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Immunohistochemical and ultrastructural changes in the brain in probable adult glycogenosis type IV: adult polyglucosan body disease

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Abstract

Glycogenosis type IV is caused by a deficiency of glycogen branching enzyme (α-1,4 glucan 6-transglucosylase). Adult polyglucosan body disease (APBD) may represent a neuropathological hallmark of the adult form of this storage disease of the central nervous system.
We analysed a case of a 45-year-old unconscious woman who died three days after admission to the hospital. Neuropathological examination revealed massive accumulation of polyglucosan bodies (PBs) in the cortex and white matter of the whole brain. PBs were located in the processes of neurons, astrocytes and microglial cells. The storage material in the cytoplasm of neurons and glial cells was visible as fine granules.
Ultrastructurally, PBs consisted of non-membrane-bound deposits of branched and densely packed filaments, measuring about 7–10 nm in diameter, typical of polyglucosan bodies.
APBD patients develop upper and lower neuron disease and dementia, probably secondary to the disruption of neuron and astrocyte functions.

Key words: *adult polyglucosan body disease, glycogenosis type IV, ultrastructure.*

Introduction

Glycogen storage disease type IV (GSD IV) is a rare autosomal-recessive disorder due to the deficiency of glycogen branching enzyme (GBE) (α-1,4-glucan: α-1,4 glucan 6-transglucosylase). The human GBE gene is located on chromosome 3p12 and consists of 16 exons. GBE enables the last stage of glycogen synthesis by enclosing short glucosyl chains [11,19]. Therefore, GBE deficiency results in the accumulation of abnormal glycogen in a myriad of tissues.

Andersen disease is a clinical manifestation of GSD IV as classic hepatic, non-progressive hepatic (juvenile) and neuromuscular neonatal forms, as well as late-onset neuroform as adult polyglucosan body disease (APBD) [17].

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Adult polyglucosan body disease was first described in 1980 [15]. The onset of symptoms is observed in persons between the ages 40 and 60 years, and the clinical progress is associated with a wide variety of sensory, motor and cognate features, and dementia until death, within 3 to 20 years [5]. APBD is characterized by a large number of polyglucosan bodies (PBs) [4,18,20,21,25]. PBs are observed mostly in the central (the white and grey matter) and peripheral nervous system, muscles and skin [1,3,7,12,21,22,26].

In the central nervous system (CNS) polyglucosan bodies are round or spheroid, located in cytoplasm axons, astrocytes and microglia [25]. Electron microscopically, PBs are composed of irregular filaments (about 7–10 nm in diameter) and granular material [8].

Report of a case and methods

A 45-year-old woman (non-diabetic) without past medical history or neurological family history was admitted to the hospital because of not waking up after the previous night's sleep [25]. One month before entry she suffered from headache and episodes of nausea and vomiting. Death followed three days of unconsciousness progressing to coma.

Laboratory studies yielded moderate hyperglycaemia and increased levels of protein (350 mg/100), glucose (105 mg/100) and chlorides (131 mmol/l) in the cerebrospinal fluid.

The autopsy showed passive hyperaemia with pulmonary oedema and ischaemic focus in the heart. The brain (1250 g) showed a moderate oedema.

Light microscopic examination was carried out on the brain fixed in 4% paraformaldehyde in 0.1 M phosphate buffer saline, pH 7.4. Samples were collected from both cerebral and cerebellar hemispheres, as well as from the brain-stem.

Blocks from the brain were paraffin embedded and stained with histological methods (H&E, PAS, PAS-dimedon, Klüver-Barrera, lectin-RCA1), and IHC reactions, including reaction with antibodies: glial fibrillary acid protein (GFAP), ubiquitin, synaptophysin (SY), HLA-DP and anti-neurofilaments (NF).

For electron microscopic evaluation, small fragments of the brain were taken from paraffin blocks. After deparaffinizing and washing in water, the material was fixed in 2.5% glutaraldehyde with post-fixation in 2% osmium tetroxide and routinely processed to Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, then examined with a transmission electron microscope (Opton DPS109).

Results

Microscopic examination of all specimens of both cerebral and cerebellar hemispheres and brain-stem revealed a great many deposits of polyglucosan bodies (PBs). PBs were found within neuronal and glial processes, especially in the white matter and slightly less in the grey matter (Fig. 1A-B). A larger number of PBs were seen on the brain surface (between the pia matter and astroglia borderline) (Fig. 2A). They also showed some tendency to concentrate around the vessels, in the subependymal layer and in the depth of sulci (Figs. 2B, 3A).

The majority of PBs looked like round, spherical, sharply demarcated structures. Some of them had an irregular, mulberry-like outline, but elongated thread-like forms also occurred, especially in the grey matter (Figs. 3B, 4A-B).

Moreover, PAS-positive deposits were observed in the cytoplasm of neurons in the cerebral cortex, thalamus, basal ganglia and brain-stem nuclei. They were most frequently present in neurons with a large cytoplasm, such as Betz cells, pontine nuclei neurons and neurons in the hippocampus hilus (Fig. 5A-B). The loss of neurons was not significant.

Using H&E and PAS methods, three types of large PBs could be identified: light oval or spherically shaped bodies, dark bodies, and bodies with a dark core and/or two light outer laminae (Figs. 2-3). At the ultrastructural level, PBs also demonstrated differentiated morphology. Some of them were composed of both filaments and an amorphous electron-dense core of varied size and shape (Fig. 10A-C), while others showed only irregular, branched filaments about 7 to 10 nm in diameter (Fig. 10D).

Immunohistochemically, PBs showed a positive reaction with ubiquitin (Fig. 6A). Immunohistochemical reaction with GFAP showed numerous PBs surrounded by glial fibres (Fig. 6B-C). In the white matter, around the vessels and ventricles, as well as subpially, the number of astrocytes increased. Some of them were less ramified, or their processes were short and fragmented (Fig. 6D). Ultrastructurally, numerous large PBs were in close relation with gliofilaments in astrocyte processes (Fig. 11). Small deposits of irregular and loosely packed filaments, typical of PBs, were located probably in the cytoplasm of astrocytes (Fig. 12).

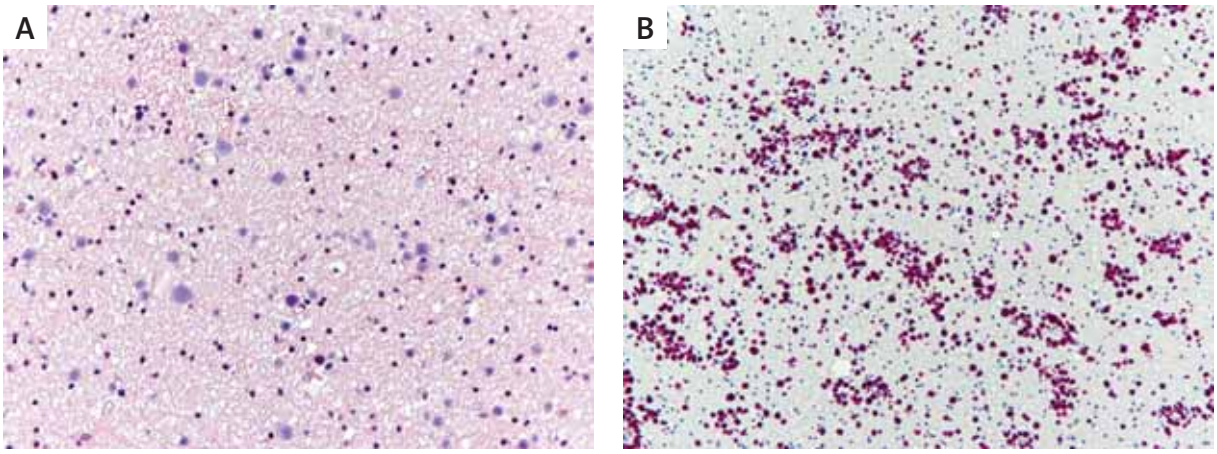

Fig. 1. Hemispheric white mater. Accumulation of PBs. A. H&E ×200; B. PAS ×200

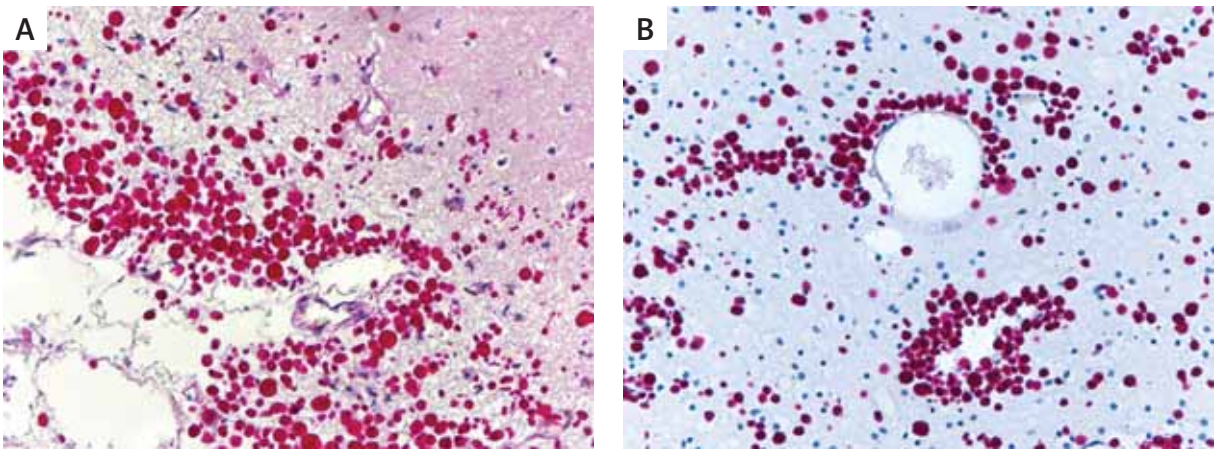

Fig. 2. A. Subpial cerebral cortex with PBs. PAS ×400; B. Perivascular PBs. PAS ×400

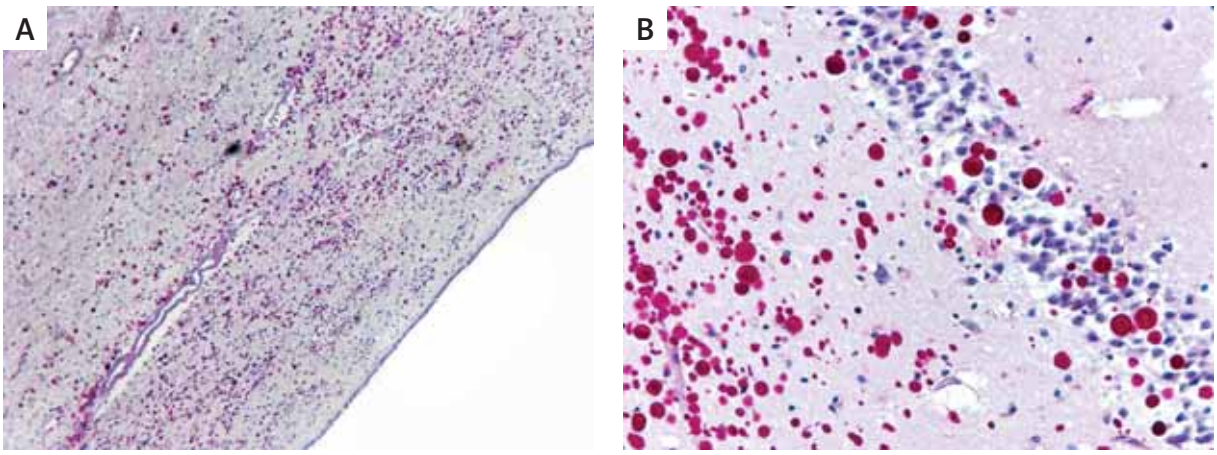

Fig. 3. A. Subventricular concentration of PBs. PAS ×100; B. Accumulation of PBs in the hippocampus. PAS ×200

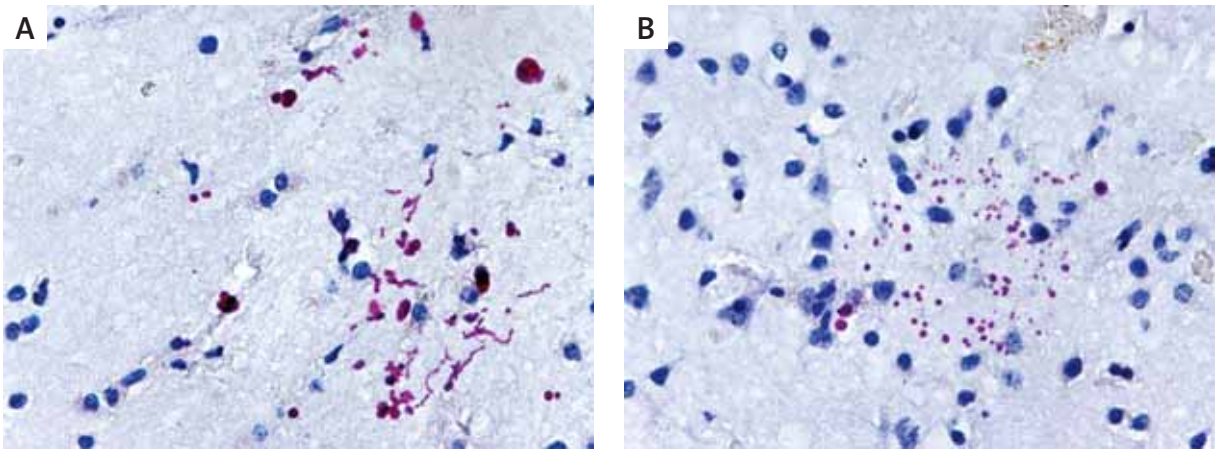

Fig. 4. A. Elongated deposits of PAS-positive material around the vessels. PAS ×200; B. Small PBs in the frontal cortex. PAS ×200

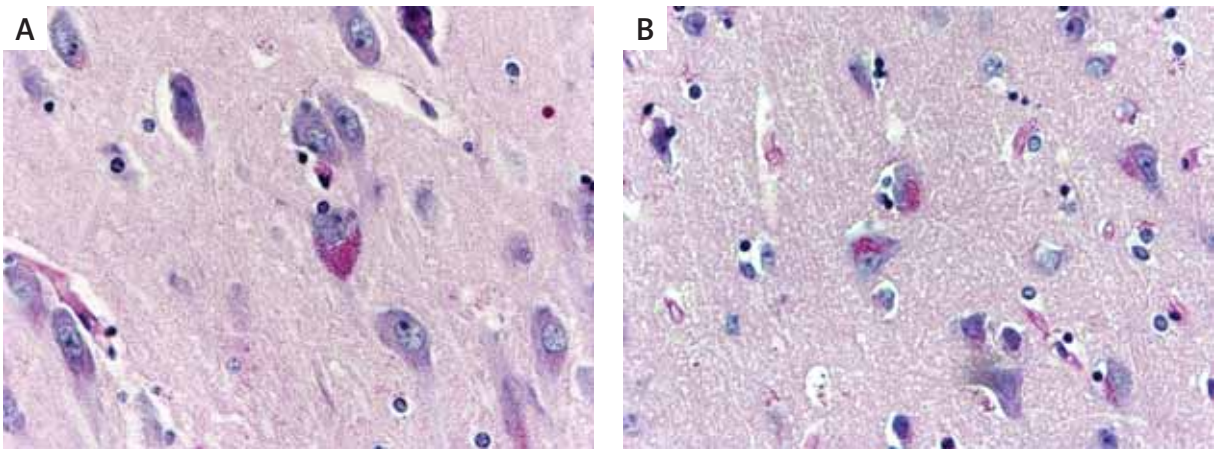

Fig. 5. A. PAS-positive deposits in the frontal cortex neurons. PAS ×400; B. PAS-positive deposits in the cytoplasm of neurons. PAS ×400

IHC reaction with anti-NF antibodies showed intra-axonal PBs as they were surrounded by neurofilaments (Fig. 7A-B). With electron microscopy, PBs were seen to occupy all the cytoplasm of axons with thin myelin sheaths (Fig. 13). Reaction with lectin RCA-1 and HLA-DP revealed that some PBs were within processes of microglial cells (Fig. 8A). Sometimes, microglia almost completely devoid of processes and cytoplasm were visible. Poor expression of HLA-DR (major histocompatibility complex class II) on the surface of microglial cells was noted (Fig. 8B). The ultrastructure of microglial cells with abundant cytoplasm filled with various vacuoles resembled macrophages (Fig. 14).

In the regions where the majority of PBs were observed, the synapses were not visible (Fig. 9A). IHC reactions of antibody CD34 with endothelial cells were moderately positive (Fig. 9B). The ultrastructural picture of endothelial cells was normal (Fig. 15).

Discussion

In the presented case, deposits of polyglucosan bodies of varied size and form were diffusely distributed in the whole brain, including both white and grey matter.

Morphologically, PBs are similar to two forms of deposits: Lafora bodies (LBs), described in progressi-

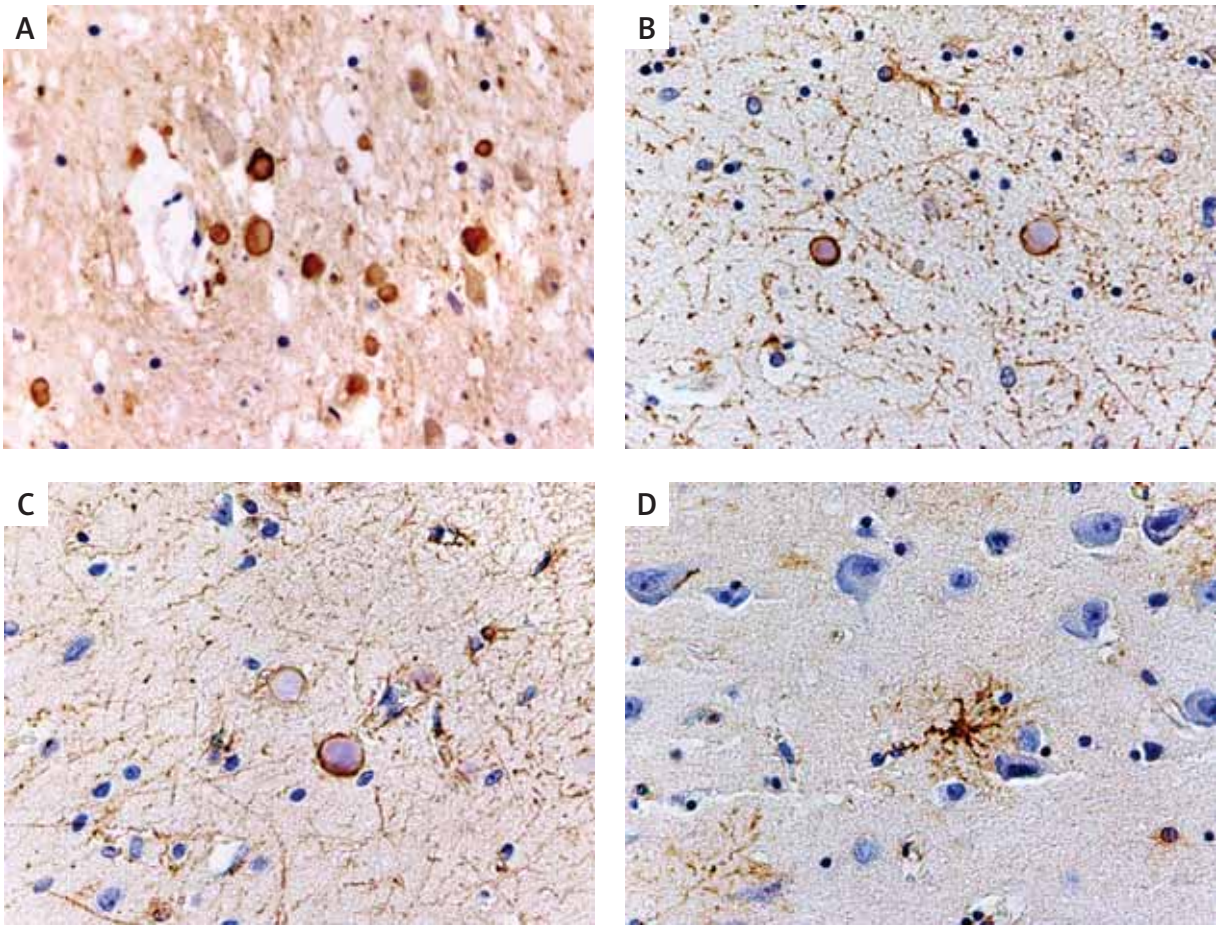

Fig. 6. A. Positive PB reaction with ubiquitin. ×400; B-C. PBs surrounded by glial fibres. GFAP ×400; D. Glial cells with damaged processes. GFAP ×400

ve myoclonic epilepsy, and corpora amylacea bodies (CABs), in disturbed metabolism, a large quantity of cerebrospinal fluid and disturbances in the barrier function [18,23]. CABs usually accumulate around blood vessels, on the brain surface and in the subependymal layer, while PBs predominate in the lower and upper layers of the cerebral cortex, as well as in the white matter and basal ganglia [16]. LBs were described in progressive myoclonus epilepsy of Lafora type (Lafora disease) and Baltic myoclonus (Unverricht-Lundborg disease) with a typical onset in teenagers followed by decline and death usually within 10 years [23,27], whereas our patient was 45 years old and had no past epilepsy in medical history. The diagnosis of APBD in this case was based on the neuropathological picture [10,15,25].

Light and electron microscopic and immunohistochemical findings corresponded with those found in APBD cases with GBE deficiency.

Adult polyglucosan body disease may or may not be associated with GBE deficiency [14]. However, neuropathological abnormalities are of the same morphological nature. The pathogenesis of glucosan deposits in CNS in cases without GBE deficiency is not known [2]. Maybe APBD with a normal GBE level is associated with the absence and/or deficit of enzymes other than GBE.

Biochemically, PBs are composed of glucose polymers with 1,4 α-D-glucoside linkage configured as branched polysaccharides [5]. Accumulation of smaller quantities of PBs can be seen within peripheral nerves of patients with various clinical disorders,

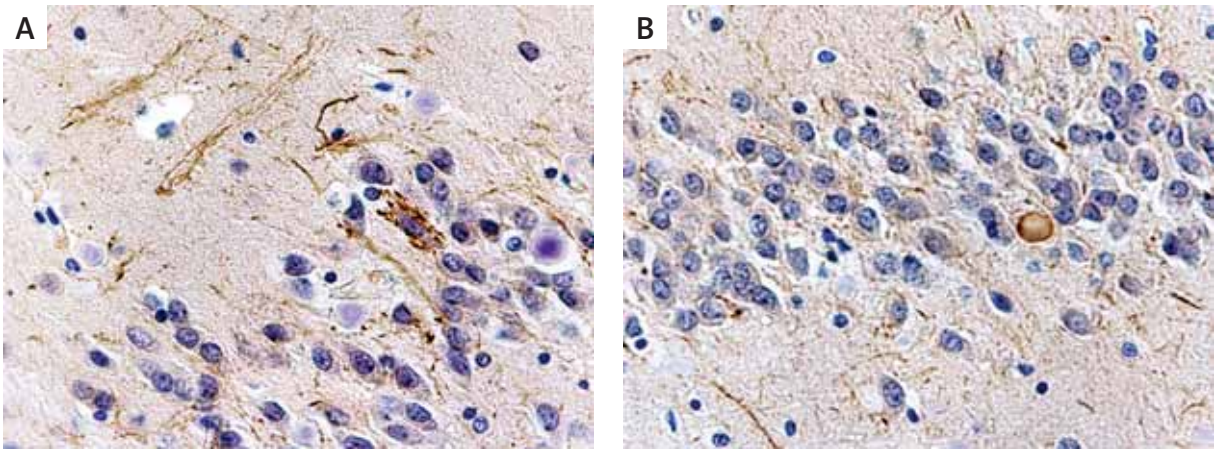

Fig. 7. A. Hippocampus, gyrus dentate; B. PBs surrounded by neurofilaments. NF ×400

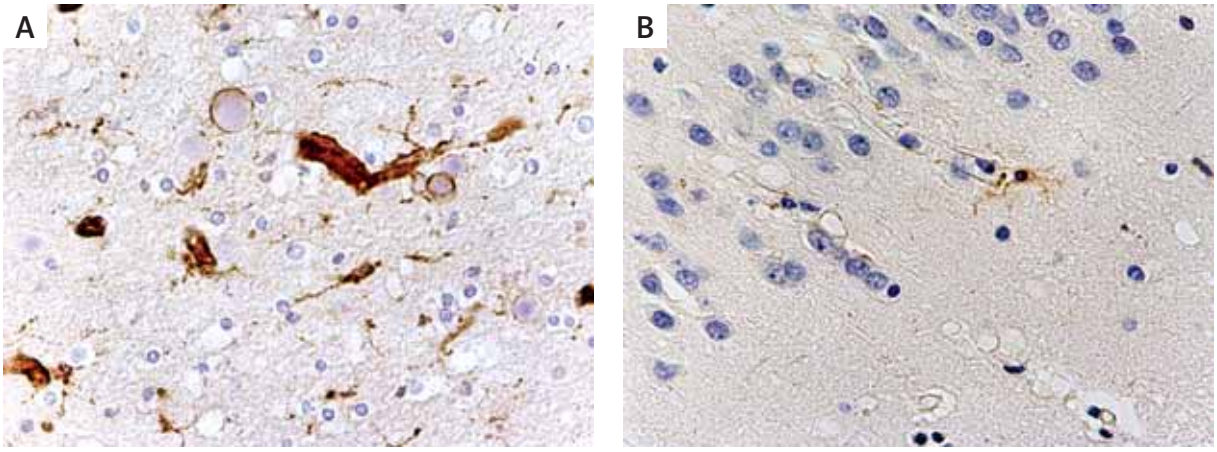

Fig. 8. A. PBs within processes of microglial cells. RCA-1 ×400; B. Poor expression of HLA-DP with microglia. ×400

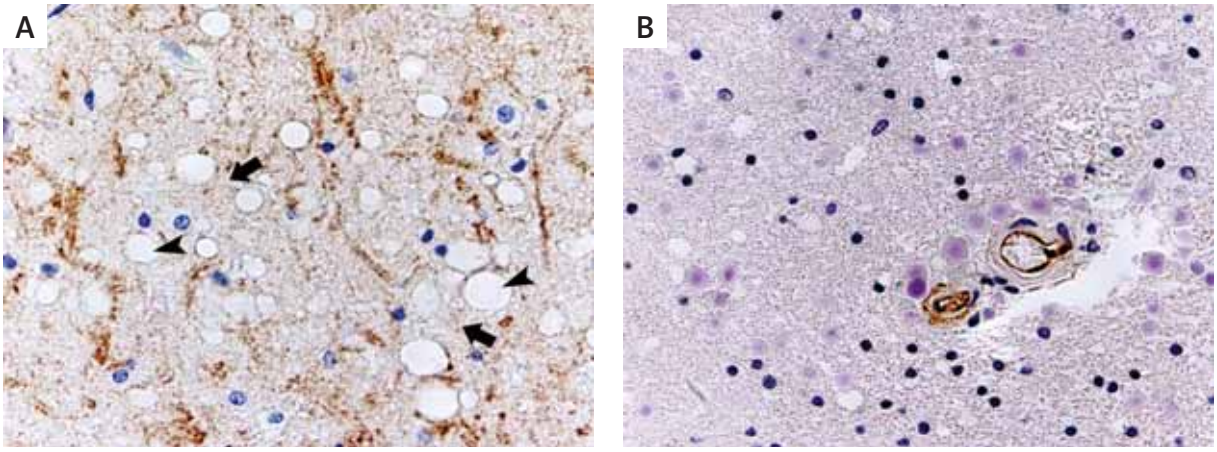

Fig. 9. A. Decreased number of synapses in neuropil (arrows) between PBs (head arrows), SY ×400; B. Endothelial cells. Positive reaction with CD-34. ×400

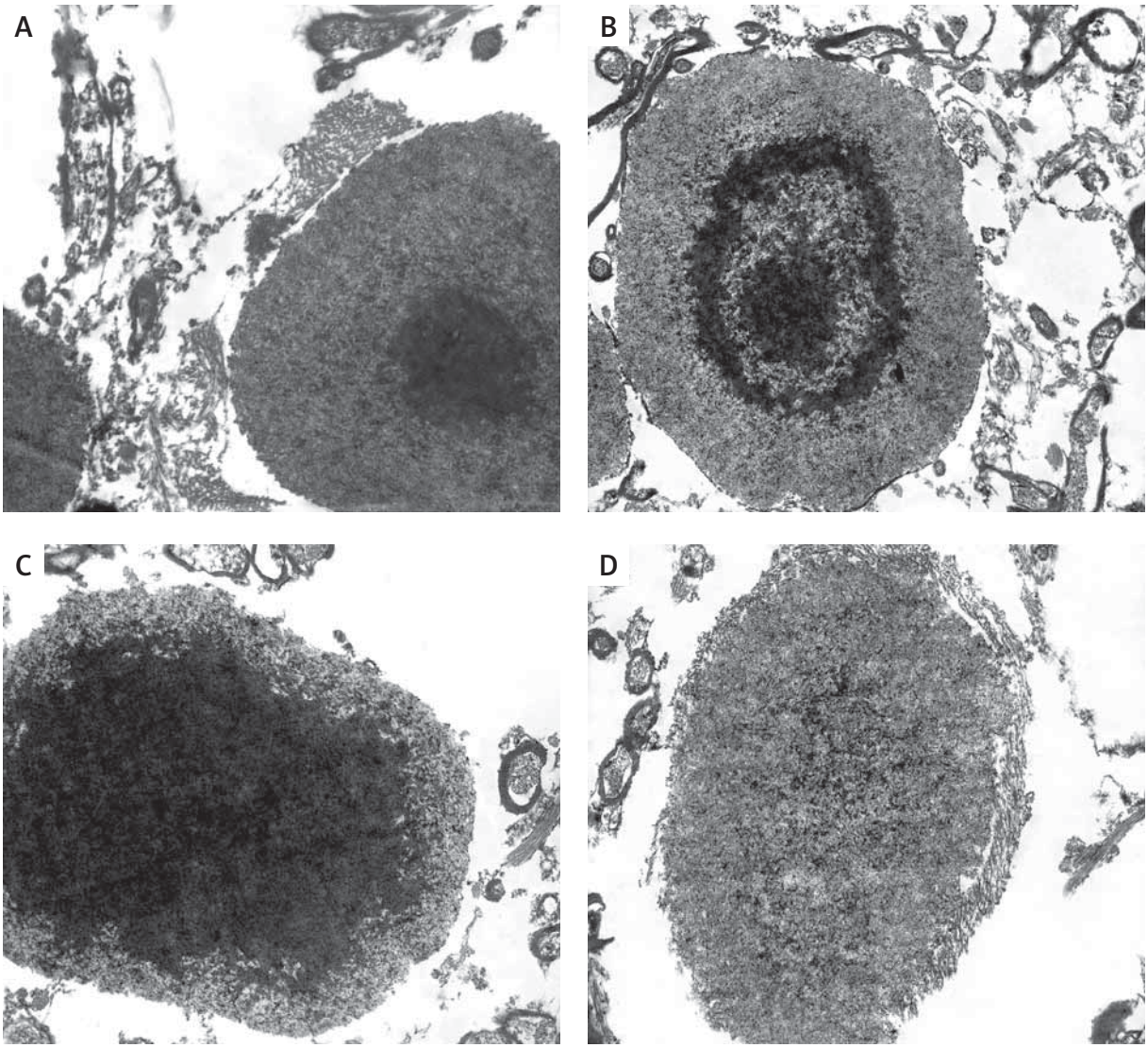

Fig. 10. A. Polyglucosan body with a small dense core within damaged astrocyte processes. Original magnification ×7000; B. Polyglucosan body with the central dense core surrounded by a thin rim the same nature as core. Original magnification ×4400; C. Large dense core in PB. Original magnification ×7000; D. PBs composed of typical filaments only. Original magnification ×7000

such as diabetes mellitus, motor neuron disease, late cerebellar-cortical atrophy and familial spastic paraparesis, but an abundance of them diffusely distributed in the whole brain appears only in APBD [1,13]. Deposits of polyglucosans in placenta, skeletal muscles and skin have been observed in GSD IV, as well as in GSD II (Pompe disease) [6,9,12,21,24]. Therefore, the presence of occasional deposits of polyglucosans and PBs within peripheral nerves, skin and muscles does not provide an evident basis for the diagnosis of APBD.

The increased frequency and size of PBs in biopsy specimens, associated with clinical symptoms and abnormalities in MRI scanning, support the diagnosis of APBD. However, neuropathological examination of the brain is required to confirm this diagnosis.

The presence of polyglucosans in the cytoplasm of neurons, axons, astrocytes and microglial cells remains unclear. It is likely that deposits of polyglucosans are trapped within perikarya since many enzymes involved in glycogen synthesis are located in the cytoplasm. Then they flow down from perikarya

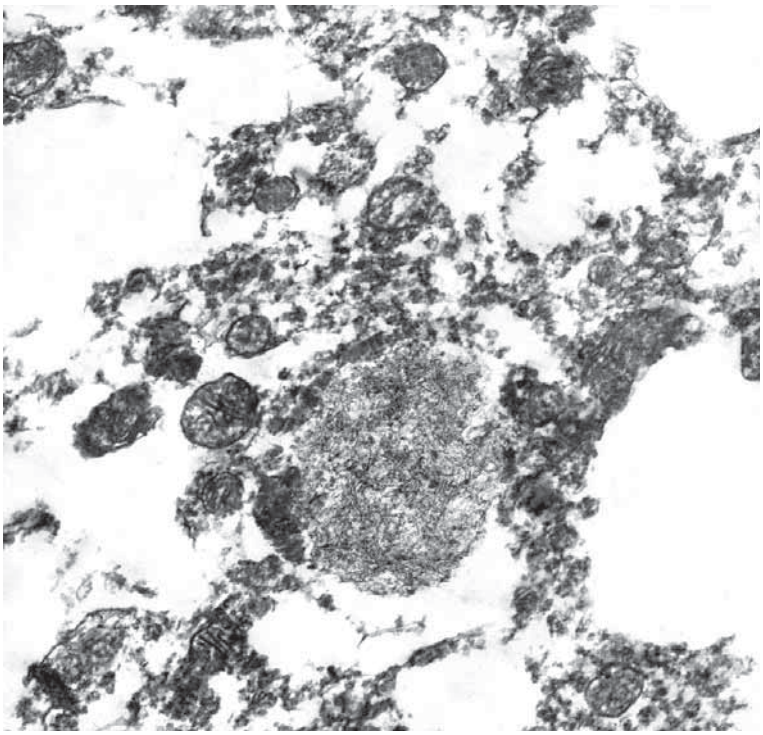

Fig. 11. Small polyglucosan body composed of loosely packed filaments in the cytoplasm of astrocyte. Origin. magn. ×7000

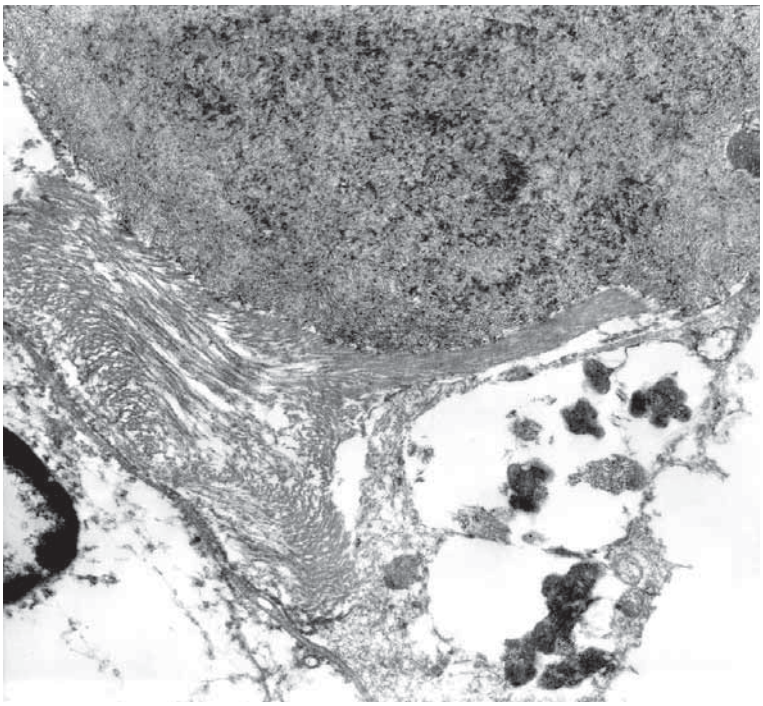

Fig. 12. Close relation between the polyglucosan body and gliofilaments of astrocytic process. Origin. magn. ×12 000

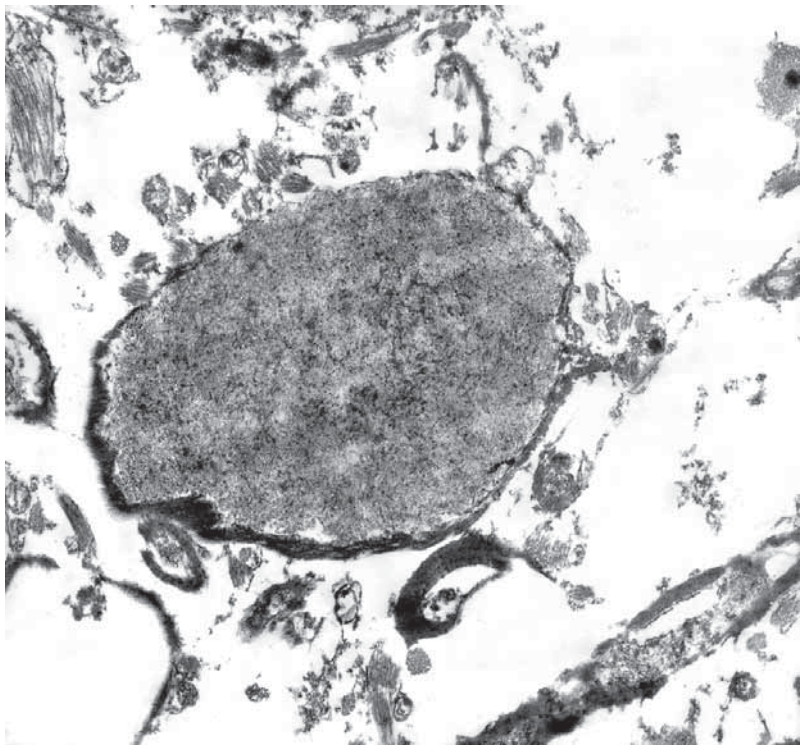

Fig. 13. Polyglucosan body within the myelinated axon. Origin. magn. ×7000

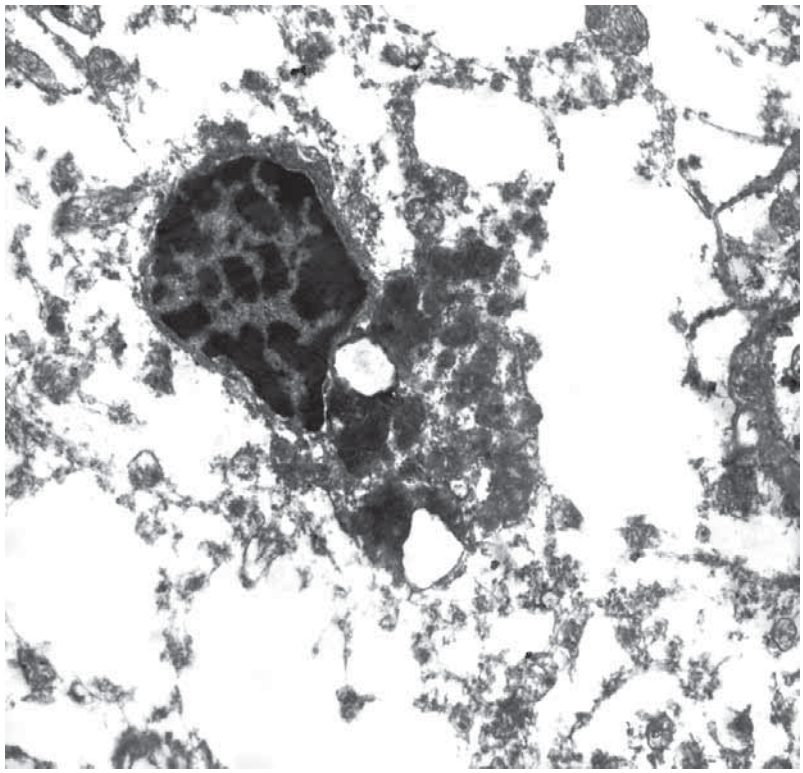

Fig. 14. Microglial cell showing macrophage morphology. Origin. magn. ×7000

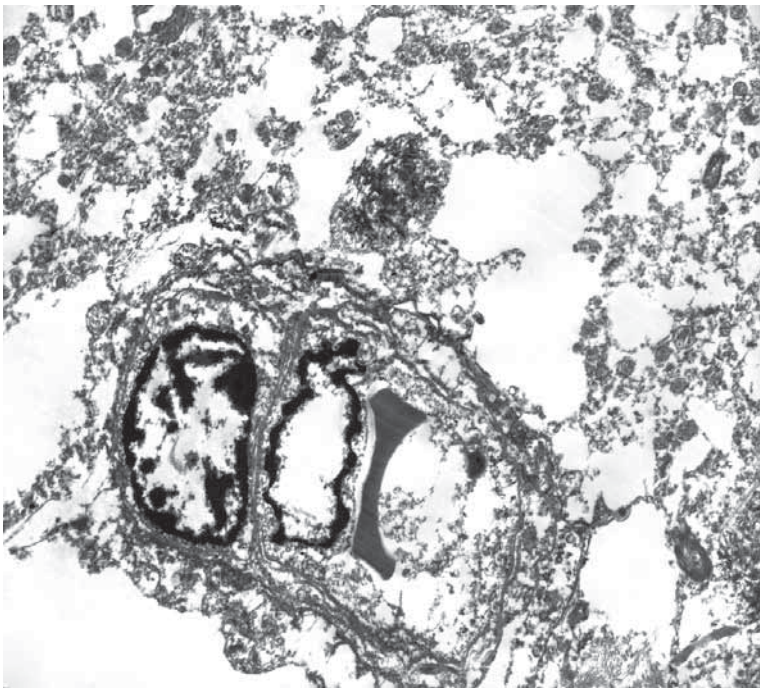

Fig. 15. Small PB in the vicinity of normal vesicle. Origin. magn. ×4400

to the processes. The destruction of microglial cell processes may cause immunological deficiency.

Massive accumulation of PBs in astrocytic processes may impair the metabolic exchange between the parenchyma and the blood. Our ultrastructural investigations revealed that the majority of PBs were located in astrocytic processes in close contact with gliofilaments or in their vicinity. The structure of these astrocytes was destroyed and only remnants of astrocytic filaments were seen. Similarly, myelinated axons showing PBs were damaged. PBs usually occupied the entire cytoplasm of axons, and their myelin sheaths were thin or broken.

APBD patients develop upper and lower neuron disease and dementia, probably secondary to the disruption of neuron and astrocyte functions.

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