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Ultrastructure of amyloid fibrils in Alzheimer's disease and Down's syndrome

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Summary. Amyloid fibrils in brains of patients with Alzheimer's disease and Down's syndrome were examined by light and electron microscopy. In addition, replicas of amyloid fibrils produced by a quick freezing method from the brain of a patient with Down's syndrome were examined by electron microscopy.

The amyloid fibrils were shown to consist of hollow rods. These were composed of filaments arranged as a tightly coiled helix, each turn of which consisted of five globular subunits. This structure appears to be similar to the prion filament observed in Creutzfeldt-Jakob disease (CJD). The possibility therefore arises that amyloid fibrils in Alzheimer's disease and Down's syndrome may be related to the transmissible agents responsible for diseases such as CJD, kuru and Gerstmann-Sträussler Syndrome (GSS).

Key words: Ultrastructure of amyloid fibril – Quick freezing method

Introduction

Amyloid fibrils are observed in the brain in Alzheimer's disease, senile dementia and Down's syndrome in deposits around blood vessels, in blood vessel walls and in senile plaques. These findings suggest that amyloid fibrils are an important component of the pathological changes characterizing these disorders.

Previous reports of the ultrastructure of amyloid fibrils and senile plaques in Alzheimer's disease and senile dementia include those of Terry et al. 1964; Schlote 1965; Wisniewski et al. 1973 and Miyakawa et al. 1974, 1979, 1982. In the present study, replicas produced by a quick freezing method (Heuser et al. 1979) have been examined in detail in the electron microscope.

Materials and methods

The material consisted of five cases of Alzheimer's disease and 1 case of Down's syndrome.

Parts of the cerebral cortex of the temporal lobes and hippocampal formation were re-

moved immediately after death, cut into small pieces and immersed in 3% glutaraldehyde in phosphate buffer (pH 7.4) for 2 h. They were washed in phosphate buffer (pH 7.4) for 10 min and immersed in 2.5% osmium tetroxide in phosphate buffer (pH 7.4) for 2 h. The tissues were dehydrated in alcohol and embedded in epon. Thick sections were stained with toluidine blue for light microscopy. Thin sections 300–500 Å were stained with uranyl acetate, lead acetate or alkaline-bismuth, and examined with a Hitachi 12A (100 kv) electron microscope. Serial sections were cut from paraffin blocks of each case and stained by periodic acid schiff (PAS) for examination by light microscopy. The reason for using the PAS stain was that it is suitable for observing both amyloid and capillary blood vessels.

Fresh material from the brain of a patient with Down's syndrome was cut into small pieces and immersed in 2% formaldehyde for 12 h and used for producing replicas by a quick freezing method (Heuser et al. 1979). The replicas of amyloid fibrils around capillaries forming the cores of typical senile plaques were examined with a JEOL 2000 EX (200 kv) electron microscope.

Results

Observing PAS-stained preparations by light microscopy, deposits of amyloid were seen around small blood vessels and forming the central cores of senile plaques (Fig. 1). By electron microscopy these amyloid masses showed numerous amyloid fibrils around capillaries (Fig. 2). Magnifying the pictures of the amyloid fibrils, revealed that they consisted of hollow rods (Fig. 3). By high resolution, amyloid fibrils consisted of paired filaments with width of 7–10 nm, composed of a granular substance (Fig. 4).

Examining replicas made by the quick freezing method, the amyloid fibrils were periodically arrayed at intervals of 5–6 nm in a longitudinal direction (Fig. 5). Numerous amyloid fibrils showed a geometric structure consisting of numerous granules arranged longitudinally (Fig. 6). After magnification each fiber was seen to have a hollow structure and width of 13–15 nm diameter. This consisted of a tightly coiled helix, each turn of which appeared to be composed of five or six globular subunits. Some subunits were of low electron density and each subunit had a width of 3–5 nm (Fig. 7a). However, when observed in an oblique direction, amyloid fibrils appeared to be oval-shaped and had five globular subunits with occasional gaps (Fig. 7b). The subunits were attached to each other and arranged in a helix (Fig. 8). From the above findings, the amyloid fibril apparently consists of a filament composed of many subunits and arranged in a helix each turn consisting of five globular subunits (Fig. 9). The actual width of the filament and its globular subunits is likely to be smaller than that recorded because a coat of metals was used in producing the replicas.

Discussion

The ultrastructure of amyloid fibrils in organs such as the liver, spleen and kidney has been described in detail (Bladen et al. 1966; Shirahama and Cohen 1967; Glenner et al. 1968). Bladen et al. (1966) discribed the presence of two particles, one a 100 Å-wide rod, the other a small pentagonal structure (unit structure) 90 Å in diameter. Narrow electron dense bands divided the rods at regular intervals into smaller segments so that they

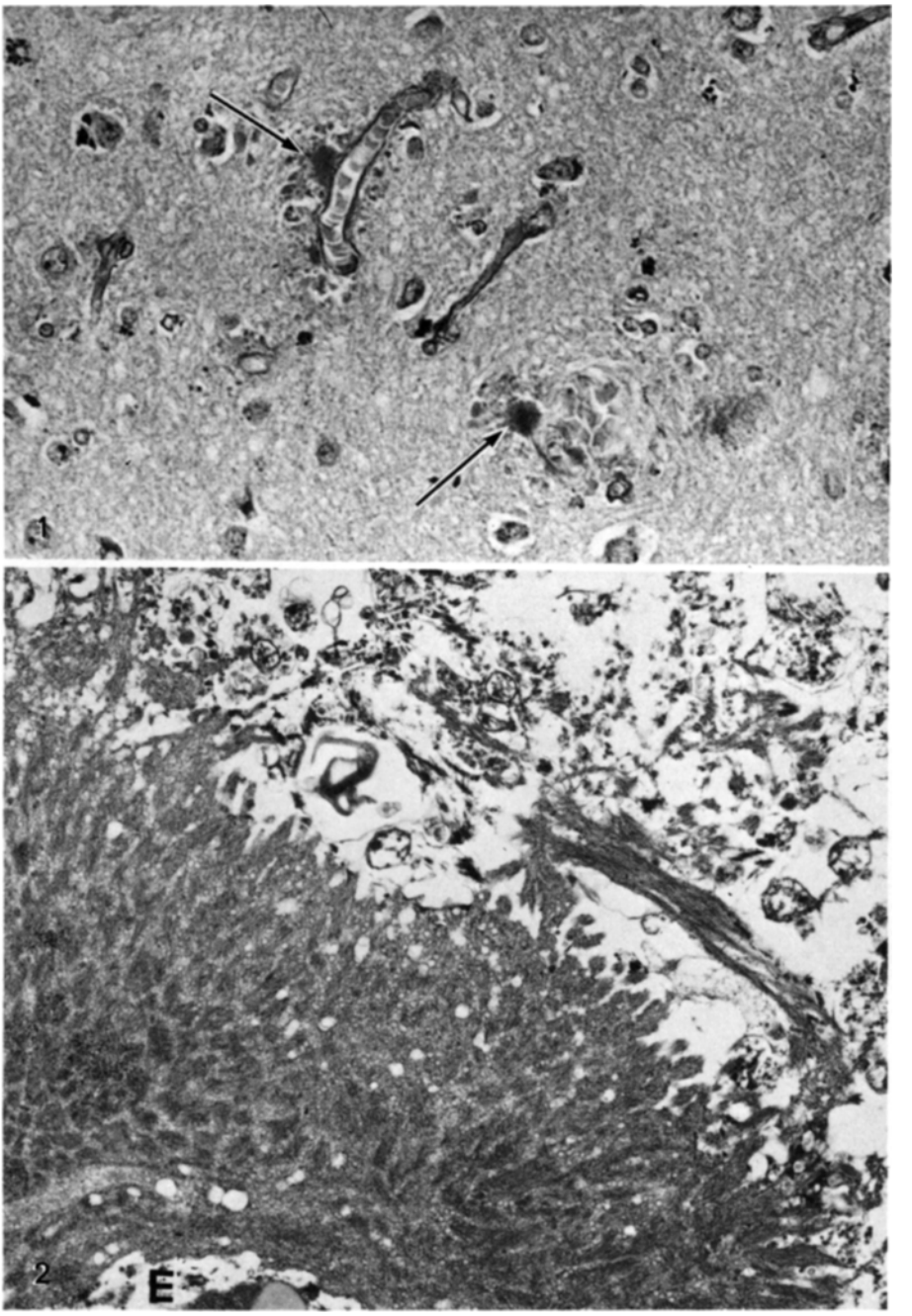

Fig. 1. Central cores of amyloid deposits (*arrows*) around blood vessels. PAS stain. × 280

Fig. 2. Numerous amyloid fibrils spreading from wall of degenerate blood vessel. (E) endothelial cell. × 7,300

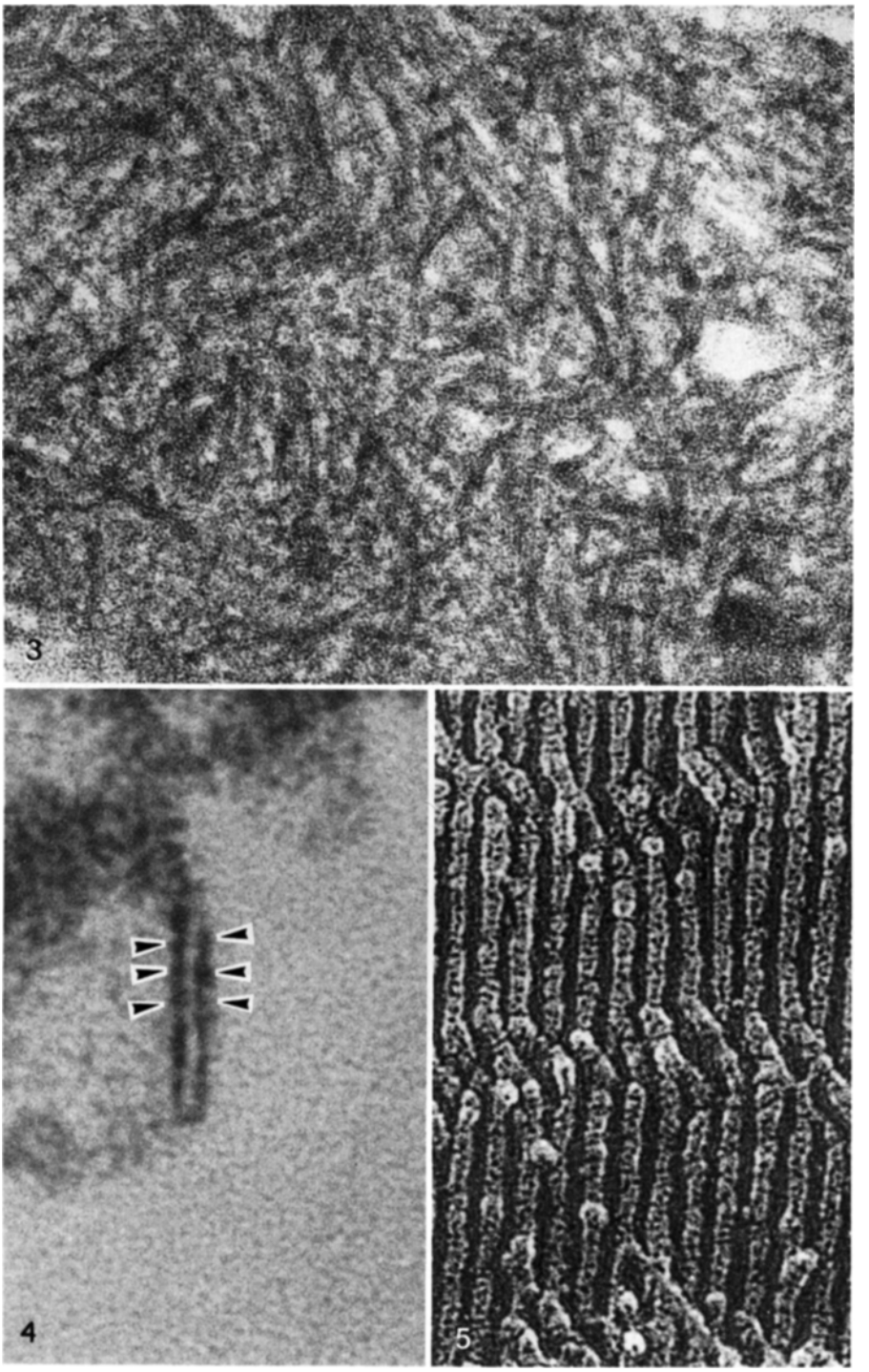

Fig. 3. Amyloid fibrils appearing as hollow rods. × 120,000

Fig. 4. An amyloid fibril showing paired arrays of filaments with a width of 10 nm, and consisting of granular substance (*arrowheads*). × 510,000

Fig. 5. Replica made by a quick freezing method. The amyloid fibrils are periodically arrayed at intervals of 5–6 nm in a longitudinal direction. × 254,000

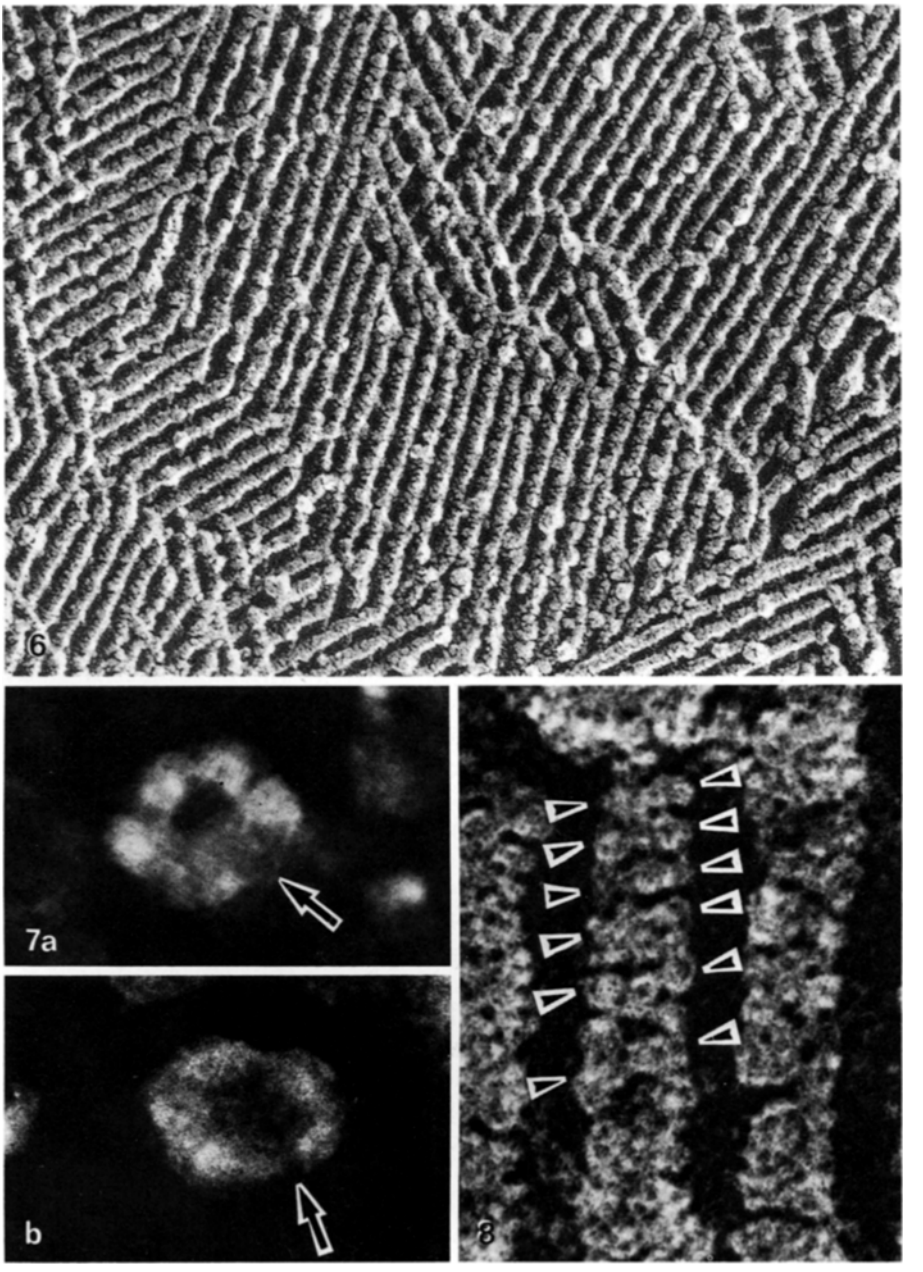

Fig. 6. Replica of amyloid fibrils in a longitudinal direction. They show a geometrical structure and consist of granular substance. × 160,000

Fig. 7. a Replica of amyloid fibril in a transverse direction. One turn of helix (*15 nm diameter*) consists of about six globular subunits. Globular subunits have width of 3–5 nm diameter. Two of the subunits show low electron density (*arrow*). × 1,610,000, **b** Replica of amyloid fibril in an oblique transverse direction. One turn of helix (*13 nm diameter*) consists of five globular subunits and appears oval-shaped. Part of one turn of the helix reveals a gap showing no electron density (*arrow*). × 1,610,000

Fig. 8. Replica of amyloid fibrils in a longitudinal direction. Each subunit (*13 nm diameter*) is arrayed in a helical form (*arrowheads*). × 1,344,000

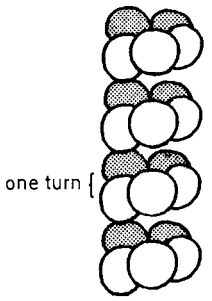

Fig. 9. Model of amyloid fibril. Filament consisting of many subunits helically winds

appeared cross striated with a periodicity of approximately 40 Å. Shirahama et al. (1967) reported that the amyloid fibril, that is the fibrous component of amyloid seen by electron microscopy of thin tissue sections, consists of a number of filaments aggregated side-by-side. These amyloid filaments are approximately 75–80 Å in diameter and consisted of five (or more likely six) subunits (amyloid protofibrils) which are arranged parallel to each other, longitudinally or slightly obliquely to the long axis of the filament. Glenner et al. (1968) examined the human spleen, liver and kidney containing amyloid deposits and detected the two morphologic components, the periodic rod and the fibril. 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 periodic rods.

There have also been a number of studies of amyloid fibrils in the brain in Alzheimer's disease (Terry et al. 1964; Schlote 1965; Wisniewski et al. 1973; Miyakawa et al. 1982). Terry et al. (1964) reported that the central cores of senile plaques consisted of stellate masses of interwoven fibrils, each 70 to 90 Å wide. The individual fibrils had a triple density indicating a hollow center. The central portion was about one third of the total thickness and periodicity in the longitudinal dimension was lacking. There were no formed elements between the fibrils, nor was there a ground substance of appreciable density. Schlote (1965) reported that a plaque-like angiopathy resulted from the infiltration of vessels by certain plasma proteins; electron micrographs of affected vessels demonstrated amyloid fibrils arranged in the form of "brush like structures" on the adventitial surface.

As mentioned above, however, the ultrastructure of individual amyloid fibrils has not yet been elucidated at the molecular level because of the great difficulty in defining detailed ultrastructure from the thin sections.

The brains of all patients with Down's syndrome over 40 years of age show histopathological changes similar to those seen in Alzheimer's disease (Epstein 1983). We therefore used fresh material from the brain of a patient with Down's syndrome to produce replicas of amyloid fibrils. As is well known, replicas obtained by quick freezing is the most accurate method for defining their structure. Using this technique we demonstrated that amy-

loid fibrils consist of hollow rods composed of filaments arranged in a tightly coiled helix. Each turn of the helix consisted of an array of five globular subunits.

This conflicts slightly with our previous report (Miyakawa et al. 1986), which indicated that each turn of the helix consisted of 5–7 globular subunits, a conclusion which needs to be corrected in the light of the present study.

Plaque-like amyloid fibrils are observed in the brain in infectious diseases such as Creutzfeldt-Jakob disease (CJD), kuru and the Gerstmann-Sträussler syndrome (GSS). (Gajdusek 1977; Masters et al. 1981). The slow infectious agents that cause the above diseases appear to differ from viruses but are similar to the agent that causes scrapie, a neurologic disorder of sheep and goats. The term "prion" has been introduced to denote this slow infectious agent, with its unusual properties (Prusiner 1982). The prion filament fulfils all the requirements for classification as amyloid both ultrastructurally and histochemically (Glenner 1980; Cohen et al. 1982; Prusiner et al. 1983) and it is therefore possible that amyloid fibrils in Alzheimer's disease and Down's syndrome may be similar to the prion filaments of these transmissible diseases. Prusiner et al. (1983) reported that the prion filament consists of rods measured 10 to 20 nm in diameter and 100 to 200 nm in length by negative staining.

Bockman et al. (1985) reported that purified fractions from the brains of two patients with CJD contained proteins which reacted with antibodies raised against the scrapie prion protein PrP 27–30. In addition, rod-shaped particles were found that were similar to those isolated from rodents with either scrapie or experimental CJD. Thus the amyloid plaques may be composed of paracrystalline arrays of prions similar to those in prion-related disease in laboratory animals. De Armond et al. (1985) identified prion proteins in the brains of scrapie-infected hamsters using immunoelectron microscopy. The structures exhibited a uniform diameter of 16 nm. Rarely, the filaments had a twisted appearance, raising the possibility that they are flattened cylinders or are composed of helically wound protofilaments. The prion filaments possessed the same diameter and limited twisting as the shorter rod-shaped particles observed in purified preparations of prions. The ultrastructural features of prion filaments are similar to those of amyloid, and as described above, the ultrastructure of prion filaments obtained from tissue specimens seems to be similar to those of amyloid fibrils presented in our study.

Although in Alzheimer's disease and Down's syndrome, no infectious pathogen is transmissible to animals, the structural similarities between prion filaments and amyloid fibrils obtained from the brain in Alzheimer's disease and Down's syndrome lead us to speculate that a transmissible agent may be involved in these disesases.

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