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Ultrastructural studies of experimental scrapie and Creutzfeldt-Jakob disease in hamsters. I. Alterations of myelinated axons

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Abstract. Classical and ultrastructural neuropathology of prion diseases are generally well described. Here we report that alterations of myelinated fibres in hamsters infected either with polioencephalopathic strains of scrapie or panencephalopathic strains of CJD (Echigo-1) are virtually identical and differ only quantitatively. In contrast, mice infected with the panencephalopathic Fujisaki strain of CJD exhibited much more elaborate changes of myelinated fibres.

Key words: scrapie, Creuzfeldt-Jakob disease, prions, ultrastructures

This paper is dedicated to the memory of Dr Clarence Joseph (Joe) Gibbs, Jr.

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INTRODUCTION

The transmissible spongiform encephalopathies (TSE) or prion diseases (Gajdusek 1977, Prusiner 2001) are a group of neurodegenerative disorders which include kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker (GSS) syndrome and fatal familial insomnia in man (Budka et al. 1995, Liberski and Gajdusek 1997), natural scrapie in sheep, goats and moufflons (Dickinson 1976, Wood et al. 1991), transmissible mink encephalopathy in ranch-reared mink (Hartsough and Burger 1965), chronic wasting disease of mule deer and elk in the USA (Williams and Young 1980, 1982, 1992, 1993), bovine spongiform encephalopathy or "mad cow" disease (Wells et al. 1987) and its analogues in several exotic species of antelopes and felidae (Wells and Wilesmith 1995). These diseases are caused by an as yet incompletely understood pathogen predominantly referred to as "prion" (Prusiner 2001) or rarely as "virino" (Kimberlin 1991). These names reflect generally different views on the molecular structure of the pathogen and, by the same token, our limited knowledge of its challenging nature (Liberski 1995). Those who prefer to view this pathogen as composed "predominantly or exlusively" of an abnormal isoform (scrapie isoform or prion protein, PrP^{Sc}) of a normal cellular glycoprotein (PrP^{c}), use the term "prion" hence the term "prion diseases". PrP^{Sc} differs from PrP^{c} neither by covalent structure nor by posttranslational modifications but by its conformation: PrP^{Sc} is mostly in a β-sheeted (or β-helical) conformation while PrP^{c} is chiefly α-helical (Caughey et al. 1991, Cohen and Prusiner 1998). A substantial proportion of investigators believe that such abnormal PrP^{Sc} (an abnormal conformer) converts PrP^{c} (the normal cellular protein) into more PrP^{Sc}. This may be accomplished either in a process where PrP^{Sc} acts as a template to convert PrP^{c} into PrP^{Sc} or in a nucleation process where PrP^{Sc} first forms a "seed" which attracts PrP^{c} and converts the normal isoform into PrP^{Sc} (Weissmann et al. 1996). Hence, prion diseases belong to a wide group of "conformational disorders" or "diseases of protein misfolding". The prion theory is almost totally accepted now and honoured by a Nobel prize (Prusiner 1997). Having said this, the theory remains to be formally proved and, despite its enormous success, it is still difficult to reconcile with the variation of strains of scrapie (Dickinson and Outram 1988, Liberski 1995).

Irrespective whether PrP^{Sc} is a part or the whole of the scrapie agent, it accumulates in the brain early in the course of disease either as amyloid plaques or as diverse extracellular deposits (synaptic, perineuronal or perivacuolar types) (Budka et al. 1995, Ironside 1998). However, some pathological phenomena – like apoptosis, may be at least in part dissociated from PrP^{Sc} accumulation (Lasmezas et al. 1997). Along these lines, it is still important to search for early pathogenetic phenomena, irrespective whether associated or not with the PrP^{Sc} accumulations.

Classical and ultrastructural neuropathology of prion diseases are basically well described (Jeffrey et al. 1995, DeArmond and Prusiner 1998, Liberski and Jeffrey 2000). It comprizes spongiform change, astrocytic gliosis and neuronal loss brought about probably by the apoptotic process. The majority of cases of the Creutzfeldt-Jakob disease (CJD) are classified as polioencephalopathic - i.e. affecting grey matter; some two dozen cases have met the criteria of the panencephalopathic type in which white matter is predominantly affected and its degeneration does not result from Wallerian degeneration (Liberski et al. 1989, 1990). In a series of previous studies we thoroughly evaluated the only available model of panencephalopathic type of CJD, the Fujisaki strain of CJD passaged in mice (Tateishi et al. 1978, Liberski et al. 1989, 1990, 1993, Kordek et al. 1996). In that model, we described two types of vacuoles and suggested that "intramyelin" vacuoles are brought about by lymphokines secreted from activated microglial cells and astrocytes. Indeed, we subsequently showed that both TNF-α peptide and mRNA are upregulated towards the terminal stage of disease (Kordek et al. 1996) and that this cytokine injected into the anterior chamber of an eye, produces lesions in the optic nerve indistinguishable from those seen in the panencephalopathic type of CJD (Liberski et al. 1993). The exact role of TNF-α is unknown. However, mice with disruption of the gene for TNF-α ("knock-outs") are resistant to scrapie infection, albeit only following peripheral inoculation (Mabott et al. 2000). In conclusion, we suggested that the myelinated axons undergoe true demyelination in the panencephalopathic types of disease (the Fujisaki strain of CJD) while in the polioencephalopathic strains of scrapie in hamsters it is destroyed in a process of Wallerian degeneration (Liberski and Gajdusek 1997). Notwithstanding, this conclusion suffered from the fact that the panencephalopathic model of CJD was devel-

oped in mice while polioencephalopathic scrapie models in hamsters. To bypass this, we developed another panencephalopathic model, the Echigo-1 strain of CJD passaged in hamsters (Mori et al. 1989, Liberski and Mori 1997, Liberski et al. 1998). Thus both pan- and polioencephalopathic models of TSE may be directly compared. We report here that alterations of myelinated fibres in hamsters infected either with polioencephalopathic strains of scrapie or panencephalopathic strains of CJD (Echigo-1) are virtually identical and differ only quantitatively. In contrast, mice infected with the panencephalopathic Fujisaki strain of CJD exhibited much more elaborate changes of myelinated fibres.

METHODS

Animals and strains

Ten Syrian hamsters from a local colony were inoculated with the 263 K or 22C-H strains of scrapie (Liberski et al. 1989). These strains are widely used as experimental tools primarily because of relatively short incubation periods which, for mice, rang from 16 to 18 weeks and for hamsters from 9 to 10 weeks for the 263K strain and 24-26 weeks for the 22C-H strain.

In addition, hamsters were inoculated with the Echigo-1 strain of CJD (Liberski and Mori 1997, Liberski et al. 1998). The Echigo-1 strain of CJD was isolated by Mori and colleagues (1989) from a case of 33 years-old female with a panencephalopathic type of CJD. The inoculum prepared from a CJD patient's brain passaged to guinea pigs with incubation periods (IP) of 728 days at the primary and approximately 400 days at subsequent passages. A substrain with substantially reduced IP (254 days) was isolated from an animal which exibited active running and an excessive response to external stimuli. This strain was re-isolated in hamsters with IP of 141 days at the 3rd passage. An incubation period following intracerebral inoculation of hamsters with 10% cleared suspension of the Echigo-1-affected brain was approximately six months. Appropriate control animals were sham-inoculated with saline.

Electron Microscopy

Following intracardiac perfusion with 2.5% gluteraldehyde and 1% paraformaldehyde in cacodylate buffer (pH 7.2), the brains were removed and rinsed in cold fixatives overnight. Samples [1 mm^3] of the right parietal cortex and adjacent corpus callosum were dissected, rinsed in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated through a graded series of ethanols and propylene oxide and embedded in Embed (Electron Microscopy Sciences, Ft. Washington, PA). Ultrathin sections were stained with lead citrate and uranyl acetate, and specimens were examined using Zeiss 109 or JEM 100 C transmission electron microscopes.

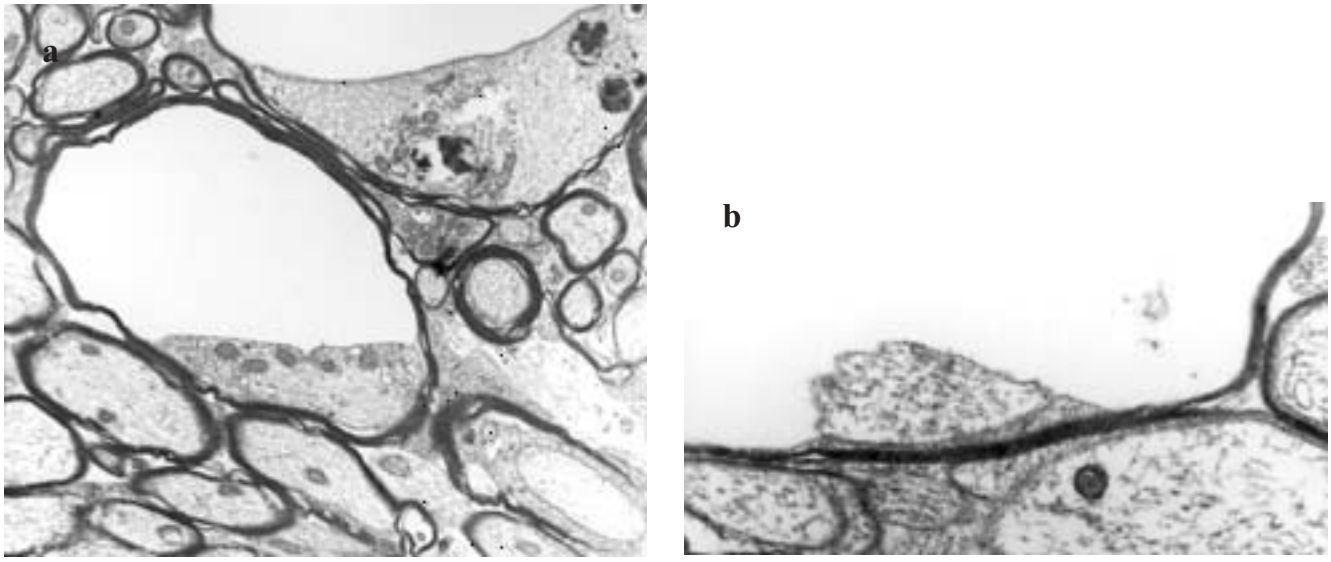


Fig. 1. Low (a) and high (b) power electron micrograph of intramyelin vacuoles in hamster brain infected with 263K strain of scrapie. Note that axon is shrunken and filled with neurofilaments and scanty electron-dense bodies. Original magnifications, (a), x 7,000; (b), x 20,000.

RESULTS

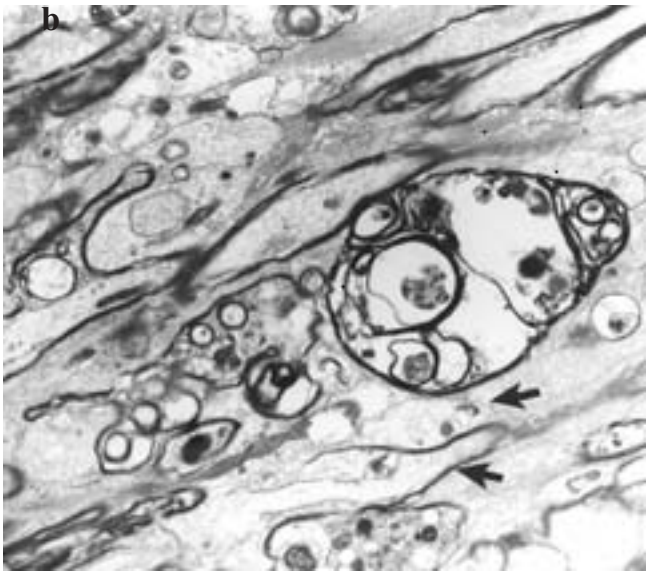
Scrapie-infected hamster brains

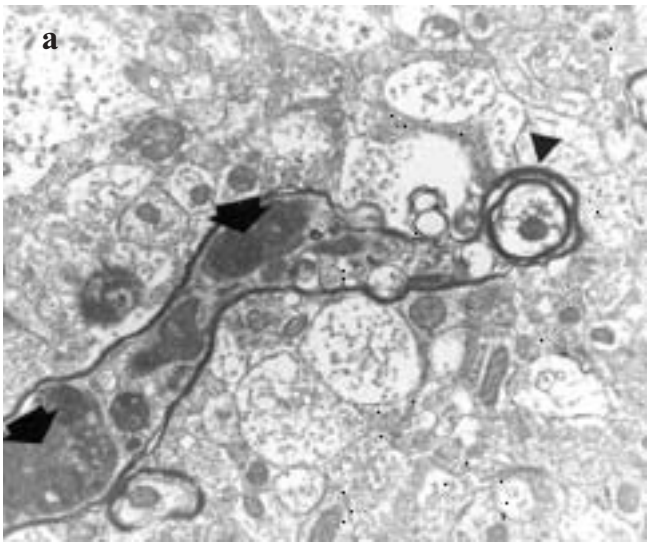
INTRAMYELIN VACUOLES

In hamsters inoculated either with 263K or 22C-H strain of scrapie, intramyelin vacuoles were readily visible even at low power (Fig. 1a, b). They were several times larger than the diameter of average mylin fibre and looked "empty". Shrunken axons were observed within distended myelin sheaths, but many bullous swellings contained no axons. Some axons looked normal but others were filled with neurofilaments and scanty electron-dense bodies. Still other axons were attached to the innermost myelin lamellae by a thin "neck" probably a mesaxon.

WALLERIAN DEGENERATION

Numerous myelinated fibres underwent typical Wallerian degeneration. A typical picture consisted of a myelinated fibre divided into "chambers" filled with electron-dense amorphous masses or "empty" spherical spaces containing myelin debris (Fig. 2). Some fibres were transformed into connected collection of smaller and larger membrane-bound spherical spaces containing myelin debris. Of note, longitudinal sections of some fibres clearly revealed that their compartment containing dark masses were contiguous with those that appeared empty. Some fibres retained part of myelin sheath but axons were shrunken, divided into compartments and electron-dense (Fig. 3). Some myelinated





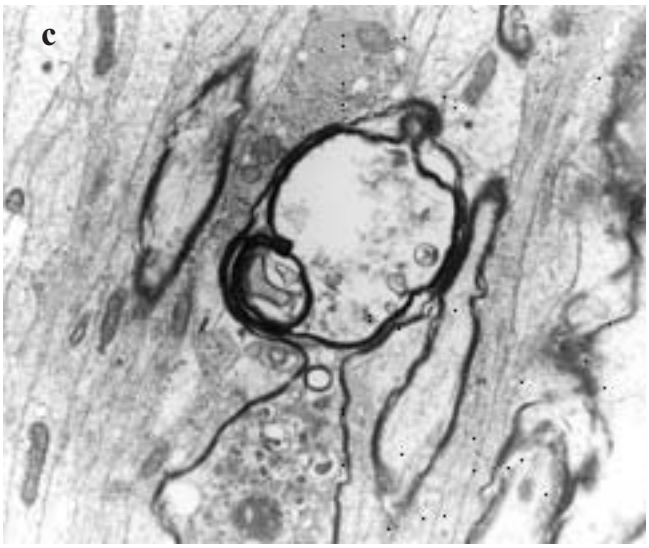


Fig. 2. Myelinated fibers damage in experimental scrapie. a, tortuous myelinated fibres running between many well preserved axonal terminals in the hamster brain infected with the 22C-H strain of scrapie. Note that the axon is divided into "chambers" filled with electron-dense amorphous masses (arrows) and some "empty" spherical spaces (arrowhead); b, a longitudinal section through the myelinated fibre undergoing Wallerian degeneration. Note that the whole fibre is transformed into membrane-bound spaces of different sizes containing myelin debris. Numerous bundles of intarcellular glial filaments are visible between fibres (arrows); c, a longitudinal section of a fibre with a part containing electron-dense masses and bullous spaces apparently "empty". Note a part of the axon separated from the rest of a fibre by concentrically arranged myelin lamellae. Original magnifications: (a, b), x 7,000; (c), x 12,000.

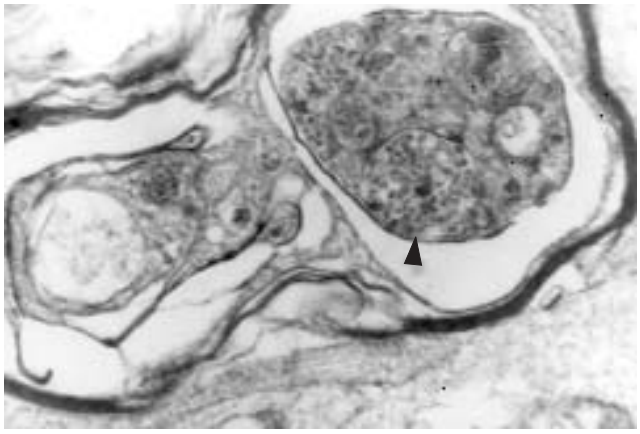


Fig. 3. A mylinated fibre undergoing Wallerian degeneration in a hamster brain infected with the 22C-H strain of scrapie. Note the relatively well preserved myelin sheath. The axon is shrunken, of increased density and divided by membraneous septae into compartments. A large multivesicular body is also visible (arrowhead). Original magnification, x 30,000.

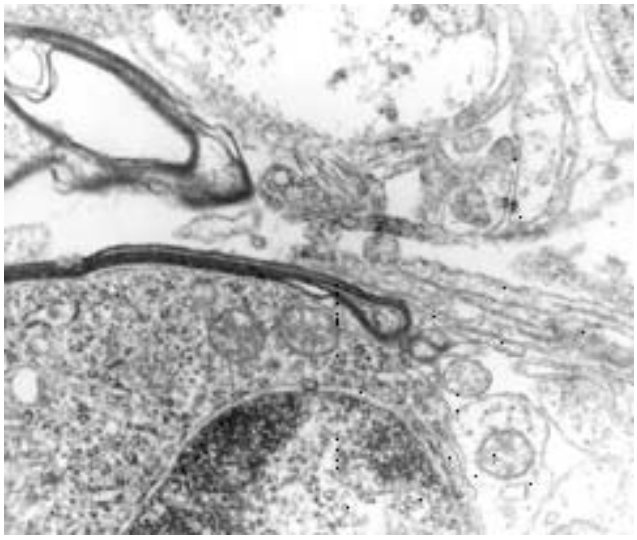


Fig. 4. A collapsed myelinated fibre with an axon apparently squeezed from the plane of the section. A hamster brain infected with the 22C-H strain of scrapie. Original magnification, x 20,000.

fibres collapsed and the axon was apparently "squeezed" from the plane of section (Fig. 4).

AXONAL CHANGES

Typical neuroaxonal dystrophy was frequently observed. Affected fibres were bullously distended and filled either mitochondria and electron-dense lysosomal bodies (Fig. 5) or masses of neurofilaments in which other organelle as mitochondria were apparently entrapped (Fig. 6). Occasionally, we observed that a part of an axon filled with electron-dense and electron-lucent bodies was separated from the apparently normally looking rest of an axoplasm by a normal my-

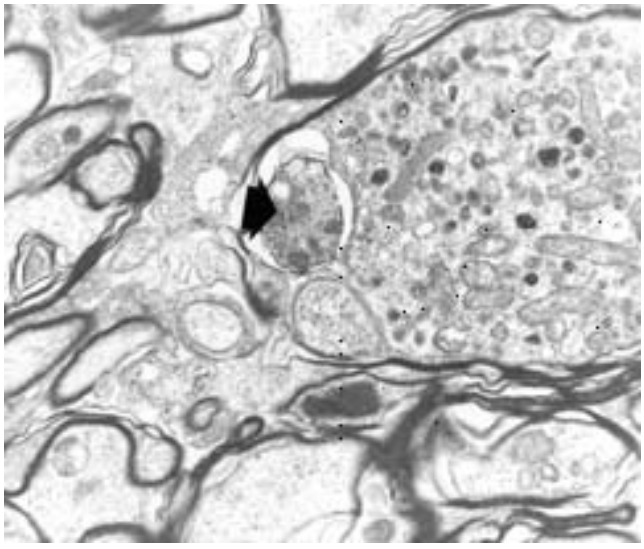


Fig. 5. A large myelinated dystrophic neurite filled with numerous mitochondria and electron-dense lysosomal bodies. Note that atypical portion of axoplasm is sequester by membranes into separate compartments which suggest autopohagic process (arrow). Hamster brain infected with the 22C-H strain of scrapie. Original magnification, x 12,000.

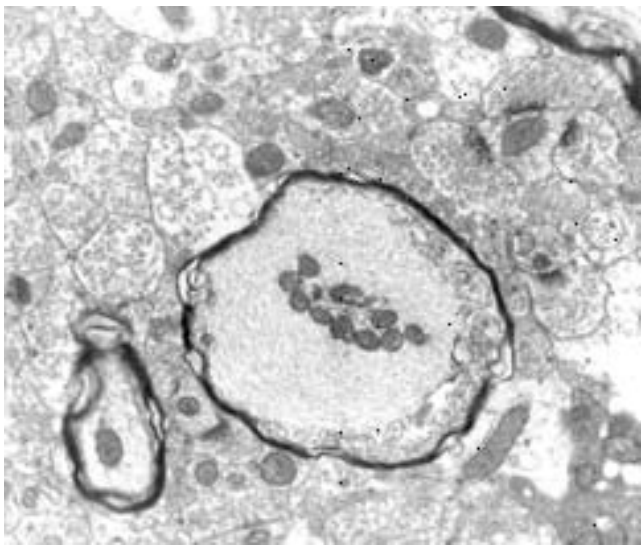


Fig. 6. A mylinated fibre filled with centrally positioned mases of neurofilaments. Some otherwise normally looking mitochondria are entrapped within these masses. A hamster brain infected with 22C-H strain of scrapie. Original magnification, x 12,000.

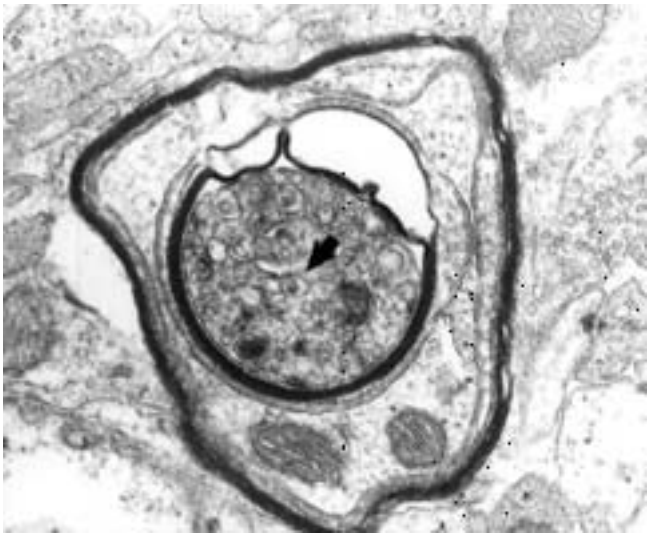


Fig. 7. A myelinated fibre from a hamster brain infected with the 22C-H strain of scrapie. Note that the central part of an axon filled with many electron-dense and electron-lucent bodies is separated from the apparently normal part by several layers of myelin. The central degenerating part is connected to the remaining part by proliferating mesaxons (arrow). Original magnification, x 30,000.

elin sheath (Fig. 7). The degenerating part of an axon was connected to the other part by proliferating mesaxons.

Echigo-1-infected hamster brains

Quantitatively, brains infected with the Echigo-1 strain of CJD exhibited changes virtually indistinguishable from both models of scrapie. They showed intramylin vacuoles (Fig. 8), mylinated fibres which underwent Wallerian degeneration (Fig. 9) and neuroaxonal dystrophy. The vast majority of neurites accumulated lysosomal dense bodies (Fig. 10).

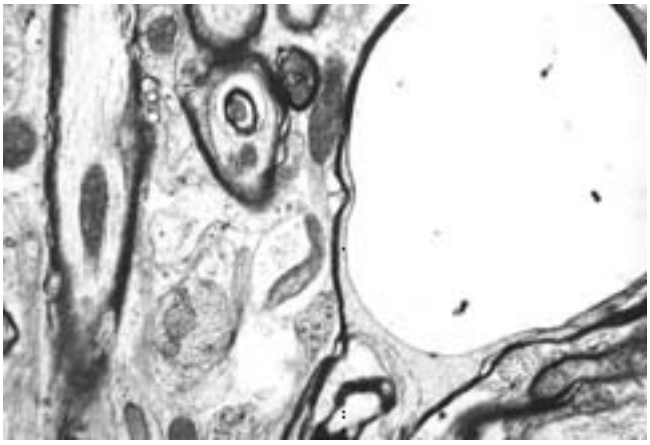


Fig. 8. Large bullous expansion of myelin sheath; an axon is missing from the plane of section. A hamster brain infected with the Echigo-1 strain of scrapie. Original magnification, x 4,400.

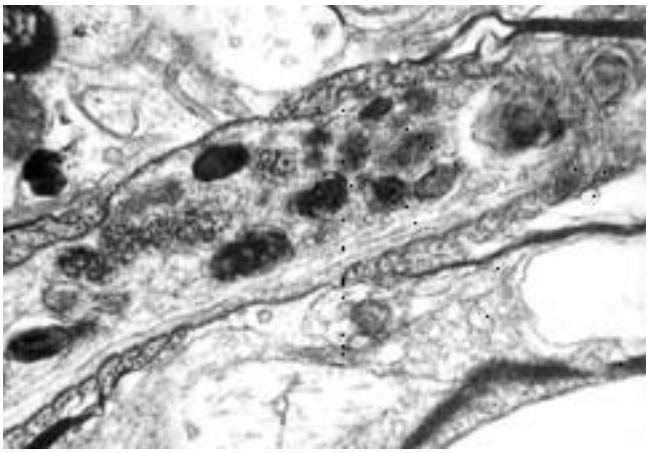


Fig. 9. A longitudinal section through a myelinated fibre showing both amorphous electron-dense masses and "empty" chambers surrounded by circular membraneous profiles. A hamster brain infected with the Echigo-1 strain of scrapie. Original magnification, x 12,000.

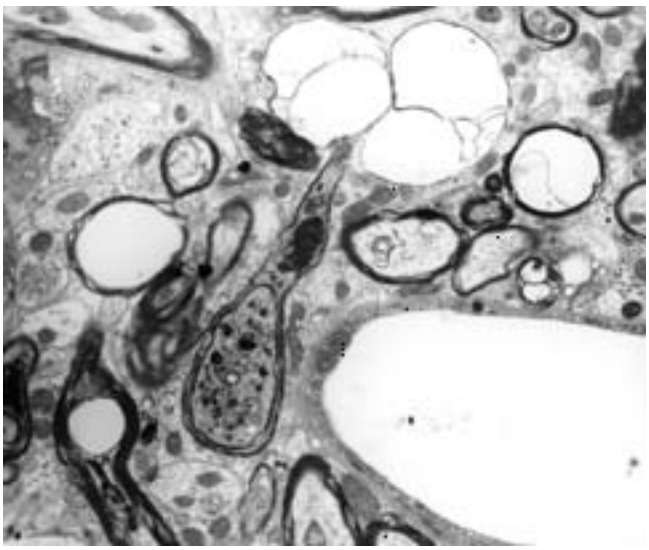


Fig. 10. A longitudinal section through an accumulation of electron-dense lysosomal and multivesicular bodies in the myelinated fibre. The section was taken from an area of the node of Ranvier; lateral loops are well preserved. A hamster brain infected with the Echigo-1 strain of scrapie. Original magnification, x 12,000.

DISCUSSION

This study evaluates the second panencephalopathic model of CJD available so far in small laboratory rodents (Mori et al. 1989, Liberski and Mori 1997, Liberski et al. 1998). It is potentially important because this is the only such model in hamsters. The Fujisaki strain of CJD was passaged in mice (Tateishi et al. 1978, Liberski et al. 1989, 1990) and, in reality, it is the Gerstmann-Sträussler-Scheinker disease strain not CJD strain). Therefore comparative studies of different strains of agent may by conduced in the same host (namely - hamster). Thus far only mouse and hamster model have been available for comparative studies and it might be suggested that described differences stem from animal species and not strain differences.

We report here that in hamsters infected either with scrapie (263K and 22C-H strains) or with the Echigo-1 strain of CJD, the alterations of myelinated fibres were virtually indistinguishable. They consisted of vacuoles within myelin sheath ("intramyelin vacuoles") and diverse alterations of axons itself: from neuroaxonal dystrophy to Wallerian degeneration.

However, in the paradygmatic Fujisaki strain passaged in mice the white matter changes are different. While they retains all the alterations reported by us for hamsters, mouse brains infected with the Fujisaki strain of CJD exhibit an additional alteration compatible with true demyelination (Raine 1985). While the latter changes have been observed simultaneously in different areas of the same sample, the following reconstruction is organised as if it followed a sequence of events. Initially, the myelin sheath was separated by cytoplasmic tongues into several concentric bands. Cellular processes penetrated between layers of myelin and lifted away the outermost lamellae. Then a complicated labyrinth of concentric cellular processes, clearly belonging to either astrocytes or macrophages invested myelinated axons. In terminal stages, axons completely denuded of myelin were seen in the centre of a concentric network of cellular processes or spirals of myelin were seen surrounded by such processes. The myelin fragments penetrated into astrocytes or macrophages where they underwent final digestion. As those changes have been seen in several different batches of animals inoculated over the period of many years, it is highly unlikely that they represent for instance a reaction to intercurrent infection. To the contrary, we strongly believe that true demyelination is an intrinsic pattern of the Fujisaki strain of CJD (GSS) passaged in mice.

From this perspective, alterations observed in hamsters seem to be "abortive". The pathological process affects mylin (vacuoles) and axons (neuroaxonal dystrophy and Wallerian degeneration) but myelin lamellae are not stripped and axons are not denuded of myelin. This situation may reflect different cellular environment in the hamster brain in comparison to the mouse brain. Indeed, the morphology of macrophages in either species is different (see the accompanying paper) and the latter fact may in turn reflect a different pattern of bioactive compounds like cytokines released from those cells. Irrespective of the cause, the differences in changes between hamster and mouse models are worthy of further studies.

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