaries and consisted of many amyloid fibrils consistently running in various directions as if forming bundles and the fibrils did not have any separated branches (Fig. 6). Examination of the ultrastructure of amyloid fibrils in a longitudinal direction, revealed rods with a width of 10–15 nm (Fig. 7A) having a hollow structure and consisting of an array of globular units in a transverse direction. The number of globular units appeared to be 5–7 (Fig. 7B). Since the replicas were prepared by coating the tissue with a 2-nm thickness of platinum and carbon, the real width of the amyloid fibrils may be 6–11 nm.

Discussion

Scholz (1938) reported plaque-like degeneration of arteries and capillaries (*drusige Entartung der Hirnarterien*) and concluded that the core of senile plaque consisted of material which permeated from

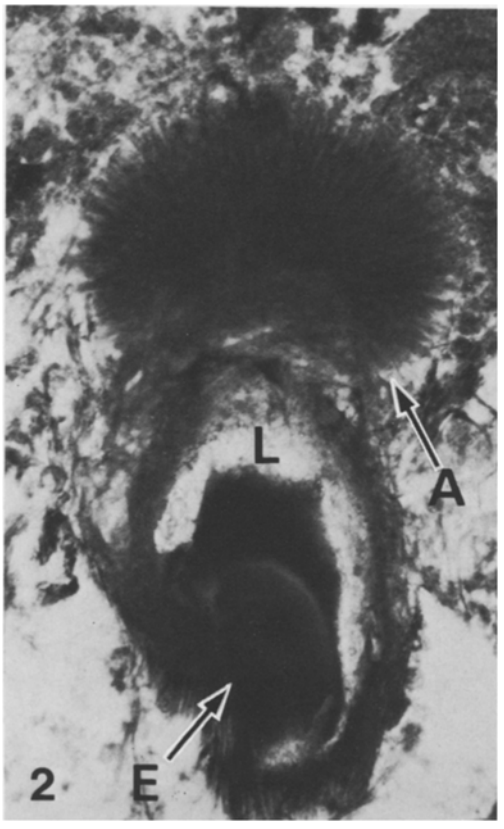

Fig. 2. Amyloid mass around a capillary (case 6). Amyloid fibrils spread from wall of the blood vessel to the parenchyma. *A* amyloid mass, *L* capillary lumen, *E* erythrocyte. × 5,000

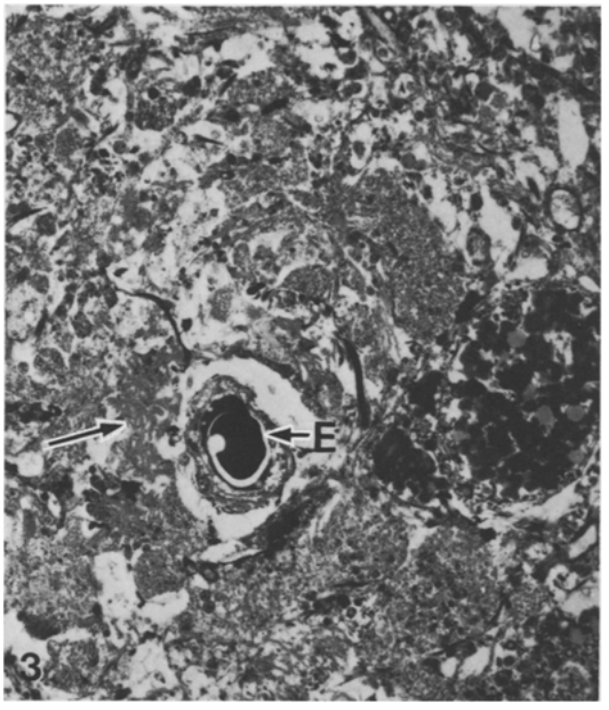

Fig. 3. A senile plaque (case 3). Amyloid fibrils (*arrow*) exist around a capillary. *E* erythrocyte. × 3,240

the blood vessels. Other authors (Morel and Wildi 1952; Pantelakis 1954; Corsellis and Briery 1954; Miyakawa et al. 1974; Miyakawa and Uehara 1979) have described similar findings. Then, Schlote (1965) reported that plaque-like angiopathy resulted from the infiltration of vessels by certain plasma proteins and electron micrographs of affected vessels exhibited amyloid fibrils arranged in the form of „brush-like structures" on the adventitial surface; a situation that could be taken to indicate a transmural flow of „precursor" substances through the vessel to the cerebral parenchyma. Hollander and Strich (1970) noted a close association between "plaque" and amyloid-involved vessels and there were undoubtedly perivascular plaques. On the other hand, Friede and Magee (1962) found 92% of plaques (probably neuritic) to be unrelated to capillaries.

Glenner (1979) reported that in a large proportion of cases of Alzheimer's presenile dementia, the major causual mechanism was an alteration of the blood-brain barrier resulting from the deposition of Congo red-positive material on the walls of small blood vessels occurring in a relatively young age group. He concluded that a partially-digested, filamentous protein (filarin) was further cleaved enzymatically by microglia cells to produce the amyloid core of the neuritic plaque.

However, Miyakawa et al. (1982) reported that all senile plaques contained at least some amyloid fibrils, and these seemed to be produced at the basement membranes of capillary endothelial cells and projected into the surrounding parenchyma. Even when the senile plaques themselves appeared to lack blood vessels, at least one degenerating capillary containing amyloid fibrils was demonstrated when serial sections were examined ultrastructurally.

Concerning the ultrastructure of the amyloid fibrils, Shirahama and Cohen (1967) examined amyloid-laden tissues (spleen and liver) and reported that the amyloid filaments are approximately 7.5–8 nm in diameter and consisted of five subunits. Glenner et al. (1968) examined the human spleen, liver and kidney containing amyloid deposits and detected the two morphologic components of human amyloid deposits; the periodic rod and fibril. The periodic rods were up to 250 nm in length, and small unit structures were approximately 9 nm in diameter. The fibrils were aggregations of 7.5–8 nm filaments devoid of a periodic rod.

In the present study, we could obtain the ultrastructural data of amyloid fibrils in the brain. In thin sections, amyloid fibrils had pairs of parallel filaments consisting of granular layers while the

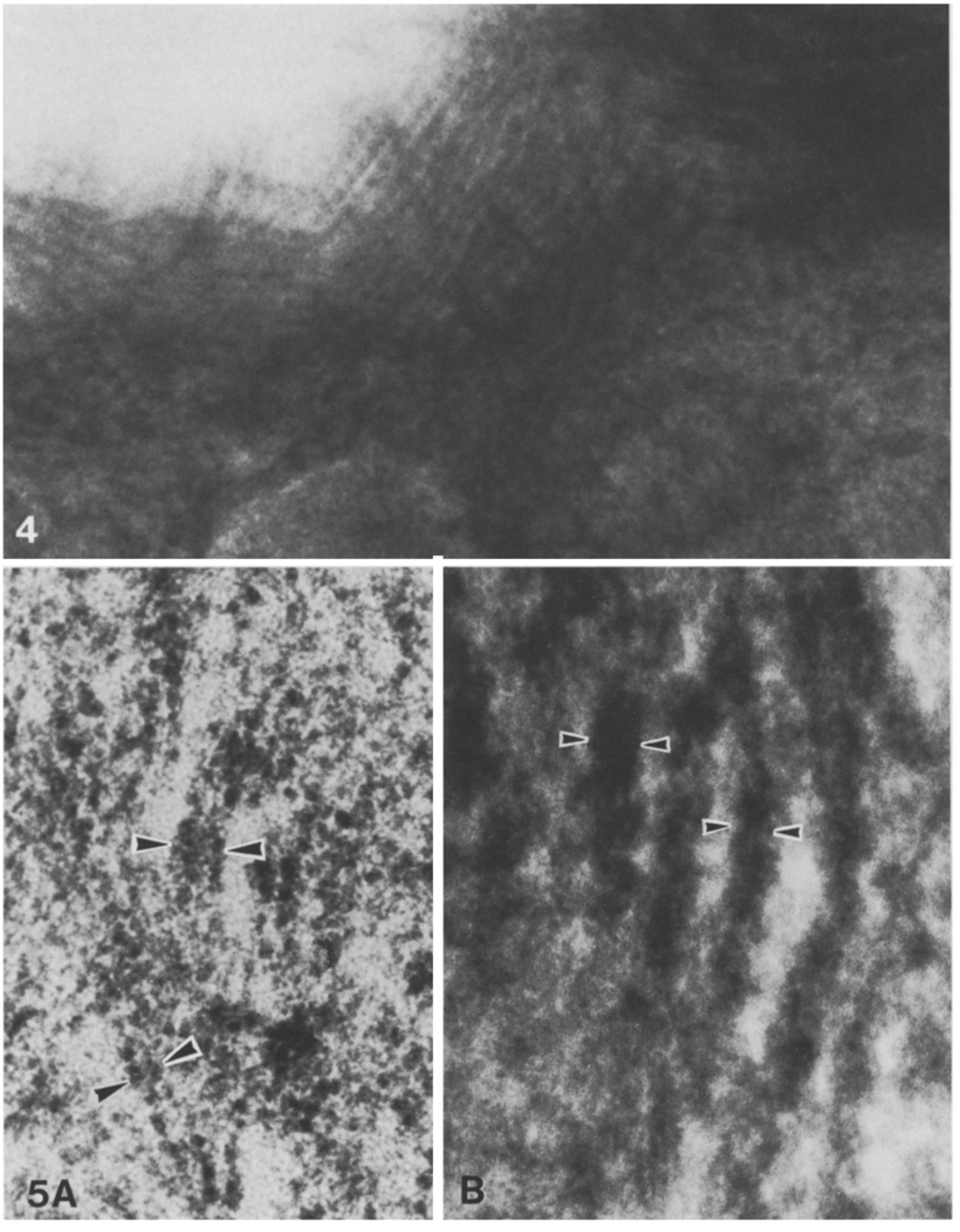

Fig. 4. In the amyloid fibrils, striped-like appearance is observed (case 7). ×67,000

Fig. 5. A Thin section of amyloid fibrils (case 1). Amyloid fibrils consist of paired filaments of granular structure. The filaments are 7–10 nm in diameter (*arrowheads*). ×600,000. **B** Amyloid fibrils have a bead-like structure (case 1). The beads are 7–10 nm wide (*arrowheads*). ×600,000

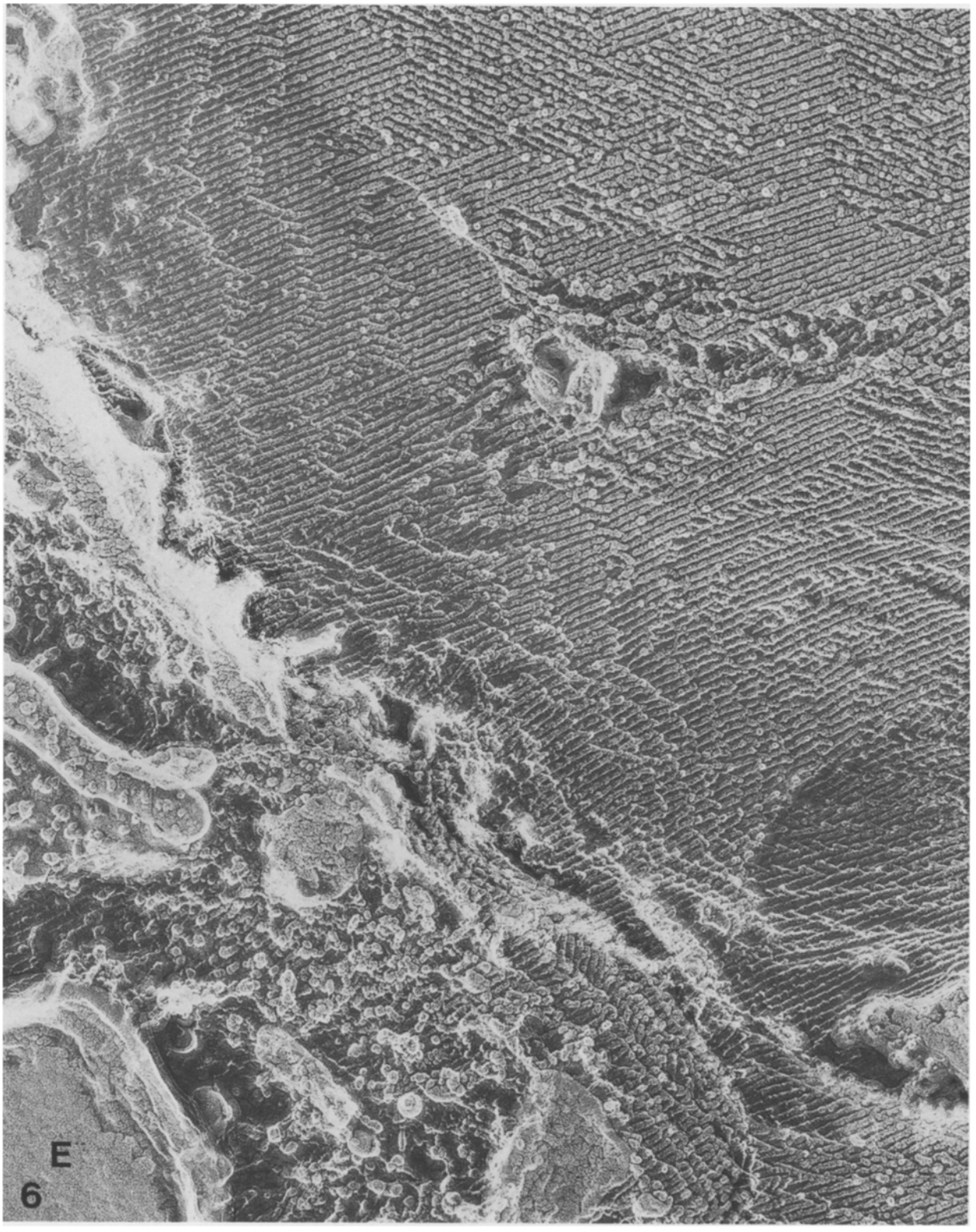

Fig. 6. Replica made by quick freezing method. Amyloid fibrils around a capillary run in various directions as if forming bundles (case 8). *E* erythrocyte. ×80,000

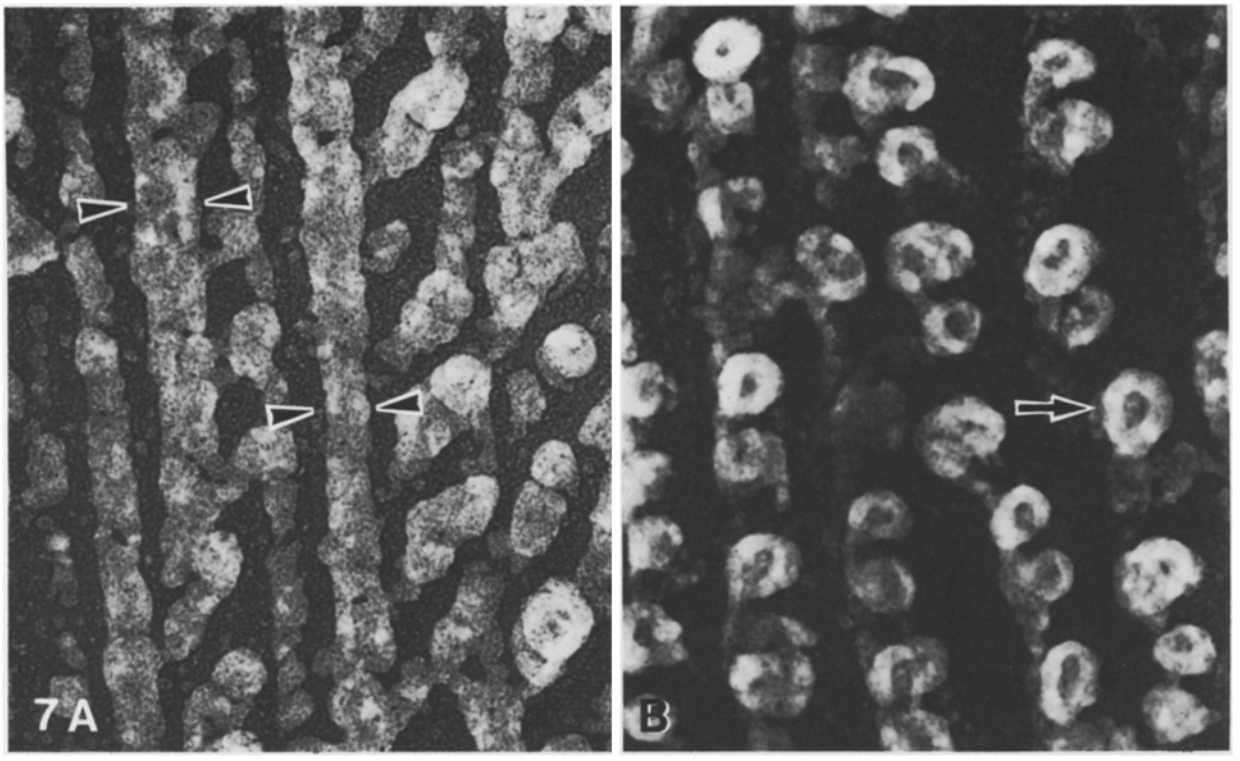

Fig. 7. A Replica of amyloid fibrils: longitudinal section (case 8). They consist of rods without a bead-like structure. The rods are 10–15 nm wide (*arrowheads*). × 550,000. **B** Replica of amyloid fibrils: transverse section (case 8). They are hollow and consist of an array of about 6 globular units (*arrow*). × 550,000

amyloid fibrils showed bead-like structures in thick sections. According to the ultrastructures obtained from replicas, the amyloid fibrils ran in various directions crossing each other as a geometrical structure and revealed rods having hollow centers. The rods seemed to consist of an array of 5–7 globular units. In support of the structures deduced from replicas, amyloid fibrils in thin sections of tissue specimens had arrays of paired filaments consisting of granular units. These findings are similar to those of the amyloid fibril structures observed in tissue specimens from the liver, spleen and kidney by Shirahama and Cohen (1967) and by Glenner et al. (1968).

The replicas in the present study were prepared with a 2-nm platinum and carbon coating. The width of amyloid fibrils' replicas obtained (10–15 nm) was, therefore, considered to have been increased by 4 nm so that the real width of amyloid fibrils might be 6–11 nm and this is similar to that of the tissue specimens. In support of the finding that the amyloid fibrils are produced at the basement membranes of the blood vessels (Miyakawa et al. 1982), amyloid fibrils in the replicas appeared to exist around small blood vessels.

Recently, Glenner and Wong (1984a) found unique cerebrovascular amyloid fibrils protein (β protein) in the serum in Alzheimer's disease and in Down's syndrome (Glenner and Wong 1984b). These findings are of great interest genetically regarding cerebrovascular changes with amyloid angiopathy. In solving the mechanism of senile plaque production, biochemical and immunological research on changes in blood serum and blood vessels are likely to be very important in the future.

Finally, De Armond et al. (1985) identified protein amyloid filaments in the scrapie-infected brain. They described ultrastructural features of the prion filaments similar to those of amyloid in many tissues including brain. These results provide the first evidence that prion protein assembles into filaments in the brain and that these accumulate in extracellular spaces to form amyloid plaques. In addition, they proposed that identification of prion filaments within amyloid deposits in experimental scrapie will provide an animal model for studying the early phase of amyloid deposition in non-transmissible cerebral amyloidosis like Alzheimer's disease. As the structure of amyloid fibrils observed in the present study resembled prion filaments, this indicates the importance

of also considering the pathogenesis of senile plaque induced by amyloid fibrils.

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