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Case report

Creutzfeldt-Jakob disease with severe involvement of cerebral white matter and cerebellum

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Summary. We describe a patient with Creutzfeldt-Jakob disease (CJD) of the ataxic and panencephalopathic type. Postmortem examination revealed the characteristic lesions of CJD in the grey matter and profound white matter involvement was seen with immunocytochemical techniques. Ultrastructural white matter lesions were identical to those described in experimentally transmitted CJD. There was marked loss of cerebellar granule cells with virtual disappearance of parallel fibres, but Purkinje cells were only slightly reduced. Electron microscopic studies revealed extensive degenerative changes including cytoplasmic vacuoles in both cell types. Silver methods disclosed massive impregnation of white matter and striking abnormalities of Purkinje cells consisting of hypertrophy and flattening of thick dendritic branches, reduction in the number of terminal branchlets, segmentary loss of spines and polymorphic spines. These findings show the extensive involvement of all three cerebellar cortical layers and the reactive plasticity of Purkinje cells to deafferentiation. They favour the hypothesis that demyelination represents a primary lesion of the white matter.

Key words: Creutzfeldt-Jakob disease – Cerebellar degeneration – Leucoencephalopathy

Introduction

Creutzfeldt-Jakob disease (CJD) is a progressive and transmissible illness due to degeneration of the nervous system, occurring in middle age and usually fatal within a year of onset (Brown et al. 1986; Kirschbaum 1968). Clinical variations are numerous but the common picture includes dementia, rigidity of muscles and myoclonic jerks. CJD affects the grey matter predominantly with widespread neuronal loss and gliosis accompanied by spongy changes of the affected regions (Kirschbaum 1968; Lampert et al. 1972; Masters and Richardson 1978; Kim and Manuelidis 1983a). Several forms of CJD have been distinguished on clinical and neuropathological grounds. The panencephalopathic type, a rare variant of the disease, is characterized by extensive white matter involvement (Tateishi et al. 1979; Park et al. 1980; Mizutani et al. 1981; Macchi et al. 1984). In the ataxic type, which represents between 10% and 17% of all cases, conventional light microscopic studies have shown, together with other characteristic lesions of spongiform encephalopathy, selective degeneration of cerebellar granule cells (Brownell and Oppenheimer 1965; Gomori et al. 1973; Jellinger et al. 1974). By using immunocytochemical, reduced silver impregnations and ultrastructural studies, we present additional pathological data in a patient with the ataxic and panencephalopathic type of CJD.

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Case report

In May 1986, a 61-year-old woman farm manager developed progressive ataxia. She was able to continue with her professional activities. The patient was examined by a neurosurgeon who found truncal ataxia and suspected a posterior fossa tumour. A CT scan revealed no apparent abnormalities. Three months later, rapid intellectual decline appeared. On admission in September 1986 her vital signs and the results of general physical examination were normal. She did not respond to verbal stimuli. Her voice was so dysarthric that language was unintelligible. There was marked gait ataxia and generalized muscle rigidity. Muscle tendon reflexes were increased. Occasional myoclonic jerks of the arms and of the facial muscles were seen. Her neurological status progressed and she became mute terminating in an apallic state. She died in February 1987.

Routine laboratory investigations including cerebrospinal fluid (CSF) examination were normal. Serial EEG recordings showed progressive slowing of background activity and periodic complexes

(PC). Serial sleep EEG recordings demonstrated that the physiological states of sleep were replaced by the cyclic changes previously described by us in patients without white matter involvement (Calleja et al. 1985). These consisted of phases with PC alternating with theta-delta activity, brief periods of suppression of background rhythm and slower PC. Two serial CT scans (October 1986 and January 1987) revealed discrete cerebral and minimal cerebellar atrophy, severe white matter hypodensity and enlarged ventricles.

Methods

Autopsy was performed 3 h after death. Several pieces from the superior semilunar lobule of the cerebellum were taken immediately and processed for electron microscopy, Golgi impregnation and reduced silver staining. The brain was then fixed in 10% formalin for 3 weeks. Coronal sections of both cerebral hemispheres, cerebellum and brain stem were cut and embedded in paraffin. The following stains were used: haematoxylin and eosin (H&E), Luxol fast blue/Kluver Barrera (LBKB), Nissl and periodic acid-Schiff. Polyclonal antibodies to glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) were used from selected areas with the avidin-biotin technique.

For electron microscopy cerebellar tissue blocks were fixed in 1% paraformaldehyde, 1% glutaraldehyde in 0.12 M phosphate buffer (pH 7.2) overnight. Then the blocks were postfixed by immersion in 0.12 M phosphate-buffered 2% osmium tetroxide solution (pH 7.2), stained in block with uranyl acetate, dehydrated in graded solutions of acetone and embedded in Araldite. Sagittal sections of cerebellar folia were used in this study.

For the rapid Golgi procedure, cerebellar slices of 3 mm thickness were fixed in a mixture of 0.2% osmium tetroxide and 2.4% potassium dichromate for 5 days, followed by 0.75% silver nitrate impregnation for 24 h at 37° C. For the Golgi-Hortega method, cerebellar slices were fixed in 1% paraformaldehyde and 1% glutaraldehyde in 0.12 M buffer phosphate. Afterwards the pieces were immersed in a mixture of 5% potassium dichromate and 5% chloral hydrate for 8–15 days. The fixed tissue was then impregnated in 0.75% silver nitrate solution for 24 h. Sections of 100–200 μm in thickness were used.

The cerebellar pieces used for silver impregnation were processed with the reduced silver method of Ramón y Cajal, formula no. 117 (Ramón y Cajal and De Castro 1933).

Results

The brain (fixed weight 1040 g) was of normal external appearance. On section both cerebral and cerebellar cortex were thin. The lateral, third and fourth ventricles were markedly dilated. The cerebral white matter was soft but otherwise unremarkable. The spinal cord was not examined.

Microscopically, widespread loss of neurons, spongy changes and hyertrophy of astrocytes were observed in the cortex, caudate nuclei, putamen and thalami. These lesions were of variable intensity, being predominant in the frontal, parietal and occipital areas. The hippocampus was the least involved area. Deeper layers of the cortex were more affected than superficial. Spongy lesions were present throughout the brain stem, including all three cerebellar peduncles. Although many areas, in particular the pontine base and middle cerebellar peduncles, showed moderate gliosis, no apparent cell depopulation was seen in substantia nigra, red nucleus, locus ceruleus, pontine or inferior olivary nuclei.

In the cerebral white matter, there was marked loss of myelinated fibers, severe spongiosis, and astrocytosis mainly of gemistocytic type (Fig. 1). Foamy macrophages were occasionally noted. The degree of demyelination in myelin preparations varied in different areas and in parts of the same area. MBP and GFAP-stained sections demonstrated extensive demyelination and gliosis even in structures that appear hardly involved in LBKB preparations, such as internal capsule (Fig. 1 B).

There were no neuritic plaques or neurofibrillary tangles. Lacunes or hyaline arteriosclerosis were not seen.

In the cerebellum study of formalin-fixed paraffin-embedded tissue and semithin sections from vermis and hemispheres revealed identical lesions. Both molecular and granular layers were thinner than normal, and there was marked spongiosis and severe loss of granule cells. Purkinje cells were slightly but significantly reduced (3.63 ± 1.08/mm versus 5.01 ± 2.1 in control at the superior semilunar lobule; $P < 0.01$). GFAP-stained sections showed prominent hypertrophy of velate astrocytes and Bergmann glia. The white matter was severely demyelinated; myelinated fibres crossing the granular layer were hardly seen. The dentate nuclei were generally well preserved. Scattered and perivascular lipid-laden macrophages were observed everywhere. Kuru-like plaques were not observed.

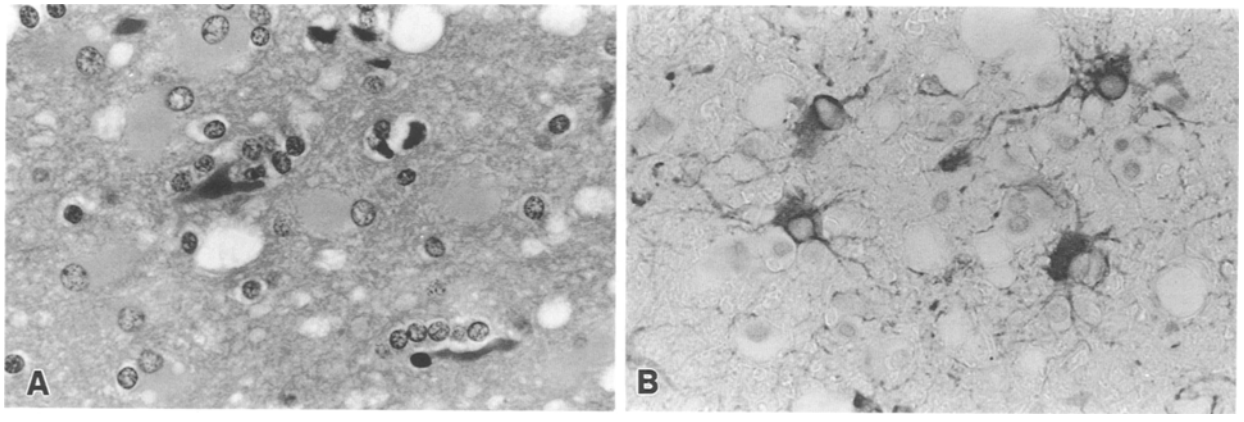

Fig. 1 A, B. Frontal subcortical white matter. There is spongiosis, and gemistocytic gliosis stained with glial fibrillary acidic protein (GFAP) antibody. **A** H&E ×380; **B** GFAP ×380

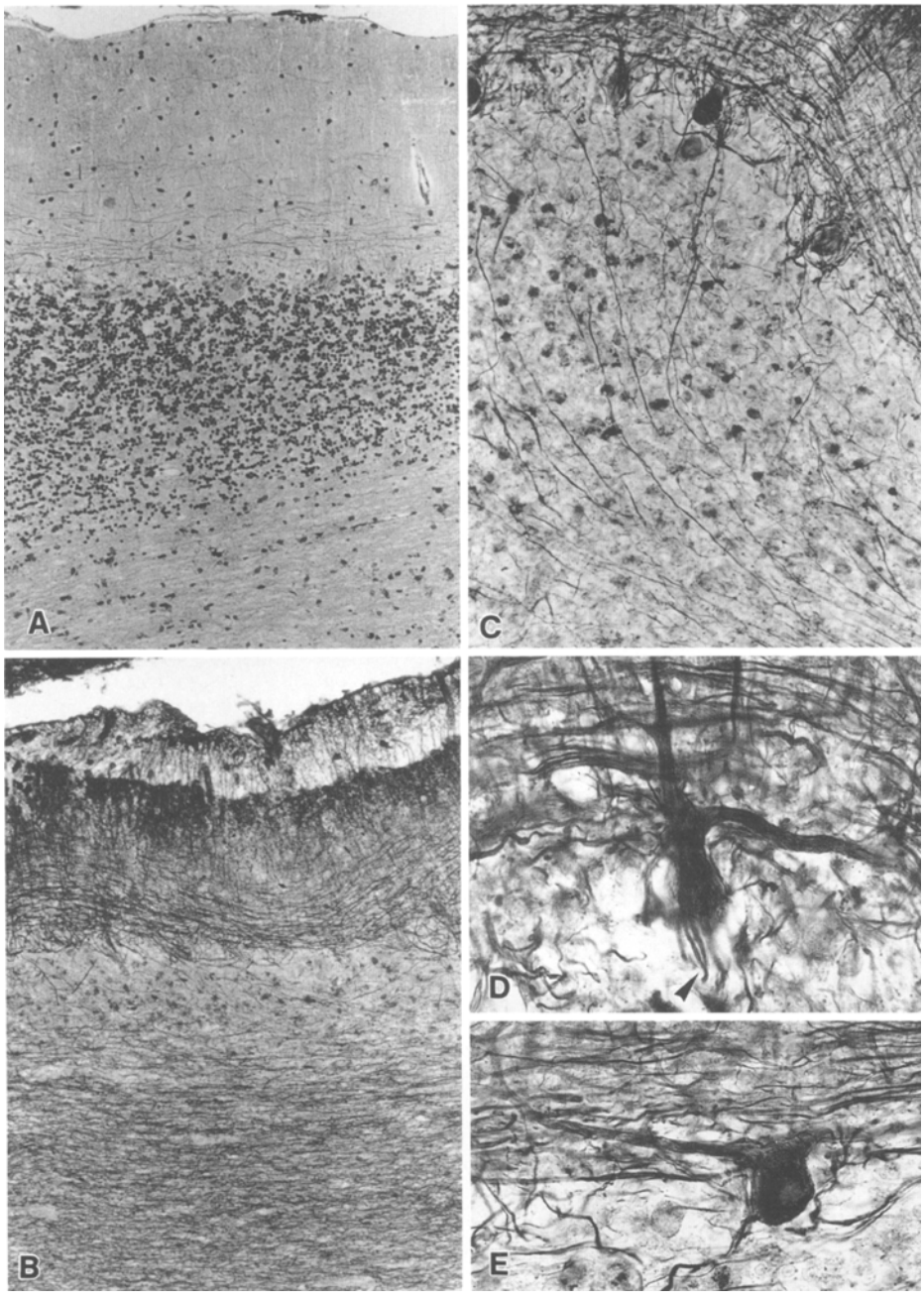

Fig. 2A–D. Cerebellar cortex preparations from control (**A**) and patient (**B–E**) stained with the reduced silver method.
B Compared to the control, there is severe spongiosis of upper half and proliferation of neuronal processes of inner half of the molecular layer, severe reduction of the granular layer and massive impregnation of the white matter.
C impregnation of Purkinje cell axons.
D, E Purkinje cells illustrating horizontalization and hypertrophy of dendritic trunks. Note the nest of basket cell axon and the pinceau (*arrowhead*). **A, B** ×140; **C** ×230; **D** ×470

The reduced silver method (Fig. 2) revealed conspicuous neuronal processes in the inner half of the molecular layer due to hypertrophy and flattening of Purkinje cell dendritic trunks, while the terminal branchlets were drastically reduced (Fig. 2B–E). The pericellular nest axons, the pinceau of basket cells (Fig. 2D) and Golgi cells were preserved. Fibres in the white matter and axons of Purkinje cells appeared massively impregnated (Fig. 2B, C, E). Some impregnated recurrent collaterals of Purkinje cell axons could be followed ascending into the supraganglionic plexus (Palay and Chan Palay 1974). Impregnated Purkinje cell axons showed very occasional swellings and "torpedoes". By means of Golgi procedures, Purkinje cells revealed the following changes (Fig. 3): multipolar cell bodies with several primary dendrites emerging from perikarya; horizontal arrangement of the thick dendritic branches; notable reduction in the number of terminal branchlets and segmentary loss of spines; descending dendritic branchlets; dendritic varicosities, polymorphic spines and rare dendritic blebs. Golgi preparations showed again hypertrophy of Bergmann glia and astrocytes.

Electron microscopic study revealed widespread spongy appearance of the neuropil, extensive degenerative changes of neurons and astroglial processes containing prominent bundles of intermediate filaments. There was massive loss of parallel fibres, synaptic contacts with Purkinje cell dendrites being drastically reduced and exclusively identified in deeper molecular layer. Loss of granule cells produced a virtual disappearance of the

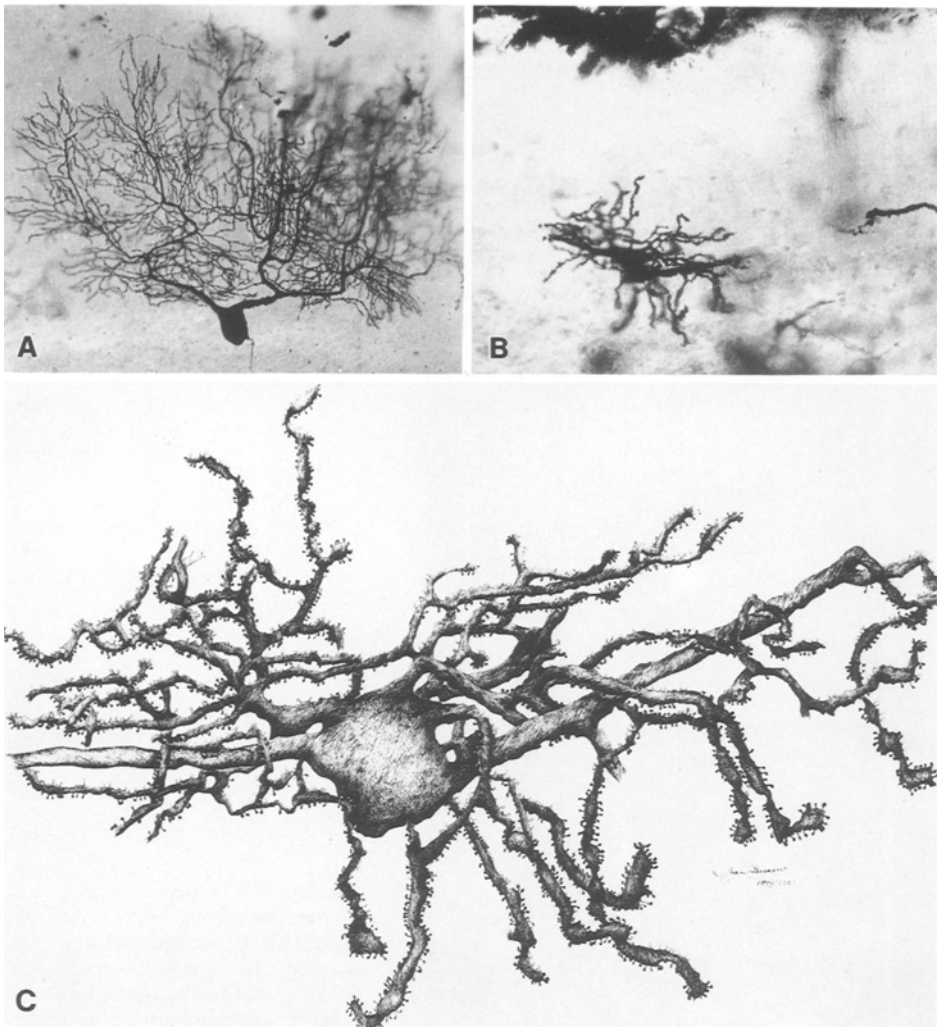

Fig. 3A–C. Golgi-Hortega preparations illustrating the global morphology of Purkinje cells from control (**A**) and patient (**B**). **C** Camera lucida drawing of the Purkinje cell showed in **B**. Note multipolar cell body, horizontal arrangement of the thick dendrites, descending dendritic branches (like "weeping willow branches"), drastic reduction of spiny branchlets, dendritic varicosities of terminal branches with segmentary loss of spines, and polymorphic spines. **A** ×200; **B** ×250

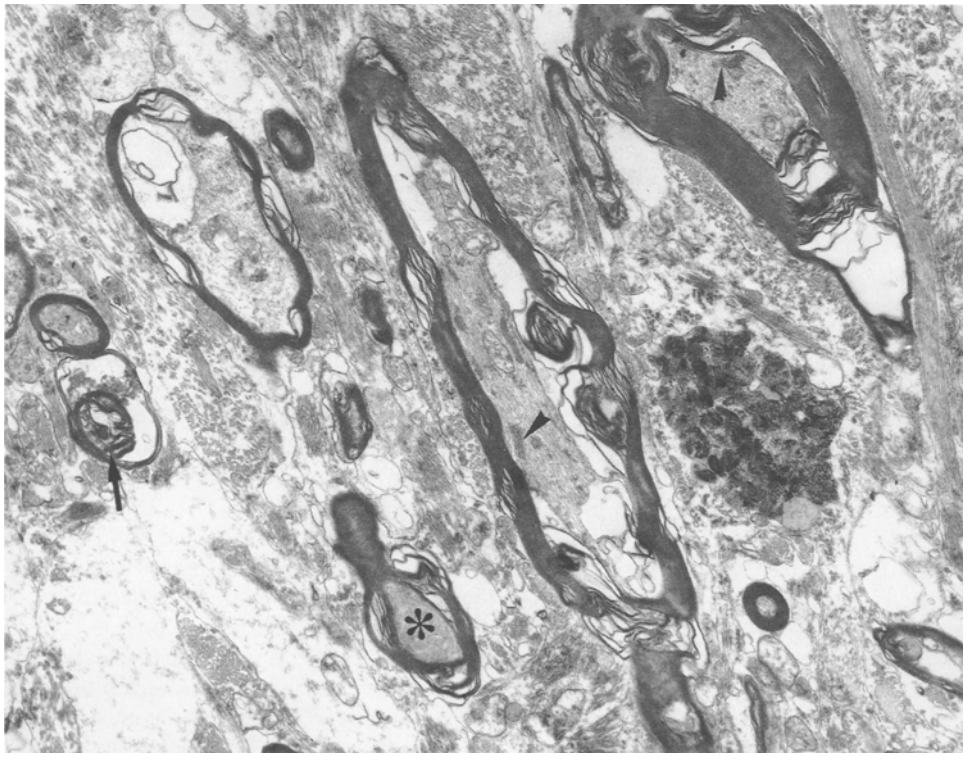

Fig. 4. Electron micrograph of the cerebellar white matter illustrating myelin lesions consisting of vacuolation and splitting. There is also axonal densification (*asterisk*) and wallerian degeneration (*arrow*). Typical hypolemmal complexes are recognized (*arrowheads*). Note the network of astroglial processes containing bundles of filaments. ×8500

characteristic cell rosettes and synaptic glomeruli. In the white matter there was loss of myelinated fibres, axonal densification, occasional images of wallerian degeneration and myelin lesions consisting of vacuolation and splitting of myelin (Fig. 4).

Discussion

The characteristic spongiform changes in the cerebral grey matter in this case were accompanied by extensive whtite matter involvement. Conventional histological methods demonstrated widespread and irregular demyelination, spongiform changes and gemistocytic gliosis (Macchi et al. 1984; Cruz-Sánchez et al. 1987). Immunocytochemical techniques revealed that white matter lesions were ubiquitous, including areas such as the internal capsule, which appeared normal in LBKB preparations. Although internal capsule sparing has been reported (Cruz-Sánchez et al. 1987; Mizutani et al. 1981), involvement of the corticospinal tract as seen here was a conspicuous finding in a panencephalopathic case of CJD and in small rodents to which the disease was transmitted (Tateishi et al. 1979, 1980; Sato et al. 1980). Ultrastructural study of cerebellar white matter demonstrated myelin lesions comparable to those described in experimental spongiform encephalopathy (Tateishi et al. 1980). Additional evidence of white matter involvement in our case comes from massive impregnation of the cerebellar white matter and Purkinje cell axons with the reduced silver method. According to Ramón y Cajal (Ramón y Cajal 1972; Ramón y Cajal and De Castro 1933), the appearance of myelin sheaths during development or regeneration makes the axon refractory to impregnation with the reduced silver method. Both mossy and climbing fibres in the white matter of cerebellar folia and Purkinje cell axons are heavily myelinated (Palay and Chan Palay 1974), that is to say silver impregnation of these fibres with Cajal's reduced procedure strongly suggests demyelination. The population of Purkinje cells and neurons in the pontine and inferior olivary nuclei in this case was either slightly reduced or normal, reinforcing the suggestion that cerebellar white matter lesions are not a dying-back phenomenon but a primary myelinopathy (Mizutani et al. 1981; Macchi et al. 1984; Cruz-Sánchez et al. 1987, 1989).

Cerebellar degeneration is found in between 50% and 82% of CJD cases at autopsy (Jellinger et al. 1974; Hauw et al. 1981; Macchi et al. 1984); lesions occur in varying combinations in all three cerebellar cortical layers and nucleus dentatus (Hauw et al. 1981). The pathological substratum of the ataxic form of CJD comprises two different patterns. The first, mainly reported in Japan, is characterized by spongiform changes, widespread kuru-like plaques, cortico-cerebellar degeneration often with predominant Purkinje cell loss, and possible white matter involvement (Mizutani et al. 1981; Yagishita et al. 1989). The second, as described here, is characterized by spongiform changes, absence of kuru-like plaques, cortico-cerebellar degeneration with predominant granule cell loss, and possible white matter degeneration (Brownell and Oppenheimer 1965; Gomori et al. 1973; Jellinger et al. 1974). In conventional, semithin and ultrathin sections, our study demonstrated massive loss of granule cells, the remainder showing nuclear pyknosis and sometimes cytoplasmic spongy changes. Mossy fibres were also massively degenerated. A consequence of granule cell loss is the extensive degeneration of parallel fibres and thinning of the molecular layer. In fact, synapses between parallel fibres and Purkinje cell spines were drastically reduced and restricted to deeper part of the molecular layer. Ultrastructural abnormalities observed in most Purkinje cells were comparable with those described in other neurons (Gonatas et al. 1965; Lampert et al. 1971, 1972; Landis et al. 1981; Kim and Manuelidis 1983a, b). Even though Purkinje cell loss was only discrete, these findings demonstrate their widespread involvement in the disease and indicate that protein synthesis was reduced (Gonatas et al. 1965). Spongiform changes in the cerebellar neuropil were ubiquitous but the degenerative process was so advanced that it was impossible to locate the vacuoles either in dendrites or axons (Sato et al. 1980; Kim and Manuelidis 1983b). Throughout the cerebellum there was marked astrocytosis with prominent bundles of intermediate filaments, but without spongiform changes (Gonatas et al. 1965). Intranuclear vacuoles were not observed (Kim and Manuelidis 1983b).

Golgi impregnations of cerebral cortex from patients with CJD have demonstrated considerable alterations in neuronal architecture (Case records MGH 1980; Ferrer et al. 1981; Landis et al. 1981; Kim and Manuelidis 1989). These are as follows: reduced calibre and irregular contour of dendrites of pyramidal neurons; decreased number of dendritic spines and dendrites; tortuous shafts of apical dendrites; and focal swellings, either semi-translucent or darkly impregnated, which involve both axons and dendrites. In a case of CJD involving the cerebellum, Ferrer et al. (1988) described depletion of distal branches and spiny branchlets and a reduced spine density in most Purkinje cells. Moreover, with reduced silver methods thickening and terminal sprouting of Purkinje cell dendrites and axonal "torpedoes" have been reported (Brownell and Oppenheimer 1965; Jellinger et al. 1974; Case records MGH 1980; Tiller-Borcich and Urich 1986). In our study Purkinje cells exhibited a unique pattern consisting of hypertrophy and flattening of dendritic arbor with reduction of spiny branchlets, dendritic spines being unusually long (Landis et al. 1981). Abnormally large and polymorphic dendritic spines have been reported in Purkinje cells which have differentiated in absence of parallel fiber innervation (Hamori 1969; Lafarga et al. 1986) and in an experimental model of mossy fibre lesion which produces secondary transneuronal degeneration of parallel fibres (Anderson and Flumferfeldt 1984). In agreement with Anderson and Flumferfeldt (1984), we suggest that Purkinje cells increase the length of their dendritic spines in order to attempt appropriate synaptic contacts after the massive pathological degeneration of parallel fibres. Since there was massive loss of parallel fibres, selective disappearance of outer Purkinje cell dendritic arbor was probably due to deafferentation. Although ultrastructural

study revealed impairment of Purkinje cells, hypertrophy of neuronal processes in the supraganglionic plexus proved the reactive plasticity (Tiller-Borcich and Urich 1986) of these cells to compensate for the loss of their main afferent input. In any case, we are not certain whether abnormalities observed in the basal dendritic arbor imply a primary degenerative change in dendrites, whether degeneration of parallel fibres led to deafferentation and subsequent resorption of spines, or whether both processes occur (Landis et al. 1981).

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